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

letin 754

Moisture in Molasses as a Factor in the Heating of Feeds

ber 1952

The TEXAS AGRICULTURAL AND MECHANICAL COLLEGE SYSTEM
GIBB GILCHRIST, Chancellor

DIGEST

Large losses occur in mixed feeds because of heating and the accompanying deterioration. Investigators are agreed that the heating is caused by the growth of molds. The differences between the levels of moisture which are safe from the growth of molds and those which are unsafe are very small. In many instances this difference is less than 1.0 percent.

Surveys by the Texas Agricultural Experiment Station show that the loss is greatest in feeds containing molasses. To determine the factor in molasses responsible for the heating, 75 samples were collected from the storage tanks of feed manufacturers over the State during the summer 1952, and analyzed for total sugars after inversion, moisture, Brix and ash. Moisture was determined by the vacuum oven drying method and ranged from 19 to over 31 percent. Over 71.0 percent of the samples contained 26 percent or more of water. If 10 percent of molasses was added, the moisture content of 71.0 percent of the feeds would be increased at least 2.6 percent.

No definite value is recommended as a standard for the moisture content of molasses. This should be a minimum because if the other ingredients used in a feed contain a normal amount of water, the extra water added in the molasses might raise the moisture content to an unsafe level.

Different values for solids were obtained by the Brix and the vacuum oven drying methods. Brix is an unreliable measure of the actual moisture content of a crude mixture such as molasses.

Total reducing sugars after inversion and ash were calculated to a 22 percent moisture basis. The values for sugars ranged from 35 to 54 percent, and those for ash from 6 to 16 percent. These differences in sugars and ash could be due to natural variations in the raw materials and in the processes used in the refineries.

Moisture in Molasses as a Factor in the Heating of Feeds

L. R. Richardson and John V. Halick*

TEXAS FEED MANUFACTURERS have reported large losses in mixed feed as the result of heating and deterioration. A study of the problem was undertaken by the Texas Agricultural Experiment Station in an attempt to find ways and means of preventing spoilage in feed ingredients and mixed feeds.

Investigators are agreed that the heating and accompanying deterioration processes are caused by the growth of molds.

Almost every ingredient used to manufacture feeds is contaminated with mold spores. It would be neither practical nor desirable to remove or inactivate these spores. It also would be impossible, under present manufacturing conditions, to prevent mold spores from re-entering the raw materials and the manufactured products. Therefore, conditions should be maintained in the mills and in the feed products which will be unfavorable to mold spore germination and growth. The most important of these is the amount of available water in the ingredients and in mixed feeds.

Mold spores simply can not germinate and grow without moisture. Differences in moisture content as small as 0.5 to 1.0 percent may determine whether molds will grow. One material may contain 14 percent or more moisture and be safe from mold growth, while another with only 7 percent may be very susceptible to spoilage. It depends entirely on the availability of the water in an ingredient rather than the specific amount present. For example, the maximum safe moisture content of wool is twice that of cotton.

Other factors which should be taken into consideration under practical conditions are variations in temperature, relative humidity of the atmosphere, and the length of time and conditions of storage.

---

*Respectively, professor and technician, Department of Biochemistry and Nutrition, Texas Agricultural Experiment Station, College Station, Texas.

INFLUENCE OF MOISTURE AND TEMPERATURE ON GROWTH OF MOLDS

Examples showing the critical nature of the moisture content of feeding materials which are safe and unsafe from the growth of molds, as reported by Snow, *et al.*, (1944), are summarized in Table 1. Molds appeared in oats with a moisture content of 16.5 percent in 19 days, but there was no growth in 1,300 days when the moisture was only 14.0 percent. Molds developed or did not develop in linseed cake, bone meal and wheat bran at differences in moisture values of from 0.5 to 1.5 percent.

