

## Relation of Interspace Relative Humidity to Growth of Molds and Heating in Feed Ingredients and Feed Mixtures

B. D. WEBB, MARY E. BAYLISS, and  
L. R. RICHARDSON

Department of Biochemistry and  
Nutrition, Texas Agricultural Experi-  
ment Station, Texas Agricultural  
and Mechanical College, College  
Station, Tex.

The relation of interparticle relative humidity to the growth of molds and/or spontaneous heating was determined in various feed ingredients and mixed feeds stored at 10°, 21°, and 32° C. Increasing moisture or increasing temperature increased the relative humidity, but when the relative humidity was measured at the same temperature as the storage temperature, no feed ingredient or mixed feed became moldy or heated during 6-week storage at relative humidity of 72.0% or less. Safe relative humidity was less variable than safe moisture content; therefore, humidity can be used to determine safe storage conditions for feed ingredients and mixtures.

MOST INVESTIGATORS agree that growth of molds is primarily responsible for the heating and accompanying deterioration in agricultural products during storage. Several recent reviews (1,4,5,9,10) summarize the literature in this field. Reports cited in these reviews show that the amount of available moisture or the relative humidity of the interparticle air is the most important condition that determines the growth of molds on stored grains. Comparatively few studies have been made to determine the conditions for the safe storage of mixed feeds or the ingredients used in mixed feeds. Snow, Crichton, and Wright (11,12) determined the growth of molds and the water uptake of oats, linseed cake, bone meal, and bran at different humidities. At the same relative humidity, wide variations existed in the moisture content of the different ingredients. Waite (13) showed that molds developed in 19 days on hay with 15.7% and in 280 days with 12.9% moisture. The critical moisture level of 32 feed ingredients was reported from this laboratory (6,7). The ingredients tested included grain by-products, protein concentrates, dehydrated alfalfa meals, bone meal, and distiller's dried grains. Generally the critical moisture level is referred to as the moisture content that is in equilibrium with a relative humidity of approximately 75% and for most cereal grains a moisture content of 13 to 15% is in equilibrium with this relative humidity. Similar studies on the nature of heating in simple and complex feed mixtures (14) showed that the moisture content of a mixture did not reliably indicate whether the feed was safe or unsafe from heating. Heating in a feed mixture depended on the moisture content and the concentration of each ingredient in the mixture.

In view of previous reports that the relative humidity of the interparticle

air largely determines whether molds grow on stored grains, relative humidity was investigated as a measure of the moisture conditions for safe storage of mixed feeds and feed ingredients at 10°, 21°, and 32° C.

### Materials and Methods

All feed ingredients were purchased locally and the moisture contents were below the critical level, so that no mold growth or heating occurred in the ingredients before they were used in the tests. In each test the ingredient was adjusted to a given moisture content by the addition of calculated amounts of water. The moist samples were thoroughly mixed and then allowed to equilibrate in a cold room at 7° C. for approximately 48 hours.

Equilibrium relative humidities of the feeds were measured with an electric hygrometer indicator and single humidity sensing elements. This equipment is essentially the same as that used previously for determining the relative humidity of corn (3) and the absorption and desorption isotherms of wheat (8).

Briefly, the following procedure was used in this laboratory. Approximately 150 grams of feed or ingredients were placed in a 250-ml. extraction flask immersed to the lip in a constant temperature water bath. A single narrow range humidity sensing element was inserted through the rubber stopper in the extraction flask to the surface of the feed. The sensing element was selected to cover the expected range of humidity as indicated from the moisture content. A constant hygrometer reading was obtained in approximately 2 to 5 hours. The exact time depended on the material and the temperature. In order to run relative humidities on several feeds in one day with only one hygrometer indicator, it was necessary to use 10 to 12 sensing elements and to allow the tem-

perature of the feeds in the flasks to equilibrate with that of the water bath before the readings were started. In most of this work the flasks were allowed to stand overnight in the constant temperature water bath before any readings were made. Regardless of the preliminary treatment, the hygrometer reading which remained constant for three successive 15-minute intervals was used to obtain the relative humidity from the calibration charts supplied by the manufacturer. According to the manufacturer, relative humidity values obtained by this instrument are accurate to  $\pm 1.5\%$ . A schematic diagram of the apparatus is shown in Figure 1.

