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• *Studies on Feed Spoilage . . .*

- *Heat Inhibiting Activity of Various Compounds and Commercial Products*

*November 1957*

## DIGEST

Several compounds which offered some promise as inhibitors of heating and the growth of molds in mixed feeds and feed ingredients were tested for their fungistatic activity in corn meal containing various amounts of moisture. Calcium propionate and sorbic acid at a level of 0.3 percent prevented heating completely in all feed ingredients tested. Smaller amounts of the inhibitors only delayed heating. Propionic acid and propionic anhydride, each at a level of 0.1 percent, prevented heating. Sodium propionate was not effective as a fungistatic agent at a level of 0.6 percent. Propionamide and propionanilide also were not effective at a level of 0.1 percent which was the maximum level tested.

Short-chain fatty acids—butyric, valeric and caproic acids—inhibited or delayed heating when they were added to corn meal at a level of 0.1 to 0.2 percent. The heat-inhibiting activity of the fatty acid decreased as the number of carbons in the fatty acids increased. Citric and succinic acid did not inhibit heating. Acetyl acetone and benzyl mucochlorate inhibited heating when added to corn meal at a level of 0.1 percent. Compounds which inhibited heating were more effective when the moisture content of the ingredient was only slightly above the critical level. They were less effective at higher moisture contents.

Four products which the manufacturer claimed inhibited heating were tested for their fungistatic activity. None of these materials was active at a level several times that recommended by the manufacturer as being effective.

## ACKNOWLEDGMENTS

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# Studies on Feed Spoilage . . .

## Heat Inhibiting Activity of Various Compounds and Commercial Products

L. R. RICHARDSON and JOHN V. HALICK\*

THE DISCOVERY OF A LOW-COST compound that would inhibit the growth of molds and the accompanying heating, which would not decrease the value of grain or feed for animal consumption, would have great economic importance in the grain, feed, animal and human food industries.

Milner, Christensen and Geddes (1947) tested approximately 100 compounds for their mold inhibitory action on wheat stored with a moisture content of 16 to 25 percent. On the basis of low toxicity to seed with a moisture content of 20 percent and a fair to high toxicity to molds, eight compounds were tested extensively as mold inhibitors. The order of their decreasing activity were: 8-hydroxyquinoline sulfate, thio-urea, p-aminobenzoic acid, sulfonilamide, benzene sulfonamide, 2-aminothiazole, chloramine B and calcium propionate.

Wolford (1945) used sodium propionate to reduce the growth of micro-organisms in fruits and vegetables, and Lenhart and Cosens (1949) used propionic acid to inhibit the growth of molds in sugar solutions.

Dubos (1950) reported that short-chain organic acids, such as acetic, propionic, butyric, caproic and capric, inhibited the growth of mammalian tubercle bacilli to a marked degree. More recently sorbic acid has been used extensively to inhibit the growth of molds in various foods, Phillips and Mundt (1950), Jones and Harper (1952), Smith and Rollin (1954a) (1954b); Melnick, Luckmann and Gooding (1954); Deuel, Alfin-Salter, Weil and Smyth (1954); Boyd and Tarr (1955); Beneke and Fabian (1955), Melnick, Valteich and Hackett (1956).

It has now become common practice for commercial bakeries and other food processors to use inhibitors to prevent the growth of molds in various food products. Sorbic acid has been used recently in human foods to a greater extent than propionate because it is a more effective inhibitor and it does not impair the flavor of foods as much as propionate.

Halick and Richardson (1953) used calcium propionate to prevent heating and the growth of molds in corn meal that contained 17.4 percent

moisture. These studies were carried out at a temperature of 90° F and a relative humidity of 70 percent. Corn meal with 0.3 percent calcium propionate did not heat in 42 days. Without propionate and stored under the same conditions, it started to heat in 2 days and reached a maximum temperature of 126° F in 3 days.

The studies using various inhibitors to prevent heating in feed ingredients were continued and the results are reported in this bulletin.