One study with cured hay (Waite, 1949), which had been cut into half-inch lengths, showed that molds developed in 19 days with 15.7 percent moisture and at 200 days with 12.9 percent. These values were obtained when the storage temperature was approximately 68° F. In all probability, molds would have appeared much sooner at a higher temperature. The influence of storage temperature on the time required for molds to appear on oats is shown in Table 2. When the oats were high in moisture, the effect of temperature was relatively small, but with a moisture content of 15.5 percent, which is on the borderline between being safe and unsafe, an increase in the storage temperature of from approximately 60° to 72° F. decreased the time required for molds to develop from 77 to 41 days. The molds would have developed sooner at higher

Table 1. Influence of moisture content of a feed ingredient on the time required for mold growth to appear at a storage temperature of approximately 68° F.

Ingredient	Moisture content and days for molds to appear	
	Moisture %	No. days
Oats[1]	16.5	19
	15.5	41
	14.0	1300 no growth
Linseed cake[1]	13.0	52
	12.0	1300 no growth
Bone meal[1]	9.5	52
	8.0	1300 no growth
Bran[1]	15.5	32
	14.0	1300 no growth
Hay[2]	15.7	19
	12.9	200

[1]Data abstracted from charts of Snow, et al. (1944).
[2]Waite (1949).

Table 2. Influence of storage temperature on the time required for mold growth to appear on oats containing different amounts of moisture[1]

Moisture %	Days required for molds to appear at two temperatures	
	59.9° F.	71.6° F.
18.0	14	10
16.5	22	19
15.5	77	41

[1]Data from Snow, et al. (1944).

temperatures, even with the moisture content of the feed the same.

Temperature is a very important factor in mold development. Many feeds manufactured in Texas are loaded into cars when the temperature is approximately 80° F. Frequently, the temperature of the car increases to 120 or 130° F. while it is in transit. If the amount of water in a feed was borderline between a safe and unsafe level when it was loaded, it would be unsafe while it was in transit. Under the latter conditions, molds would develop and grow rapidly.

Snow, *et al.*, (1944), determined the moisture content of feeding stuffs which are safe from mold growth for 3 months and for 2 to 3 years at storage temperatures of 60 to 70° F. Davenport, *et al.*, (1950), showed that a moisture content above 12 percent was too high for safe storage of sorghum grains under conditions existing in Texas. Examples of these data are summarized in Table 3. While they show an average

Table 3. Moisture levels below which feeding stuffs may normally be safe from mold growth for 3 months and 2 to 3 years

Feeding stuff	Moisture % for safe storage	
	3 months	2-3 years
Wheat[1]	15.7	14.6
Maize[1]	14.8	13.7
Barley[1]	14.8	13.6
Oats[1]	14.5	13.4
Middlings[1]	14.4	13.1
Bran[1]	14.4	12.8
Soybeans[1]	13.3	11.0
Linseed cake[1]	12.3	11.1
Artificially dried grass[1]	13.7	11.1
Hay[1]	12.6	11.0
Fish meal[1]	11.5	9.9
Meat and bone meal[1]	10.3	8.7
Bone meal[1]	9.5	8.7
Sorghum grain[2]	10-12	

[1]Snow, et al. (1944).
[2]Davenport, et al. (1950).

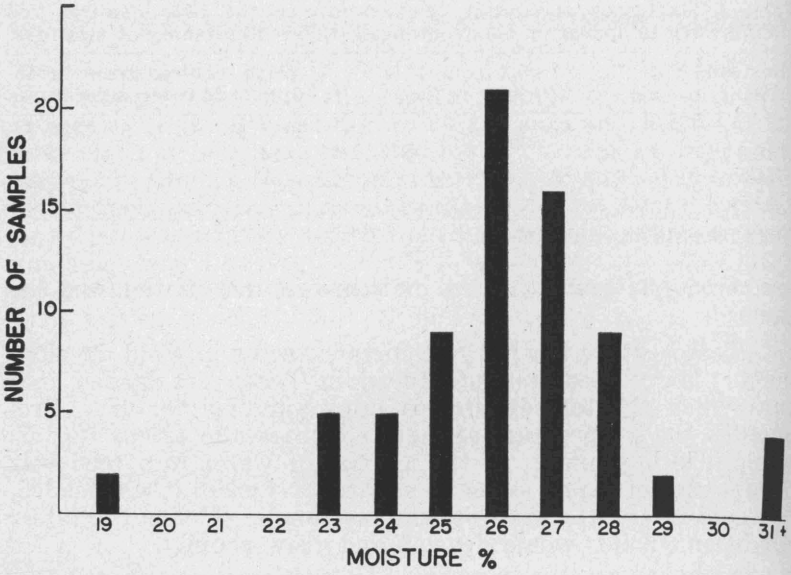

Figure 1. Number of samples of molasses with different amounts of moisture.