Relative humidity was determined on each lot of feed or feed ingredient at 10°, 21°, and 32° C. (approximately 50°, 70°, and 90° F.). Heating properties of the same feeds were determined by the procedure described by Halick and Richardson (6). In these tests the inside of the heating apparatus was maintained at 32° C. and 70.0% relative humidity. Growth of molds in the samples stored at 10° and 21° C. was determined by visual inspection after a storage of 6 weeks. Moisture content was determined in triplicate by the official vacuum oven procedure for grain and stock feeds (2).

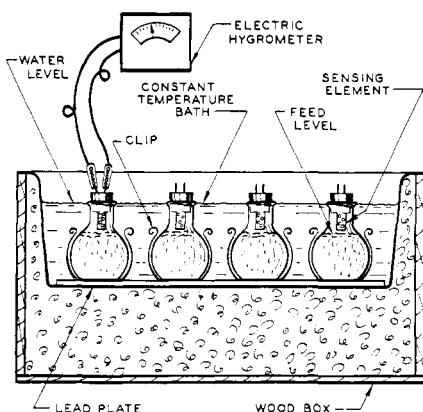
### Results and Discussion

**Storage Temperature and Interspace Relative Humidities of Feed Ingredients.** The relationship of moisture content and interspace relative humidities of seven feed ingredients at three storage temperatures are summarized in Table I and Figure 2.

The data for milo gluten meal in Table I show that relative humidity is affected by both storage temperature and moisture content of the feed. When the temperature was constant, the humidity increased with increase in moisture

**Table I. Relative Humidities of Milo Gluten Meal Containing Different Amounts of Moisture at 10°, 21°, and 32° C.**

Moisture, %	Relative Humidity, %		
	10° C.	21° C.	32° C.
9.9	51.4	58.9	66.1
11.0	56.7	61.0	70.4
11.5	57.5	63.7	71.5
12.0	61.4	65.8	73.5
12.5	63.8	68.9	75.8
13.2	64.7	69.8	78.7
13.5	66.3	71.9	79.9
14.5	69.2	74.9	81.5
14.9	71.3	75.1	82.6
16.1	73.0	77.8	83.4
16.8	74.5	79.6	84.5
18.3	74.8	80.0	86.4



**Figure 1. Apparatus for determining relative humidity of feed samples**

and when the moisture was constant, it increased with increase in temperature. Similar results were obtained with other feed ingredients. Relative humidities of ground corn, ground milo, steamed bone meal, soybean oil meal, dehydrated alfalfa leaf meal, and cottonseed meal containing different amounts of moisture were determined at 10°, 21°, and 32° C. for each ingredient at moisture contents corresponding to humidities ranging from 45 to 95%. Sections of curves illustrating the moisture and relative humidity relationship are shown in Figure 2 for relative humidities ranging from 60 to 85%. This humidity is the range primarily involved in safe and unsafe storage conditions for feeds.

These curves show that the relative humidity at a given per cent moisture varies widely with different ingredients. The change in humidity per unit change in moisture was largest in steamed bone meal and least in dehydrated alfalfa leaf meal.

**Relation among Moisture Content, Relative Humidity, and Growth of Molds and Heating in Feed Ingredients Stored at Various Temperatures.** The relative humidity and moisture content of seven feed ingredients which were safe and unsafe from the growth of

**Table II. Safe and Unsafe Relative Humidities and Moisture Contents of Feed Ingredients Stored at 10°, 21°, and 32° C.**