### EXPERIMENTAL

#### Propionates as Inhibitor of Heating

Since earlier tests have shown that calcium propionate prevented heating in corn meal, a test was carried out to determine whether other propionates also were effective inhibitors. The propionates tested were propionic acid, propionic anhydride, calcium propionate, sodium propionate, propionamide and propionanalide. The compounds were mixed with the corn meal and heating studies were carried out in the apparatus previously described by Halick and Richardson (1953). The effect of adding various amounts of these propionates on the growth of molds and the accompanying heating in corn meal is summarized in Table 1. Propionic acid and propionic anhydride at a level of 0.1 percent inhibited the

TABLE 1. EFFECT OF VARIOUS PROPIONATES ON HEATING AND THE GROWTH OF MOLDS IN YELLOW CORN MEAL

Moisture content of corn meal, %	Inhibitor	Percent added	Started to heat, days	Mold count, 10,000 per gm
15.2	Propionic acid	None	7	525.0
15.1	" "	0.1		1.8
14.9	Propionic anhydride	None	7	360.0
14.8	" "	0.1		
15.2	Calcium propionate	None	7	525.0
15.1	" "	0.1	7	525.0
15.0	" "	0.3		3.5
14.8	Sodium propionate	0.1	7	565.0
14.8	" "	0.3	7	245.0
13.4	" "	0.6	14	184.0
15.6	Propionamide	0.1	5	800.0
14.4	" "	0.3	6	665.0
15.9	Propionanalide	0.1	6	650.0
15.8	" "	0.3	11	205.0

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growth of molds and heating in corn meal containing approximately 15 percent moisture. The same meal without an inhibitor started to heat in 7 days and reached a maximum temperature of 120° F in 10 days. Calcium propionate inhibited the growth of molds and heating at a level of 0.3 percent, but was not effective at a level of 0.1 percent. Sodium propionate did not inhibit the growth of molds and heating at a level of 0.6 percent. Both propionamide and propionanilide were not effective as mold inhibitors at a top level of 0.3 percent under the conditions used in these experiments. Why calcium propionate was more effective than sodium propionate is not understood. It is well known that propionates are more effective inhibitors of the growth of molds in an acid environment and it is possible that calcium propionate may provide a more acid environment than the sodium salt.

### Heat-inhibiting Action of Various Compounds

Short-chain fatty acids of the same homologous series as propionic acid have been reported to inhibit the growth of various microorganisms. In view of these reports, several fatty acids and

TABLE 2. EFFECT OF VARIOUS COMPOUNDS ON HEATING AND THE GROWTH OF MOLDS IN YELLOW CORN MEAL

Moisture content of corn meal, %	Inhibitor	Percent added	Started to heat, days	Mold count, 10,000 per gm
15.2	Butyric acid	None	11	525.0
15.2	" "	0.10		60.0
15.2	" "	0.30		0.3
18.3	" "	None	2	1550.0
18.9	" "	0.10	12	650.0
18.0	" "	0.30		
14.0	Valeric acid	None	8	
14.0	" "	0.10		
13.4	Caproic acid	None	12	
13.4	" "	0.10	22	
13.4	" "	0.20		
16.3	Sorbic acid	None	3	
16.3	" "	0.10	17	
16.3	" "	0.20	33	
16.3	" "	0.30		
15.2	Acetyl acetone	None	9	100.0
15.2	" "	0.02	10	360.0
15.2	" "	0.05	24	325.0
15.2	" "	0.10		5.0
15.2	Benzyl mucochlorate	None	10	550.0
15.2	" "	0.02	12	235.0
15.2	" "	0.05	19	155.0
15.2	" "	0.10		1.5
15.7	Citric acid	None	6	
15.6	" "	0.25	7	
15.5	" "	0.50	7	
15.3	" "	0.75	7	
15.5	" "	1.00	7	
15.4	Succinic acid	None	7	
15.6	" "	0.25	7	
15.5	" "	0.50	7	
15.6	" "	0.75	7	
15.7	" "	1.00	7	

other compounds which offered some promise were tested as inhibitors of heating in corn meal. The results are summarized in Table 2.

Butyric acid at a level of 0.1 percent prevented heating in corn meal containing 15 percent moisture. When the moisture content of the meal was increased to 18 percent, 0.1 percent of butyric acid delayed the onset of heating, but 0.3 percent was required to prevent it completely. Valeric acid at a level of 0.1 percent also prevented heating in corn meal that contained 14 percent moisture. This acid was not tested with meals that contained a larger amount of moisture. Caproic acid did not prevent heating at a level of 0.1 percent in corn meal containing 13.4 percent moisture. At a level of 0.2 percent, however, it prevented heating. Caproic acid was less effective than butyric or valeric acid. The heat inhibiting activity of these acids appeared to decrease as the number of carbons in the chain increased.

Sorbic acid at a level of 0.3 percent prevented heating for at least 42 days when the corn meal contained 16.3 percent moisture. At a level of 0.1 and 0.2 percent, sorbic acid delayed heating from 3 to 17 and 33 days, respectively. Acetyl acetone and benzyl mucochlorate, each at a level of 0.1 percent, prevented heating and the growth of molds completely in corn meal containing 15.2 percent moisture. The latter two compounds were not effective inhibitors of heating at a level of 0.02 or 0.05 percent. Citric and succinic acids did not prevent heating and the growth of molds when added to corn meal at a level of 1.0 percent.