difference of only 1.5 percent between safe and unsafe levels of moisture, these data emphasize again the narrow range of the critical moisture value.

COMPOSITION OF MOLASSES USED IN TEXAS FEEDS

Feed manufacturers report more heating and molding in feeds containing molasses than in feeds containing any other ingredient. Molasses have a higher percent of moisture than grain and other feed ingredients. As a result, a feed containing molasses is higher in moisture than one mixed with the same ingredients without molasses. In our preliminary studies, the moisture content of four samples of molasses ranged from 22 to 33 percent. A feed mixed with 10 percent molasses containing 22 percent moisture would have approximately 1.0 percent less water than one mixed with a high moisture molasses. Consequently during 1952, 75 samples of molasses were collected by inspectors of the Feed Control Service from the storage supplies of feed manufacturers located in every section of Texas. The following determinations were made on each sample: moisture, Brix, Baume, ash and total invert sugars. The methods used for these determinations are described under "Methods."

Moisture Content of Molasses

The number of samples with different amounts of moisture is shown in Figure 1. The moisture content ranged from 19 to over 31 percent. Two samples had 35 and 36 percent moisture, respectively. Only a few samples had moisture content below 26 percent. If 10 percent of such molasses was added to a feed, the moisture content of 71.0 percent of the feeds would be increased at least 2.6 percent.

With present standards of moisture for the ingredients and for molasses, and under the conditions of temperature and humidity to which feeds are exposed, it would be practically impossible to mix a feed containing 10 percent of the high moisture molasses which would be safe from the growth of molds and the accompanying heating and deterioration. The density of blackstrap molasses as they come from the centrifuges in the refineries ranges from 85° to 92° Brix; and the total solids by vacuum drying (Spencer and Meade, 1945) range from 77 to 84 percent.

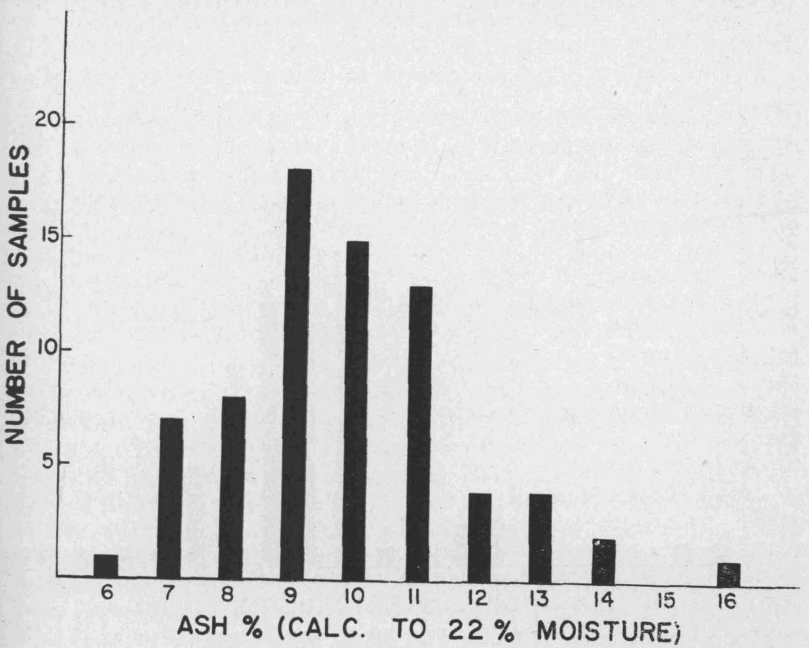

Figure 2. Number of samples of molasses with different amounts of ash.