Ingredient	Mold Growth or Heating <sup>a</sup>	10° C.		21° C.		32° C.	
		Moist., %	RH, %	Moist., %	RH, %	Moist., %	RH, %
Ground corn	—	16.2	67.7	15.0	71.0	13.7	71.6
	+	17.0	72.5	15.4	73.7	14.1	74.1
Ground milo	—	17.3	73.1	15.3	71.3	14.1	72.5
	+	17.9	75.0	15.7	73.8	14.3	73.6
Soybean oil meal	—	18.6	70.3	15.3	70.8	14.0	73.1
	+	20.6	72.5	16.8	73.0	14.4	74.2
Cottonseed meal	—	16.0	71.3	14.2	70.9	12.2	70.9
	+	17.6	73.0	15.1	72.9	12.5	73.2
Milo gluten meal	—	16.1	73.0	13.5	71.9	11.5	71.5
	+	16.8	74.5	14.5	74.9	12.0	73.5
Dehydrated alfalfa leaf meal	—	18.5	68.9	15.9	71.5	13.0	71.1
	+	21.3	73.7	17.0	72.8	14.6	74.3
Steamed bone meal	—	11.2	71.5	8.4	68.4	7.7	70.0
	+	12.6	77.3	9.4	72.6	8.1	75.3

<sup>a</sup> — No mold growth and/or heating. + Mold growth and/or heating.

molds and/or heating when stored at 10°, 21°, and 32° C. (summarized in Table II) illustrate the wide variation between safe and unsafe moisture contents for different ingredients. Likewise, safe and unsafe moisture values differed widely for the same ingredient stored at different temperatures. On the other hand, differences between safe and unsafe humidity values were small regardless of the kind of feed or storage temperature, if the temperature for measuring humidity and storage were the same. No ingredient or mixed feed investigated so far has become moldy or heated in 6 weeks when the storage humidity was below 72.0%. In general, growth of molds or heating did not occur until the humidity was above 73.0%. On the basis of these data, the critical relative humidity for feed in storage for 6 weeks has been taken as approximately 72.0%. This critical relative humidity is shown graphically in Figure 2. This critical humidity may not represent the absolute minimum relative humidity at which some species of molds germinate and grow when a feed is stored for longer periods (17,13). In order to use humidity values to predict whether an ingredient or mixed feed will be safe during storage, it is necessary to determine the relative humidity at the maximum temperature which the feed is likely to encounter during storage.

**Moisture Content of Ingredients with 72.0% Relative Humidity at Different Temperatures.** The moisture content of each of seven feed ingredients with a relative humidity of 72.0% is given in Table III for 10°, 21°, and 32° C. These values were obtained from the data given in Table I and those used to plot the curves in Figure 2. As no feed with a relative humidity below 72.0% became moldy or heated, the

**Table III. Moisture Content of Feed Ingredients with a Relative Humidity of 72.0% at 10°, 21°, and 32° C.**

Ingredient	Per Cent Moisture at 72.0% RH		
	10° C.	21° C.	32° C.
Ground corn	16.9	15.2	13.8
Ground milo	17.0	15.4	13.8
Soybean oil meal	20.3	15.9	13.6
Cottonseed meal	16.3	14.6	12.4
Milo gluten meal	15.8	13.6	11.6
Dehydrated alfalfa leaf meal	20.7	16.4	13.9
Steamed bone meal	11.0	8.9	7.8

moisture values given in Table III would correspond to critical moisture values for the ingredient at each temperature. It would be possible also by using the graphs in Figure 2 to obtain the approximate critical moisture value for an ingredient for any temperature between 10° and 32° C.

**Relative Humidity and Rate of Heating.** The time required for ground corn, cottonseed meal, and steamed bone meal with relative humidities above the critical level (72.0%) to heat when stored at 32° C. is summarized in Table IV. In general, heating started within two periods. Feeds with humidities ranging from 73 to 77% usually started to heat in about 3 weeks; those with humidities ranging from 77 to 85%, in less than 2 weeks. These data show that storage periods of at least 6 weeks should be used when the moisture and relative humidity conditions are determined for safe storage of feeds.

