TEXAS AGRICULTURAL EXPERIMENT STATION
R. D. LEWIS, Director, College Station, Texas

lletin 768

LIBRARY
A. & M. COLLEGE OF TEXAS

Influence of Moisture on Heating in Feeds

0. 72
5 b
768

ust 1953

The TEXAS AGRICULTURAL AND MECHANICAL COLLEGE SYSTEM
GIBB GILCHRIST, Chancellor

DIGEST

Feed ingredients did not heat below a certain critical moisture level, which varied with different ingredients. Heating became more rapid and intense as the moisture level increased from the critical level to that where maximum heating occurred.

Ground oats, wheat and corn heated faster and to a higher temperature than the unground grains containing the same amount of moisture. The number of molds in ingredients which heated was larger in every instance than it was in those which did not heat.

Mixtures of corn meal and molasses did not heat when 15 and 20 percent of molasses containing 25.5 percent moisture were added to corn meal with 8.7 percent moisture. Corn meal containing 13.2 percent moisture heated; when this meal was mixed with 5, 10, 15 and 20 percent molasses containing 27.4 percent moisture, all the mixtures heated. Heating occurred also when the same corn meal was mixed with 5, 10, 15 and 20 percent molasses with 21.0 percent moisture. Mixtures containing molasses with 21.0 percent moisture started to heat on the 12th day, while those containing molasses with 27.4 percent moisture began heating on the 7th day.

The addition of 10 and 20 percent molasses with 21.0, 25.2 and 30.7 percent moisture delayed heating in wheat bran containing 17.2 percent moisture. Molasses with 21.0 percent moisture delayed heating longer than those with higher levels of moisture, and 20 percent of molasses delayed heating longer in every case than 10 percent of molasses.

Corn meal containing as much as 17.4 percent moisture was completely inhibited from heating for 42 days by the addition of 0.3 percent of calcium propionate. The same meal without the inhibitor heated rapidly to a high temperature. The addition of 0.1, 0.15 and 0.20 percent calcium propionate delayed heating but did not prevent it.

The problems involved in the heating processes are complex. To eliminate heating, standards for the moisture content of all ingredients used in feeds should be reevaluated. The absence of heating in molasses feeds will not be insured by establishing a standard for the moisture content of molasses alone. Standards for molasses for animal feeds should be based on the total invert sugar after inversion and on moisture. Moisture should be as low as practical. Some feed manufacturers are equipped to use molasses containing as little as 20 to 22 percent moisture, especially during the summer, but others are not equipped to use them with less than 24 to 26 percent moisture at any time. Probably the only way a workable standard for molasses can be established is by joint consideration of the problems involved by all the interested groups.

Influence of Moisture on Heating in Feeds

J. V. Halick and L. R. Richardson*

SPONTANEOUS HEATING in feed ingredients and mixed feeds is a major problem in many areas of Texas and other states (Richardson and Halick 1952). Spontaneous heating of various materials may be caused by: biological activity or respiration of living materials; by the metabolic heat produced as the result of the growth of microorganisms; or by chemical oxidation. The amount of heat produced by the biological activity or respiration of living materials is relatively insignificant and does not cause serious losses under practical conditions. Investigators of heating in grains and other feed ingredients are agreed that the most destructive heating and deterioration processes are caused by the growth of molds.

The early literature on the influence of microorganisms in the heating of moist grains has been reviewed by several investigators, (Carlyle and Norman, 1946; Milner and Geddes, 1946ab; Oxley, 1948; and Christensen, Olafson and Geddes, 1949) and will not be reviewed in detail in this bulletin. In a study with wheat maintained under adiabatic conditions and containing 22 percent moisture, Milner, Christensen and Geddes (1947) concluded that the increase in temperature was directly correlated to the growth of molds until a temperature of 125-131° F. (52-55° C.) was attained. At this temperature, the molds were killed and the heating ceased. With wheat of a higher moisture content, it was possible for bacterial growth to increase the temperature to 154-158° F. (68-70° C.). At this temperature, bacteria were killed and the heating ceased unless strictly controlled adiabatic conditions were maintained. Under these conditions, the temperature continued to rise spontaneously due to nonbiological or chemical oxidation.