Ash Content of Molasses

The number of samples with different percent of ash (calculated to a 22 percent moisture basis) is given in Figure 2. The samples varied in ash content from less than 6 to over 16 percent, and 84.9 percent of them contained 11 percent or less ash. The ash, as determined, represented the minerals which remained after ignition. No attempt was made to determine the amount or kind of each constituent in the ash. A high ash content is not interpreted to mean that the sample was adulterated by the addition of any material.

Molasses contain most of the non-sugar constituents of the cane juice and some of the non-extractable portion of the sucrose and non-reducing sugars in concentrated form. The composition of cane juice is quite variable and is influenced by such factors as the variety and the climatic and soil conditions. Lime, alkalies and other inorganic materials are used in the purification and clarification processes and further changes in the composition of the juice are brought about by these treatments. Other reports show that the ash content of molasses from different countries varies from 7 to 16 per-

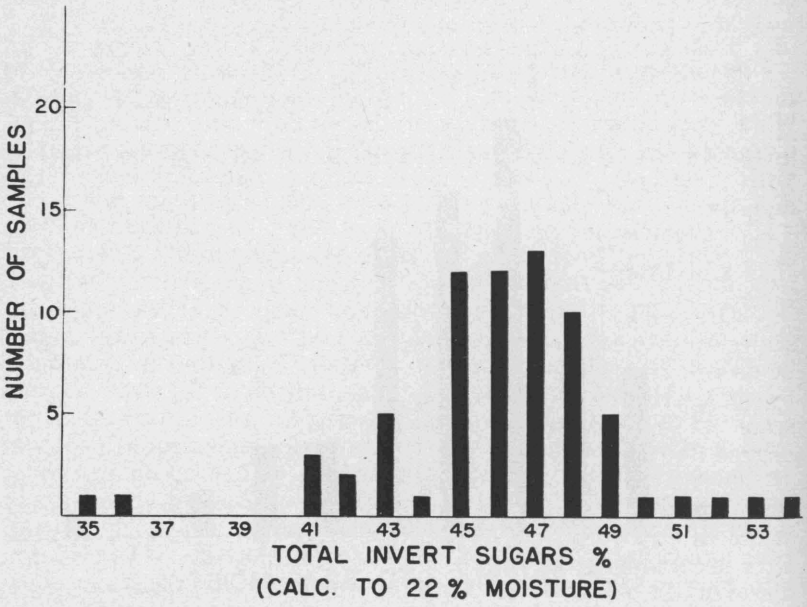

Figure 3. Number of samples of molasses with different amounts of total invert sugars.

cent or more. These examples illustrate, in a general way, the many possibilities for a wide variation in the composition of molasses, in ash content and in other constituents.

Total Reducing Sugars After Inversion

The number of samples with different percent of total reducing sugars after inversion (calculated to a 22 percent moisture basis) is given in Figure 3. Amounts varied from 35 to 54 percent, and 82.4 percent of the samples contained 45 percent or more total reducing sugars. It is not surprising, as in the case of ash, to find a large variation in the total sugars in the molasses, because all refineries do not operate with the same efficiency.

Feeding Value of Molasses

The chief feeding value of molasses is the amount of sugar available to replace the carbohydrate in an equivalent amount of grain in the ration. When molasses are used to replace grain in a ration on the same weight basis, equivalent amounts of carbohydrates are not used. The higher the moisture content, the lower is the relative feeding value when a given weight of molasses is used to replace an equal weight of grain.

Another important function of molasses is their use to increase the consumption of much of the low-quality roughage which is produced in many areas. Animals will consume large quantities of these low-quality feeds when they are mixed with molasses, probably because of the sugar content of the molasses.