In order for corn to have a No. 2

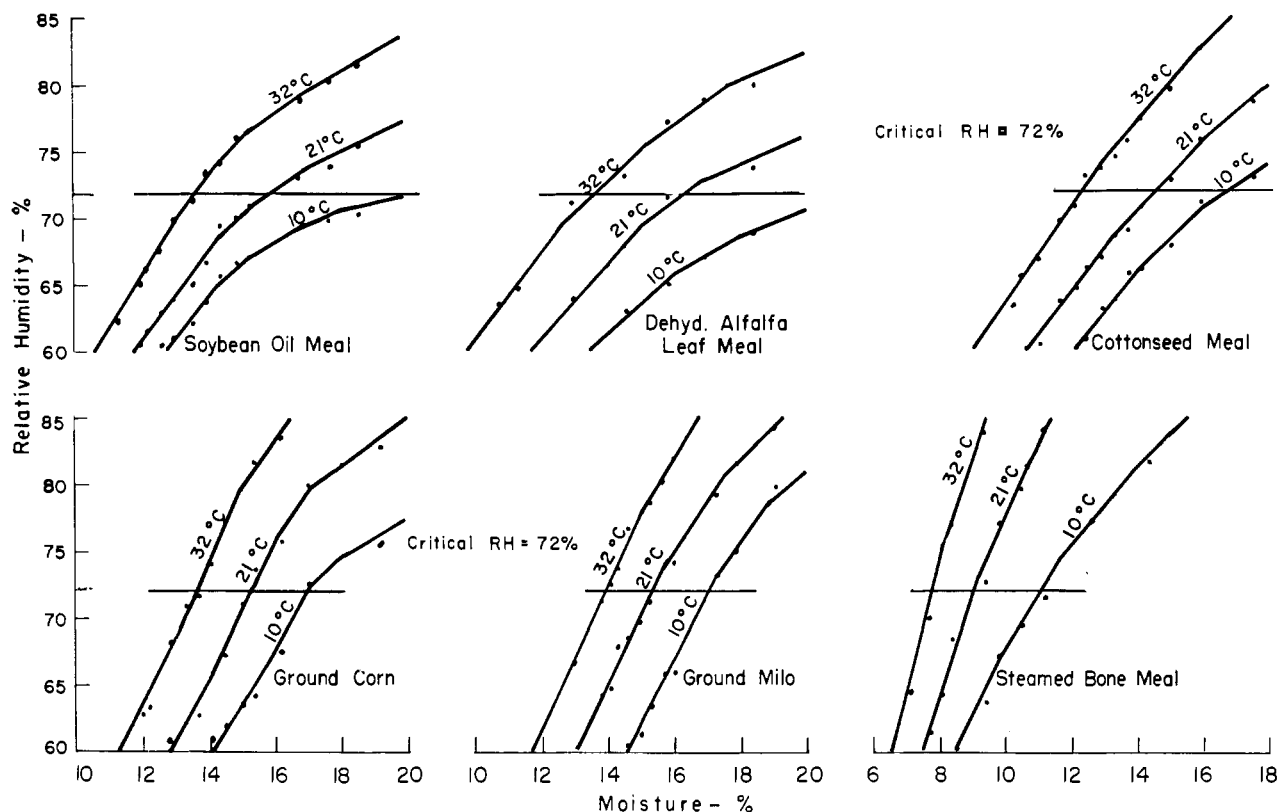


Figure 2. Relation of relative humidity to moisture content of feed ingredients at 10°, 21°, and 32°C.

Table IV. Time Required for Ground Corn, Cottonseed Meal, and Steamed Bone Meal with Different Relative Humidities to Heat When Stored at 32° C.