Causes of Deterioration in Moldy Feeds

Rapidly growing molds produce substances called enzymes, and these hydrolyze carbohydrates, fats and possibly proteins, thereby changing the composition of the feed. Some of the nutritive value of a feed is lost when it heats, but practically all the damage as the result of heating is caused by the action of enzymes produced by molds, rather than by the heat itself. Feeds which have molded may be consumed poorly or be refused by animals, but it is generally assumed that molds which usually grow on grains and feed ingredients are non-toxic.

---

*Respectively, technician and professor, Department of Biochemistry and Nutrition.

Procedure Used to Study Heating in Feeds

The apparatus used to study heating in feeds is composed of a multiple point Electronik precision indicator, vacuum flasks (Dewar) and a storage cabinet which can be controlled at a constant temperature and relative humidity. Two kilos of an ingredient are put in the vacuum flasks along with a thermocouple wire attached to *the Electronik indicator. The flask containing the ingredient and* the thermocouple is placed in the storage cabinet, which is maintained at 90° F. and a relative humidity of 70 percent. The apparatus used in these studies is shown in Figures 1 and 2. With this apparatus, it is possible to duplicate data and to determine the causes of and the conditions necessary for heating in feedstuffs with a high degree of accuracy.

Critical Moisture Level

The critical moisture level of a material is the moisture content at which it is barely safe or barely unsafe from the growth of molds and the accompanying heating. The number of days required for various ingredients containing different amounts of moisture along with the maximum temperatures, together with the number of days required to reach the maximum temperature and the number of molds per gram of material, are summarized in Table 1. In the preliminary studies, the temperature of the ingredient in each flask was recorded three times daily. However, one reading gave

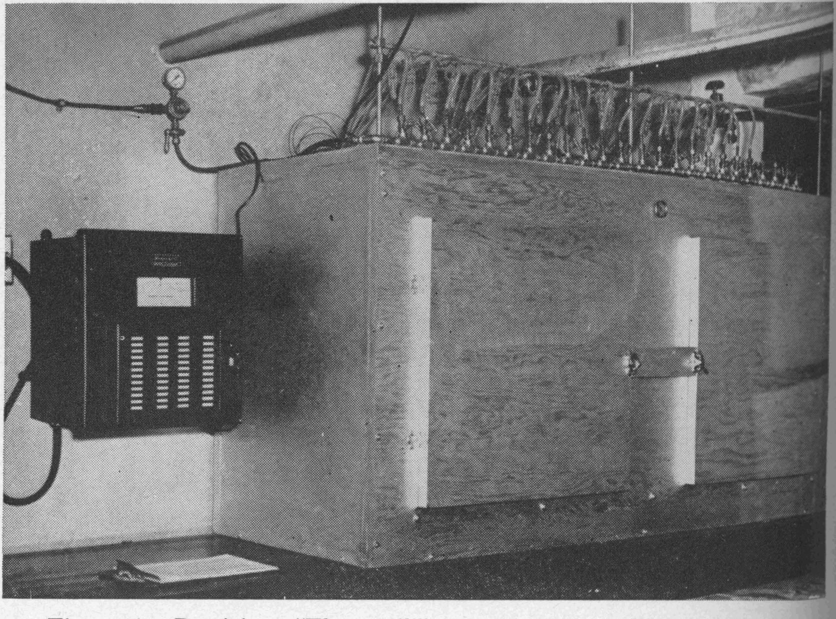

Figure 1. Precision "Electronik" temperature indicator and storage cabinet used in studies of heating in feed ingredients and mixed feeds.