Brix and Baume

Degree Brix and degree Baume refer to the graduation scales on two different hydrometers. Either hydrometer may be used to measure the density or specific gravity of a solution by replacing the Brix or Baume scale with a specific gravity scale. The relationship of degree Brix, specific gravity and degree Baume is given in Table 41.3 of the Methods of Analysis of the Association of Official Agricultural Chemists. The Baume scale has no convenient relation with the percent of solids in any sugar product. In commercial analyses, degree Brix is considered as the percent of solid matter or total solids dissolved in a liquid, but this is true only when pure sucrose is dissolved in water. It is generally recognized by sugar chemists that the Brix of a crude mixture, such as molasses

Table 4. The Brix of solutions or mixtures containing known amounts of solids

Solution or mixture	Solids added, gm. per 100 gm.	Total solids, %	Observed Brix°
Sugar (sucrose)	50	50	50.3
	60	60	60.0
	70	70	70.3
Sugar †	50		
calcium carbonate	10	60	65.1
Sugar †	60		
calcium carbonate	10	70	75.7
Sugar †	50		
salt (sodium chloride)	10	60	67.8
Sugar †	60		
salt (soduim chloride)	10	70	77.7

is invariably higher than the solids obtained in a vacuum oven, because inorganic salts and many complex compounds are present which have a higher density than the sugars.

Two sets of data are described which illustrate that Brix does not give the same value for solids as is obtained by the vacuum oven drying method. Therefore, it is not a reliable measure of the moisture content of a crude mixture. One set was obtained by the use of solutions or mixtures which were prepared in the laboratory from pure compounds and which contained a known concentration of solids. The following solutions and mixtures were used: a pure sugar (sucrose solution), a sugar solution with salt (sodium chloride) added and a sugar solution with finely-divided calcium carbonate added. These preparations were made in the laboratory to determine whether Brix could be used as a measure of the amount of solids in solutions or mixtures of known concentration. These data are summarized in Table 4.

The solids of a pure sugar (sucrose) solution by Brix were essentially the same as the solids in the original solution. When finely-divided calcium carbonate or salt was added to the sugar solution, the Brix was 5 to 7 points higher than the total solids in the original preparation. These tests show that Brix values may indicate 5 to 7 percent less water in the sample than is actually present. They show also that the inorganic salt does not have to be soluble to give a false reading. For example, when an insoluble salt, such as calcium

carbonate, was used, the difference between the solids added to the solution and the Brix was essentially the same as when the soluble salt was used.

Solids by the vacuum drying method and by Brix were determined for each sample of molasses used in this study. The data showed also that these methods do not give the same values when used on a crude mixture like molasses. Data illustrating the differences between Brix and moisture values of molasses are given in Table 5. To simplify the presentation of these data, only a few values are given here, but there were many samples of molasses with the same Brix which varied as much as 4 to 5 percent in moisture. This variation is illustrated specifically with five different samples of molasses with a Brix value of 80. Three had a moisture content of 23 to 24 percent and two had 26.2 and 27 percent moisture, respectively. A sample with a high Brix frequently contained more water than one with a much lower Brix. This is illustrated when the sample with 88 Brix and 23.6 percent moisture is compared with one with 80 Brix and 23.1 percent moisture.

Ash content seems to be associated with this variation between Brix and moisture more than any other constituent. In practically every case where the moisture was high for a given Brix, the ash content (calculated to a dry-weight basis so that the values for different samples would be expressed on the same basis) was also high.

Spencer and Meade describe several methods which have been suggested to convert Brix reading into total solids. Most

Table 5. The relationship of degrees Brix and the percent moisture and ash in molasses

Brix degrees	Moisture %	Ash %
76	28.6	15.1
	28.9	15.9
	22.5	5.3
	26.1	7.9
80	23.9	12.7
	23.3	10.2
	23.1	10.2
	27.0	19.0
	26.2	11.4
86	19.8	12.3
	18.4	12.4
88	23.6	17.7

of these calculations involved the percent of ash. These methods were useful in specific factories, but unfortunately a fixed and unvariable relationship between Brix and total solids by the vacuum drying method in such products as molasses, does not exist.