Ground Corn		Cottonseed Meal		Steamed Bone Meal	
RH, %	Heated, days	RH, %	Heated, Days	RH, %	Heated, days
72.0	..	72.0	..	72.0	..
74.1	38	73.2	35	75.3	36
77.2	23	74.5	20	77.0	23
81.6	9	77.4	10	83.8	4
86.6	5	86.0	4	86.3	5

Table V. Critical Moisture Level of Some Feed Ingredients

Ingredient	Critical Moisture Level, %	
	Reported previously <sup>a</sup>	From graph at 72% RH
Corn, ground	13.0	13.8
Milo, ground	13.0	13.8
Soybean oil meal	13.1-13.8	13.6
Cottonseed meal	11.5-12.8	12.4
Dehydrated alfalfa leaf meal	14.9	13.9
Steamed bone meal	8.7	7.8

<sup>a</sup> See Halick *et al.* (6, 7).

grade, the maximum moisture content of the whole grain must not be over 15.5%. If this corn is ground, then stored or mixed in feeds in normal

Table VI. Relation of Relative Humidity and Moisture Content to Heating in Feed Mixtures Stored at 32° and 70% Relative Humidity

Mixture No.	Added to Basal Mixture <sup>a</sup>		Mixture		Heated, Days
	Supplement	%	Moisture, %	RH, %	
1	None	...	13.0	70.8	..
		...	13.4	73.2	31
		...	12.0	69.4	..
2	Corn fermentation <sup>b</sup> solubles	2.5	12.9	72.9	36
		5.0	13.0	72.9	29
		5.0	13.1	73.2	..
		5.0	13.7	76.2	21
3	Fish solubles <sup>c</sup>	2.5	13.1	74.2	24
		5.0	13.5	75.3	18
4	None Fat <sup>d</sup>	...	13.1	71.3	..
		5.0	12.4	70.9	..
		10.0	11.7	71.4	..
5	None Fat	...	13.5	74.1	34
		5.0	12.8	73.6	33
		10.0	11.9	74.2	30

<sup>a</sup> Basal mixture. Ground corn 69, soybean oil meal 25, steamed bone meal 3.0, dehydrated alfalfa leaf meal 2.5, salt 0.5%.

<sup>b</sup> Condensed corn fermentation solubles contained 45% moisture.

<sup>c</sup> Fish solubles contained 53% moisture.

<sup>d</sup> Animal feeding fat.

amounts, the ground corn or mixed feed is susceptible to the growth of molds and heating. A maximum of 15.5% moisture for corn over a storage period of 6 weeks may be satisfactory for some areas, but ground corn or a feed containing ground corn with this amount of water is not safe in the Gulf Coast area during the warm season.

**Critical Moisture Values of Feed Ingredients.** The critical moisture con-

tent of 32 feed ingredients and the procedure used to determine the critical level have been reported (6,7). In general, these values represented a moisture content approximately 0.3% below the maximum moisture content tested which did not heat in 6 weeks. The values reported previously and those obtained from the graph at 72.0% relative humidity are given in Table V. The values by both procedures were

obtained at a storage temperature of 32° C.

In general, the moisture content of ingredients with a relative humidity of 72.0% was slightly higher than that reported previously. A maximum difference in moisture content obtained by the two methods was 1.0% each for dehydrated alfalfa leaf and steamed bone meal. From a practical viewpoint, less time is required to obtain the critical moisture level from the relative humidity measurement than by observing the time required for a feed to heat in the heating apparatus. The former procedure required only a few hours; the latter, at least 6 weeks.

**Relation of Humidity, Moisture Content, and Spontaneous Heating in Complex Feed Mixtures.** It is practically impossible to apply critical moisture levels in predicting moisture conditions that will be safe or unsafe for storage of feed mixtures. Before moisture contents can be used in judging whether a feed mixture will be safe from heating, it is necessary to know the composition, concentration, and critical moisture level of each principal

ingredient. On the other hand, the data in Table VI show that all mixtures were safe for a 6-week storage period when the relative humidity was 72.0% or less. The unreliable nature of moisture contents for determining safe storage conditions is illustrated by two mixtures with and without animal feeding fat. In this instance, heating depended upon moisture content of the hygroscopic components of the mixture. When a nonhygroscopic ingredient was added, the total moisture was less but this did not change the ability of the feed mixture to support the growth of molds.