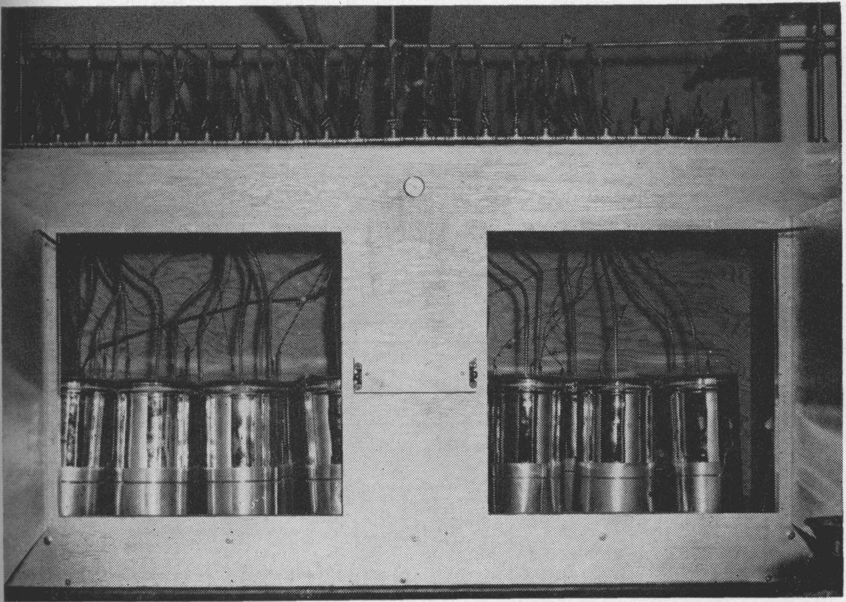

Figure 2. Inside of storage cabinet showing vacuum flasks (Dewar) which hold the ingredients and mixed feeds during studies on heating.

sufficient information for practical purposes, and only one reading was used in the later studies. Each ingredient tested so far heated to a maximum temperature which varied with its moisture content, then it cooled off gradually until the temperature had returned to that of the storage cabinet. The cooling-off process required several weeks, and tied up equipment which could be used for studying the rate of heating in other materials. A study of the rate of heating appeared to be of greater importance than the rate of cooling off; therefore, to use the equipment available at maximum capacity for heating studies, an ingredient which had heated to a maximum was discontinued after the temperature had declined continuously for 3 or 4 consecutive days. Some samples which heated were taken for mold counts and moisture content 10 days after the test was started, while others which did not heat were taken at 42 days.

Table 1 shows that an ingredient did not heat below a certain critical moisture level. It heated when the moisture content was slightly above the critical level, but the time required for the heating to start and to reach the maximum temperature was relatively long and the maximum temperature was relatively low. As the moisture level increased, an ingredient started to heat and reached a high maximum temperature in a relatively short time. For example, ground yellow corn meal with 12.3 percent moisture did not heat in 42 days. When the moisture was increased to 13.3 percent, it started to heat in 12 days and reached a maximum temperature of 108° F. in 19 days. When the moisture was increased to 15.8 percent, the corn meal started to heat in 3 days and reached a maximum temperature of 117° F. in 5 days.

Table 1 shows that the rate of heating and the maximum temperature reached depended on the moisture content of an ingredient. Even though different ingredients started to heat at different moisture levels, the overall picture for the heating cycle was essentially the same for all ingredients.

Table 1. Heating and number of molds in feed ingredients containing various amounts of moisture