METHODS USED IN THE ANALYSIS OF MOLASSES

Determination of Moisture

The method used for the determination of moisture was essentially the same as the official vacuum drying method described in the Methods of Analysis of the Association of Official Agricultural Chemists. The details of the procedure, as used in this laboratory, were: The molasses were mixed thoroughly with a glass stirring rod and 1 to 2 gm. were weighed into an aluminum dish (with cover) approximately 7.5 cm. in diameter and 2.5 cm. deep. The molasses were dried in a vacuum oven at a temperature of 60° C. with the vacuum gauge reading approximately 29. A small current of dry air was admitted to the oven to insure removal of the water vapors. It required 8 hours to obtain a constant weight, but a value was obtained at 6 hours which was never more than 0.5 to 1.0 percent less than that obtained at 8 hours. Eight to 18 hours drying are used in our laboratory because it is frequently convenient to leave the samples in the oven overnight.

A few precautions should be observed in determining the total available water in molasses. The temperature should be uniform throughout the oven and should not exceed 70° C. (preferably 60° C.). An efficient vacuum should be maintained in the oven, so that, even with a small current of air entering it, the pressure does not exceed 50 mm. of mercury. This is usually a reading of approximately 29 or more on the vacuum gauge, under the barometric pressure which exists at College Station.

Dry air should be admitted to the oven when the vacuum is reduced. The dish should be covered and placed in a dry desiccator until cool. Then it should be weighed immediately. For an accurate moisture determination, the weight, on redrying for 1 hour, should not change more than 2 mg. In our experience, it has not been necessary to use sand or pumice in moisture determinations. The dish should be large enough and the sample small enough so that the dried molasses will not fill the dish to overflowing. The dried molasses usually will occupy a larger volume than the original sample.

Determination of Ash

The carbonate ash was determined by the official method as described for sugars and sugar products in the Methods of Analysis of the Association of Official Agricultural Chemists.

Brix and Baume

Brix readings were obtained by the use of a "Streamline Silvertip" hydrometer manufactured by Wm. Hiergesell & Sons. One hundred and fifty grams of molasses and 150 gm. of distilled water were placed in an 800 ml. Berzelius beaker and mixed with a mechanical stirrer until all soluble portions of the molasses were in solution. The mixture was then transferred to a cylinder 2 inches in diameter and 15 inches long. The hydrometer was inserted and readings taken after it had come to rest. This usually required 3 to 5 minutes. When large quantities of gas were present, time was allowed for the gas to escape. A large amount of insoluble material settled out of many samples and this changed the Brix reading a few points. The reading before the insoluble material settled out was taken as the Brix of the solution. The readings were corrected for temperature by the use of the tables given for this purpose in the Methods of Analysis of the Association of Official Agricultural Chemists. The corrected value was multiplied by the dilution factor to obtain the Brix of the original molasses. Baume reading was obtained for a given Brix from the tables in the Official Methods.

Total Invert Sugars

The method used for the determination of total invert sugars was essentially the same as that described by Wildman and Hansen (1940) for the determination of sugars in plant material. The details of the procedure, as adapted for the determination of total invert sugars in molasses, is: Approximately 2 gm. of molasses were weighed accurately into a 100 ml. volumetric flask; 50 ml. of distilled water were added to dissolve the molasses and 1 ml. of saturated neutral lead acetate was added to clarify the solution. Distilled water was added to volume, and the contents were transferred to a 250 ml. centrifuge tube and centrifuged for about 4 minutes at 1,800 r.p.m. The supernatant filtrate was poured into a 125 ml. Erlenmeyer flask containing a slight excess of solid sodium oxalate to precipitate the lead, after which it was filtered through a dry filter paper into a dry beaker.

Inversion of Sucrose

A 50 ml. volumetric flask containing 25 ml. of the clarified molasses solution and 2.5 ml. of concentrated hydrochloric acid was placed overnight in an incubator set at 60° C, to invert the sucrose which was present in the molasses. The acid solution was then transferred to a 100 ml. beaker and neutralized with strong sodium hydroxide to a pH of 6.5, using a Beckman pH meter. The neutralized solution was then transferred quantitatively back to the 50 ml. volumetric flask and made up to volume.