#### Literature Cited

- (1) Anderson, J. A., Alcock, A. W., "Storage of Cereal Grains and Their Products," Vol. II, Am. Assoc. Cereal Chemists, St. Paul, Minn., 1954.
- (2) Assoc. Offic. Agr. Chemists, "Official Methods of Analysis," 8th ed., 1955.
- (3) Brockington, S. F., Dorin, H. C., Howerton, H. K., *Cereal Chem.* **26**, 166 (1949).
- (4) Christensen, C. M., *Bot. Rev.* **23**, 108 (1957).

- (5) Geddes, W. F., *Food Technol.* **12**, 7 (1958).
- (6) Halick, J. V., Richardson, L. R., Texas Agr. Expt. Sta., Bull. **768** (1953).
- (7) Halick, J. V., Richardson, L. R., Cline, M., *Ibid.*, **860** (1957).
- (8) Hubbard, J. E., Earle, F. R., Senti, F. R., *Cereal Chem.* **34**, 422 (1957).
- (9) Milner, M., Thompson, J. B., *J. Agr. Food Chem.* **2**, 303 (1954).
- (10) Oxley, T. A., "Scientific Principles of Grain Storage," Northern Publ. Co., Liverpool, 1948.
- (11) Snow, D., Crichton, M. H. G., Wright, N. C., *Ann. Appl. Biol.* **31**, 102 (1944).
- (12) *Ibid.*, p. 111.
- (13) Waite, R., *Ibid.*, **36**, 496 (1949).
- (14) Webb, B. D., Richardson, L. R., Texas Agr. Expt. Sta., Publ. **MP 383** (1959).

*Received for review November 9, 1959. Accepted February 23, 1960. Supported in part by a grant-in-aid from the Corn Products Co., New York, N. Y. Adapted in part from data obtained by B. D. Webb in partial fulfillment of requirements for the degree of doctor of philosophy from the Agricultural and Mechanical College of Texas.*

## FOOD ADDITIVES

### Preservative Effect of Sorbic Acid on Creamed Cottage Cheese

GEORGE A. PERRY and  
ROBERT L. LAWRENCE

Technical Division, Corn Products  
Co., Bayonne, N. J.

Commercially made creamed cottage cheese is a microbiologically unstable product because bacteria, yeast, and mold contaminants cause flavor changes. This deterioration is retarded, but not halted by low temperature storage, or by rigid sanitation. The incorporation of sorbic acid into this product arrests the growth of most contaminants, but not the group of microorganisms associated with flavor formation in dairy products. The efficacy of sorbic acid depended on its concentration, the storage temperature, and the intensity of the initial contamination. Levels of 0.05 and 0.07% sorbic acid in creamed cottage cheese of pH 4.9 increased microbiological and flavor stability.

MANY factors affect spoilage in creamed cottage cheese. As a result, opinion varies among investigators in the field as to the type of microflora prevailing. Actually, 17 different organisms capable of causing spoilage were isolated and identified by Bonner and Harmon (1). They grew these isolates in laboratory media at three concentrations of sorbic acid and reported that concentrations up to 0.25% were not effective in completely destroying the organisms when the pH was not reduced. They also employed a 0.1% aqueous solution of sorbic acid for curd-washing and reported an adverse effect upon

flavor. Geminder (2) showed that 0.075% potassium sorbate or sorbic acid in finished cottage cheese retarded the growth of yeasts, molds, and slime-forming bacteria. Stull (3) reported that sorbic acid at a level of 0.05% inhibited the growth of psychrophilic (cold-loving) organisms, thereby extending the shelf life of cottage cheese.

#### Materials and Methods

Portions of curd and cream dressing were taken from regular production in a large, independent dairy plant. The curd was made using the "short-set"

method which entails culturing skim milk (of 10% total solids content) at 90° F. for 4 to 4.5 hours. The curd was cut and then cooked until a desirable consistency was attained. After draining, the curd was washed three times using water containing 5 p.p.m. of chlorine, the last wash consisting of 35° F. water. To this curd, salted cream was added as a dressing.

In these studies, sorbic acid (in amounts required to give levels of 0.05 and 0.07% in the finished product) was added to the pasteurized and cooled cream dressing (80° F.) and the treated cream was held at 40° to 45° F. until used.