Moisture, %	Started heating, days	Max. temp., %	Reached max. temp., days	No. molds per gm.
		Corn, whole		
14.4	----	------	----	230,000
15.0	16	95	34	1,633,333
17.8	6	107	19	18,333,333
		Corn, ground		
12.3	----	------	----	33,670
13.3	12	108	19	3,566,000
14.7	6	115	9	3,000,000
15.8	3	117	5	19,670,000
		Milo, whole		
12.3	----	------	----	350,000
13.2	20	94	24	11,066,666
14.2	10	96	24	17,833,333
14.8	9	99	14	3,430,000
16.6	4	114	14	12,230,000
		Milo, ground		
12.7	----	------	----	36,700
13.7	18	113	24	1,223,000
14.3	11	116	14	3,000,000
14.9	7	120	9	6,800,000
		Oats, whole		
14.5	----	------	----	157,000
14.8	5	101	38	8,830,000
17.0	4	108	14	5,670,000
19.7	3	110	14	12,330,000
		Oats, ground		
11.5	---	------	----	66,670
12.6	18	109	28	5,367,000
14.9	4	117	7	8,600,000
17.5	3	120	4	14,230,000
		Steamed bone meal		
8.2	----	------	----	----------------
9.3	7	103	8	4,400
10.2	4	113	5	316,000
		Wheat, whole		
14.1	----	------	----	
14.8	4	100	7	
15.7	4	101	5	
16.7	3	105	5	
17.5	3	106	5	

Table 1. **Heating and number of molds in feed ingredients containing various amounts of moisture, continued**

Moisture, %	Started heating, days	Max. temp., %	Reached max. temp., days	No. molds per gm.
Wheat, ground				
11.9	----	------	----	22,000
12.5	20	95	25	....
13.5	12	107	15	23,000
14.0	5	112	8	93,300
14.7	3	114	5	123,000
15.3	4	118	6	5,167,000
Wheat bran				
11.6	----	------	----	16,000
13.4	26	106	41	220,000
14.0	21	109	29	3,670,000
15.3	15	111	21	22,000,000
16.2	8	114	12	15,670,000
17.8	6	120	7	109,300,000
Wheat shorts				
12.3	----	------	----	33,000
13.0	31	109	38	1,347,000
13.5	28	106	36	2,500,000
14.0	18	109	24	7,966,000
17.6	5	118	6	13,067,000
19.3	4	122	5	44,670,000

The critical moisture level of a few ingredients are summarized in Table 2. These were determined at a temperature of 90° F. to approximate the conditions that occur in many areas when heating in feeds is most frequent. A relative humidity of 70 percent was used because it was found that the moisture content of the ingredients in the vacuum flasks did not change appreciably at this relative humidity during a storage period of 42 days. These data show that ground and underground grains and grain by-products heat at levels of moisture lower than those which have been accepted traditionally as safe for grains of higher grades.

Mold and Bacterial Counts

Mold counts were made on samples which were taken when the observations were discontinued. They were made by a modification of the method described by Bottomley, Christensen and Geddes,

Table 2. Critical moisture level of feed ingredients

Ingredient	Critical moisture %
Bone meal	8.7
Corn, whole	14.7
Corn, ground	13.0
Milo, whole	12.7
Milo, ground	13.0
Oats, whole	14.5
Oats, ground	12.3
Wheat, whole	14.3
Wheat, ground	12.0
Wheat bran	13.0
Wheat shorts	12.7

1952. The modifications were: potato-dextrose agar, adjusted to a pH of 3.5 with citric acid, was used in the culture medium instead of malt-salt or malt-salt-boric acid media, which was recommended by these authors. In our experience, the potato-dextrose agar gave a count which was higher and more easily read. The plates were incubated for 3 days at room temperature, and the number of mold colonies on each dish was determined by the use of a Quebec colony counter.

For the serial dilutions, 48 ml. of a hot 0.15 percent agar solution were autoclaved in a 4-oz. screw capped medicine bottle. After cooling, each bottle contained approximately 45 ml. of the agar solution. The original dilution of a sample was made by adding 5 gms. of material to the agar solution (approximately 45 ml.) in a medicine bottle. Each dilution was thoroughly shaken to obtain a uniform suspension. Serial dilutions were made for each sample by adding 5 ml. of the original suspension and each subsequent dilution to the sterile agar solution in a medicine bottle. The number of molds was determined in 3 dilutions of each sample. For culturing, 1 ml. of the suspension was pipetted into a sterile Petri dish and potato-dextrose agar was added. Each dilution was run in triplicate and plates which contained less than 30 colonies were rejected. In general, plates which contained more than 50 or 60 colonies also were rejected, but when certain species of molds predominated, a larger number could be counted.