Ten milliliters of the inverted sugar solution and 10 ml. of distilled water were pipetted into a 50 ml. short centrifuge tube which had a conical bottom and pourout spout. Ten milliliters each of copper sulphate and alkaline tartrate solutions were added in that order by means of automatic pipettes, then the tubes were placed in a hot water bath at 80° C for 20 minutes. They were removed from the water bath, allowed to cool to room temperature and centrifuged for 4 minutes. The supernatant liquid was decanted off and the precipitate was washed and broken up with a jet of hot water from a wash bottle.

It was centrifuged a second time and the supernatant liquid was decanted off. The precipitate was then broken up with 10 to 15 ml. of hot water from the jet of the wash bottle, and 5 ml. of acid ferric ammonium sulfate were added while it was kept in suspension with a small stream of hot

Table 6. Data used to convert mg. of copper to mg. of sugar[1]

Copper	Total invert sugar	Copper	Total invert sugar	Copper	Total invert sugar
mg.	mg.	mg.	mg.	mg.	mg.
5	2.30	75	36.03	145	72.61
10	4.64	80	38.56	150	75.22
15	6.90	85	41.06	155	77.93
20	9.30	90	43.61	160	80.67
25	11.64	95	46.17	165	83.43
30	14.03	100	48.74	170	86.18
35	16.42	105	51.32	175	88.96
40	18.82	110	53.93	180	91.76
45	20.24	115	56.53	185	94.61
50	23.67	120	59.16	190	97.41
55	26.11	125	61.80	195	101.20
60	28.58	130	64.45	200	113.10
65	31.04	135	67.11		
70	33.53	140	69.80		

[1]Wildman and Hansen, 1940.

water from the wash bottle. The amount of copper in the precipitate was determined by titration with 0.1 N potassium permanganate solution, and the amount of sugar present in the sample was determined by the use of the Wildman and Hansen tables (Table 6).

Reagents Used in Sugar Determinations

1. *Fehling's* as modified by Quisumbling and Thomas.

Copper sulfate. Dissolve 69.28 gm. of reagent copper sulfate ($CuSO_4.5H_2O$) in warm water and then dilute to 1 liter in a volumetric flask. After standing several hours, the solution is filtered and transferred to a pyrex reagent bottle.

Alkaline tartrate solution. Dissolve 346 gm. of reagent sodium potassium tartrate in warm water and then transfer the solution to a 1 liter volumetric flask. A saturated solution of sodium hydroxide is then digested on a steam bath until insoluble carbonates have settled out. The exact alkalinity of the solution is determined and the amount containing exactly 130 gm. of sodium hydroxide is added to the sodium potassium tartrate solution and made to 1 liter.

2. *Ferric ammonium sulfate.* Dissolve 240.9 gm. of ferric ammonium sulfate ($Fe(NH_4)(SO_4).12H_2O$) in warm water, and add 200 ml. of concentrated sulphuric acid (H_2SO_4). After cooling, dilute the solution to 1 liter and filter.

3. *Neutral lead acetate.* Dissolve 20 gm. of neutral lead acetate ($Pb(C_2H_3O_2)_2.3H_2O$) in water and dilute to 100 ml.

LITERATURE CITED

Davenport, M. G., J. W. Sorenson, H. G. Johnston, R. A. Hall and Jack Bradshaw, 1950. Storing sorghum grain in South Texas. Texas Agri. Expt. Station Prog. Rept. 1240.

Snow, D., M. H. G. Crichton and N. C. Wright, 1944. Mould deterioration of feeding stuffs in relation to humidity of storage. Part I. The growth of moulds at low humidities. Ann. Appl. Biol. 31:102.

----------, 1944. Mould deterioration of feeding stuffs in relation to humidity of storage. Part II. The water uptake of feeding stuffs at different humidities. Ann. Appl. Biol. 31:111.

Spencer, G. L. and G. P. Meade, Cane Sugar Handbook, Eighth Edition, 1945. John Wiley & Sons, Inc., New York.

Waite, R., 1949. The relation between moisture content and moulding in cured hay. Ann. Appl. Biol. 26:496.

Wildman, Sam G, and Elmer Hansen, 1940. A semi-micro method for the determination of reducing sugars. Plant Physiol. 15:719.