### Propionate and Sorbic Acid as Inhibitors of Heating in Different Ingredients

Tests were carried out to determine whether calcium propionate and sorbic acid prevented heating in a variety of feed ingredients. Both compounds were added separately to the ingredient at a level of 0.3 percent. The flasks containing the ingredients with and without the inhibitor were then stored in the heating apparatus under the same conditions used in other studies. The ingredient used and days required for the various treatments to start heating are given in Table 3.

The data show that calcium propionate and sorbic acid prevented heating in every ingredient used when its moisture content was only slightly above the critical level. When the moisture content of an ingredient was 1 to 3 percent above the critical level, the inhibitors were much less effective. For example, meat and bone scraps have a critical moisture level of 10.0 percent. When the moisture content was increased to 11.1 percent, it started to heat in 17 days without the inhibitors, and did not heat in 42 days with either inhibitor. When the moisture was increased to 12.5 percent, the sample con-

TABLE 3. INHIBITION OF HEATING IN FEED INGREDIENTS CONTAINING VARIOUS AMOUNTS OF MOISTURE WITH PROPIONATE AND SORBATES

Ingredient	Moisture content, %	Days to start heating		
		Without inhibitor	With inhibitors	
			Calcium propionate, 0.3%	Sorbic acid, 0.3%
Wheat shorts	15.3	20		
Flaked barley	14.9	11		
Ground oats	14.9	12		
Fish meal	13.6	12		
Sorghum gluten meal	11.9	19		
Linseed meal	12.4	19		
Meat & bone scraps	11.1	17		
Meat & bone scraps	12.5	9	15	15
Soybean meal	15.0	30	30	
Cottonseed meal	12.4	21		
Cottonseed meal	14.7	8	6	10

taining the inhibitors started to heat in about the same time as the sample containing 11.1 percent moisture and no inhibitor.

The results with soybean and cottonseed meals containing large amounts of water were similar to those with meat and bone scraps. The results show that calcium propionate and sorbic acid inhibit the growth of molds and heating in practically all feed ingredients. However, the activity of both compounds depended on the amount of inhibitor present and on the moisture content of the ingredient.

### Heat-inhibiting Action of Propionate in the Presence of Sodium Acetate and Yeast Extract

It was observed in studies which were carried out in a nutrient medium with an isolated mold, *Aspergillus niger*, that the addition of sodium acetate or a yeast extract to the medium destroyed the mold inhibiting action of the propionate. As a result of this observation, sodium acetate and a yeast extract were each added to corn meal containing 0.3 percent calcium propionate. The meal contained 15.8 percent moisture and heated readily without the propionate. When the propionate was present, the mixture did not

TABLE 4. HEAT INHIBITING ACTION IN CORN MEAL BY PROPIONATE IN THE PRESENCE OF ACETATE AND YEAST EXTRACT

Moisture content of corn meal, %	Calcium propionate, %	Supplement	Started to heat, days
15.8	None	None	4
15.8	0.3	None	1
15.8	0.3	Yeast extract 1%	
15.8	0.3	Sodium acetate 1%	

No heating observed

heat even though sodium acetate or yeast extract was present. These data show that the sodium acetate or yeast extract did not destroy the inhibiting action when the propionate was added to an ingredient. These results are summarized in Table 4.

### Heat-inhibiting Activity of Commercial Products

Several commercial products which were claimed by the manufacturer to inhibit heating and the growth of molds were tested. These data are summarized in Table 5. None of the products tested was effective even at levels that would make their use prohibitive.

### Use of Inhibitors in Feeds

The question may be asked whether the use of inhibitors in mixed feeds would be practical. Calcium propionate and sorbic acid are allowed by the Food and Drug Administration to be used as fungistatic agents in human foods and presumably are permissible for animal feeds. Therefore, it appears that these two compounds could be used to inhibit heating in animal feed, provided the cost would not be too great. Whether the cost is too great can be determined only by the feed manufacturer and the manufacturer of the inhibitor. In private conversations with one of the authors (LRR), manufacturers have expressed doubt that it would be feasible to use propionates as inhibitors of heating in mixed feeds under present conditions. It appears that sorbic acid would be prohibitive at the present time for the same reason.