Thioglycolate agar (Baltimore Biological Laboratory) was used as the culture medium for the bacterial counts. The procedures used for the serial dilutions and culture of the bacteria were essentially the same as those used for the mold counts, with the exception that the dilutent was 0.1 percent tryptone in 0.15 percent agar solution. Thioglycolate medium was used for the bacterial counts because it gives the total number of bacteria, including anaerobes and aerobes (Pittman, 1946).

There were discrepancies in the values obtained for the number of molds in a few ingredients. For example, the ground yellow corn reported in Table 1 was from the same lot as the whole corn. The whole corn contained 230,000 mold spores per gram, while the same corn ground contained only 33,670. The values for mold counts were obtained on samples which had been in the heating apparatus. If an ingredient did not heat, it had been in the heating apparatus for at least 42 days before the sample was taken. It is possible that mold spores actually increased on the whole corn containing 14.4 percent moisture, even though the temperature did not increase and there was no visible evidence that mold growth had occurred. For this reason, it is not interpreted that the absolute number of molds has any important significance. The important point is that, within a series, the highest temperature and also the largest number of molds were obtained, in general, in the samples which contained the highest level of moisture.

Both bacterial and mold counts were made on two samples of bone meal. One sample contained 7.0 percent moisture and did not heat. It contained 4,000 molds and 74,500 bacteria per gram. The other sample contained 10.2 percent moisture and had heated to 113° F. in 5 days. It contained 316,000 molds and 81,500 bacteria per gram. These data indicate that the growth of molds rather than the growth of bacteria was the cause of the heating. Further evidence which supports this conclusion is that the moisture content of all the ingredients studied was lower than that usually considered necessary to support the growth of bacteria, and the temperature to which the ingredients heated was within the range normally produced by the growth of molds.

Since heating had ceased and the temperature had started to decline before the samples were taken for mold counts, it is obvious that mold spores were not destroyed. The samples with the higher levels of moisture heated to a higher temperature than those with less moisture. The temperature at which the samples stopped heating appears to be more closely related to the moisture content and physical characteristic of the ingredient than to the absolute temperature produced.

Another important point is that ingredients which did not heat still contained a large number of mold spores. This fact emphasizes the need of maintaining conditions in grains, feed ingredients and mixed feeds so that mold spores cannot germinate and grow.

Heating in Ground and Whole Grains

The fact that many feed ingredients heated at moisture levels which ordinarily would be considered safe for grains, suggested that the heating cycle in ground materials might be different from that in the unground grains. In view of these observations, the heating cycles in ground and whole oats, wheat and corn were compared. The data for oats and wheat are summarized in Figure 3. Ground oats or wheat, with essentially the same amount of moisture as the whole grains heated more rapidly and to a much higher temperature than the whole grains. Ground oats heated to above 115° F. within 5 to 7 days, while the whole oats increased only a few degrees in temperature during the test period. The course of heating in ground and whole wheat and in ground and whole corn was essentially the same as that for oats. Under practical conditions, whole corn with 14.5 to 15.0 percent moisture might be relatively safe from heating, but the same corn probably would heat after it was ground.

Heating in Mixtures Containing Molasses

Surveys made by the Texas Agricultural Experiment Station (Richardson and Halick, 1952) show that feeds containing molasses heat more frequently than those containing any other ingredient. Studies were carried out to determine the influence of the moisture

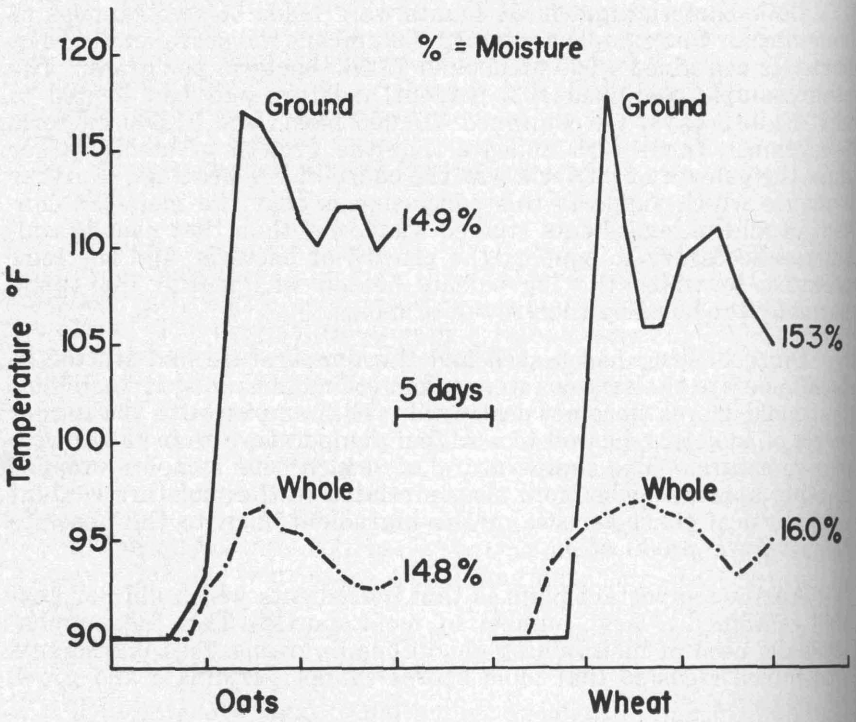

Figure 3. Ground oats and wheat heated more rapidly and intensely than the whole grains.

content of molasses on heating in mixtures of corn meal and molasses. These data are summarized in Table 3. None of the mixtures heated when 15 or 20 percent of molasses containing 25.5 percent moisture was added to corn meal containing 8.7 percent moisture. A similar study was made with mixtures of corn meal that contained 13.2 percent moisture and 5, 10, 15 and 20 percent of molasses that contained 21.0 and 27.4 percent moisture. Unfortunately, the corn meal without molasses started to heat in 18 days and reached a maximum temperature of 104° F. in 24 days, but the results show that the addition of molasses accelerated heating under these conditions. When molasses were added, the mixtures started to heat and reached higher maximum temperatures sooner than the corn meal alone. Mixtures containing molasses with 27.4 percent moisture started to heat in approximately 7 days, while mixtures containing molasses with 21.0 percent moisture did not begin to heat until the 12th day. Tests are in progress to determine the maximum moisture content of corn meal that will be safe when it is mixed with various amounts of molasses containing different amounts of moisture.

Another test was carried out to determine the influence of molasses on heating when they were mixed with wheat bran that

Table 3. Heating in mixtures of corn meal and molasses

Molasses added, %	Moisture content of mixture %	Started heating, days	Max. Temp., °F.	Reached max. temp., days
Moisture content of molasses, 25.5%				
0	8.7			
15	11.2			
20	11.5			
Moisture content of molasses, 21%				
0	13.2	18	104	24
5	13.8	13	110	16
10	14.4	11	118	14
15	14.6	12	117	15
20	14.8	13	118	17
Moisture content of molasses, 27.4%				
0	13.2	18	104	24
5	14.1	8	114	11
10	14.9	7	115	9
15	15.3	7	115	8
20	15.5	7	115	9