TABLE 5. ACTIVITY OF COMMERCIAL PRODUCTS WHICH THE MANUFACTURER CLAIMED WOULD INHIBIT HEATING

Moisture content of corn meal, %	Amount of product added	Started to heat, days
15.47	None	3
15.26	3/4 lb per ton <sup>1</sup>	4
15.51	5 lb per ton	4
15.44	1 lb per ton <sup>1</sup>	4
15.43	5 lb per ton	3
15.64	None	3
16.00	1.0%	2
16.25	1.5%	3
16.19	2.0%	3
16.64	2.5%	3
16.18	None	4
15.93	300 ppm	4
16.14	350 ppm	4
16.11	400 ppm	4
16.02	500 ppm	4
16.91	None	4
16.73	0.005%	4
16.69	0.01%	4
16.72	0.05%	4
16.55	0.10%	4

<sup>1</sup>Amount recommended as being effective.

The cost of using acetyl acetone at the rate of 0.1 percent would be approximately \$3.50 per ton and the cost of using benzyl mucochlorate would be about the same or more at the present time. Therefore, these two compounds and fatty acids would be too expensive.

It appears that the use of materials with moisture contents below the critical level is the only practical procedure to prevent heating in mixed feeds and feed ingredients. The critical moisture contents of approximately 30 feed ingredients are given in Texas Station Bulletin 860.

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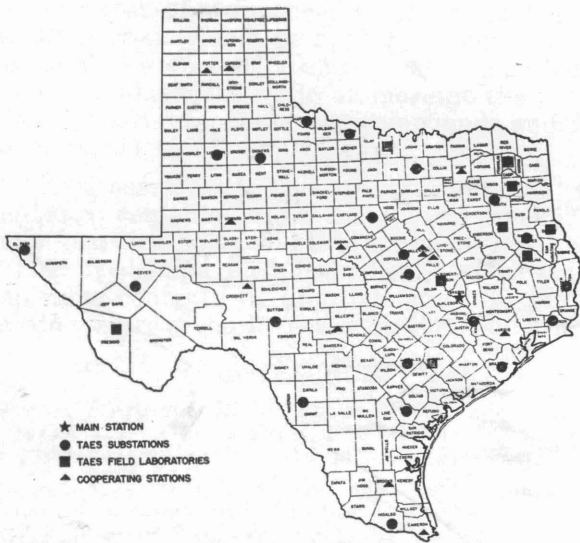
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# State-wide Research



Location of field research units of the Texas Agricultural Experiment Station and cooperating agencies



The Texas Agricultural Experiment Station is the public agricultural research agency of the State of Texas, and is one of ten parts of the Texas A&M College System

## ORGANIZATION

IN THE MAIN STATION, with headquarters at College Station, are 16 subject-matter departments, 2 service departments, 3 regulatory services and the administrative staff. Located out in the major agricultural areas of Texas are 21 substations and 9 field laboratories. In addition, there are 14 cooperating stations owned by other agencies. Cooperating agencies include the Texas Forest Service, Game and Fish Commission of Texas, Texas Prison System, U. S. Department of Agriculture, University of Texas, Texas Technological College, Texas College of Arts and Industries and the King Ranch. Some experiments are conducted on farms and ranches and in rural homes.

## OPERATION

THE TEXAS STATION is conducting about 400 active research projects, grouped in 25 programs, which include all phases of agriculture in Texas. Among these are:

- |                                      |                                 |
|--------------------------------------|---------------------------------|
| Conservation and improvement of soil | Beef cattle                     |
| Conservation and use of water        | Dairy cattle                    |
| Grasses and legumes                  | Sheep and goats                 |
| Grain crops                          | Swine                           |
| Cotton and other fiber crops         | Chickens and turkeys            |
| Vegetable crops                      | Animal diseases and parasites   |
| Citrus and other subtropical fruits  | Fish and game                   |
| Fruits and nuts                      | Farm and ranch engineering      |
| Oil seed crops                       | Farm and ranch business         |
| Ornamental plants                    | Marketing agricultural products |
| Brush and weeds                      | Rural home economics            |
| Insects                              | Rural agricultural economics    |
|                                      | Plant diseases                  |

Two additional programs are maintenance and upkeep, and central services.

*Research results are carried to Texas farmers, ranchmen and homemakers by county agents and specialists of the Texas Agricultural Extension Service*

AGRICULTURAL RESEARCH seeks the WHATS, the WHYS, the WHENS, the WHEREs and the HOWS of hundreds of problems which confront operators of farms and ranches, and the many industries depending on or serving agriculture. Workers of the Main Station and the field units of the Texas Agricultural Experiment Station seek diligently to find solutions to these problems.

# Today's Research Is Tomorrow's Progress