was high in moisture. The moisture content of the molasses used was 21.0, 25.2, and 30.7 percent, and that of the bran was 17.2 percent. These results are summarized in Figure 4. The bran without molasses started to heat on the 4th day and reached a maximum temperature of 118° F. on the 6th day. The addition of molasses delayed heating, regardless of their moisture content, but the molasses with low moisture delayed heating longer than those with high moisture. Also 20 percent of molasses at every moisture level delayed heating longer than 10 percent of molasses. It is apparent that the effect of molasses on heating in mixtures containing molasses and another feed ingredient depend to a large extent on the moisture content of the ingredient. If the moisture content of the ingredient (corn meal) is near the critical level the addition of molasses may hasten heating, but if the moisture content of the ingredient (wheat bran) is high, the addition of molasses may delay heating. In any case, these data show that a feed mixed with low moisture molasses will not heat as rapidly as one mixed with a high moisture molasses, but the use of a low moisture molasses alone will not eliminate heating entirely. In the final analysis, the moisture content of the other ingredients are just as critical a factor as that in molasses, and the moisture content of ingredients will have to be such that the total moisture in the mixed feed will be below the critical level.

The Brix values shown in Figure 2 are for this particular sample of molasses, which was adjusted to contain 30.7, 25.2 and 21.0 percent moisture. These Brix values would not necessarily be the same for another sample of molasses containing the same amount of moisture. Moisture was determined by the vacuum drying method.

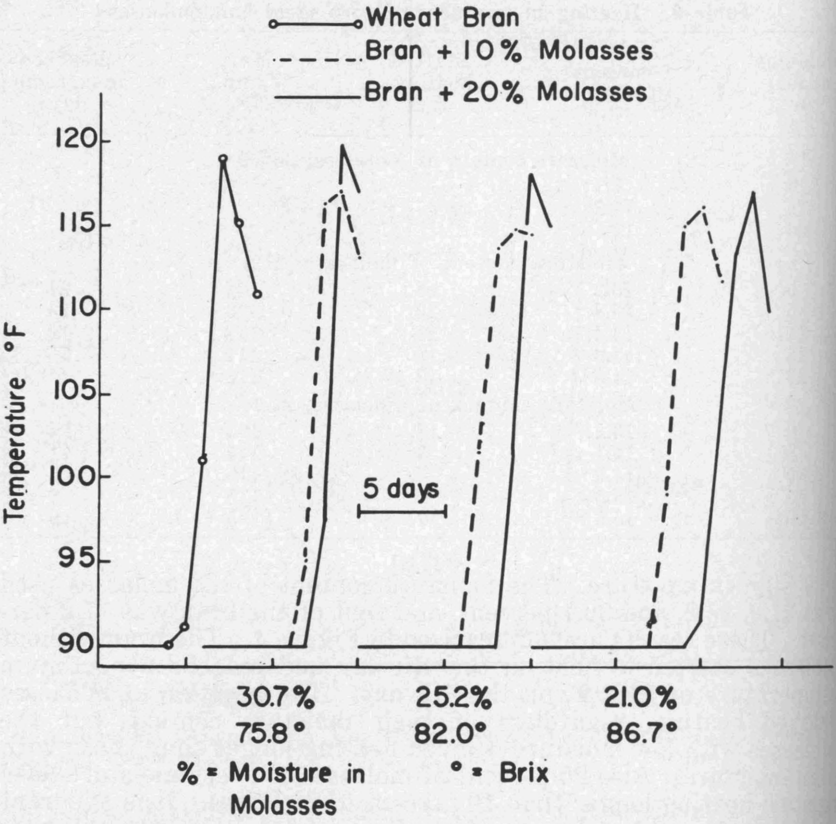

Figure 4 Heating in wheat bran containing 17.2 percent moisture was delayed by the addition of 10 or 20 percent of molasses.

Inhibitors

A few studies were carried out to determine whether inhibitors could be used to prevent the growth of molds and heating in feeds. Propionates, particularly calcium propionate, are used to delay the growth of molds in certain products. The results of studies with calcium propionate as an inhibitor of the growth of molds in corn meal are summarized in Table 4. It is seen that 0.3 percent of calcium propionate completely prevented heating in corn meal containing 13.3, 16.0 and 17.4 percent moisture for at least 42 days. Without the calcium propionate, the meal started to heat in 2 to 12 days and reached a maximum temperature in 3 to 19 days. The time required for the corn meal which did not contain propionate to heat depended on its moisture content. Calcium propionate at levels of 0.1, 0.15 and 0.2 percent delayed heating, but a level of 0.3 percent was required to prevent the growth of molds and heating. The mold counts show that the calcium propionate did not kill the mold spores, but simply prevented their germination and growth.

Table 4. Calcium propionate as an inhibitor of the growth of molds

Moisture in corn meal, %	Calcium propionate, %	Started heating, days	Max. temp. °F.	Reached max. temp., days	No. molds per gm.
13.3	0	12	108	19	3,570,000
	0.30	----	------	----	28,300
16.0	0	3	117	5	19,670,000
	0.30	----	------	----	10,000
17.4	0	2	126	3	66,670,000
	0.30	----	------	----	23,000
15.3	0.10	13	112	17	30,670,000
	0.15	25	110	32	31,000,000
	0.20	37	100	48	28,000,000

Standard for Molasses

There has been a demand during the past 2 years for a standard for molasses used in animal feeds. The data described in this bulletin illustrate the complex nature of the problems involved in the heating process. To eliminate heating, standards for the moisture content of all ingredients used in feeds should be reevaluated. The absence of heating in molasses feeds will not be insured by establishing a standard for the moisture content of molasses alone. Any standard for molasses for animal feeds should be based on nutritive value. Since carbohydrate is the principal nutrient supplied by molasses, it should be the first nutrient considered. Moisture as well as carbohydrate should be included in the standard, and the maximum should be as low as is practical. Some feed manufacturers are equipped to use molasses containing as little as 20 to 22 percent moisture, especially during the summer, while others are not equipped to use them with less than 24 to 26 percent moisture at any time. Probably the only way a workable standard can be arrived at is by joint consideration of the problems involved by all the different interested groups.

Acknowledgments

This investigation was supported in part by a grant-in-aid from the Southwestern Sugar and Molasses Company, New York, through the courtesy of A. I. Kaplan, president.

We are indebted to E. I. du Pont de Nemours and Company, Wilmington, Del. through the courtesy of F. M. Jornlin, for generous supplies of calcium propionate (Mycoban) and propionic acid.

Literature Cited

Bottonley, R. A., C. M. Christensen and W. F. Geddes, 1952. Grain storage studies. X. The influence of areation, time, and moisture content on fat acidity, nonreducing sugars, and mold flora of stored yellow corn. Cereal Chem., 29:53.

Carlyle, R. E. and A. G. Norman, 1941. Microbial thermogenesis in the decomposition of plant materials. Part II. Factors involved. J. Bact. 41:699.

Christensen, C. M., U. H. Olafson and W. F. Geddes, 1949. Grain storage studies. VII. Relation of molds in moist stored cottonseed to increase production of carbon dioxide. Cereal Chem. 26:109.

Milner, M., and W. F. Geddes, 1946a. Grain storage studies. III. The relation between moisture content, mold growth and respiration of soybeans. Cereal Chem. 23:225.

Milner, M., C. M. Christensen and W. F. Geddes, 1947. Grain storage studies. VI. Wheat respiration in relation to moisture content, mold growth, chemical deterioration and heating. Cereal Chem. 24:182.

Milner, M., and W. F. Geddes, 1946b. Grain storage studies. IV. Biological and chemical factors involved in spontaneous heating of soybeans. Cereal Chem. 23:449.

Nagel, C. M., and G. Semeniuk, 1949. Some mold induced changes in shelled corn. Plant. Physiol. 22:20.

Oxley, T. A., 1948. The scientific principles of grain storage. The Northern Publishing Co. Ltd. Liverpool.

Pittman, M., 1946. A study of fluid thioglycolate medium for the sterility test. J. Bact. 51:19.

Richardson, L. R., and J. V. Halick, 1952. Moisture in molasses as a factor in the heating of feeds. Texas Agr. Expt. Sta. Bul. 754.