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Myron George Cropsey

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Outline of Studies:

Major: Agricultural Engineering.

Minor: Mathematics, Physics.

Biographical Items:

Born January 29, 1910, Oakland, California.

Undergraduate Studies, University of California, 1929-33.

Graduate Studies, North Dakota State College, 1939-41.

Michigan State University, 1954-56.

Experience

Union Diesel Engine Co. 1936-37, Jr. Engineer.

U.S.Department of Agriculture 1937-41, Jr.Ag.Engineer.

U.S. Army 1941-46, Lt. - Lt.Col.

Oregon State College 1946-present, Assoc.Professor.

Member: Sigma Xi, Tau Beta Pi, Sigma Pi Sigma,

American Society of Agricultural Engineers,

American Society for Engineering Education,

Registered Professional Engineer, State of Oregon.

THE EFFECT OF ONE-MILLION VOLT CATHODE RAY IRRADIATION ON THE RESPIRATION RATE, MILLING TESTS, AND BAKING PROPERTIES OF WHEAT

By

Myron George Cropsey

AN ABSTRACT

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Large quantities of wheat are lost each year at moisture levels slightly above those safe for storage. Often because of damp weather farmers are not able to harvest wheat at the moisture percentages required for safe storage. Frequently wheat changes in moisture within the elevator or storage bin so that it is no longer safe in storage. The purpose of the author's experiments was to determine whether cathode ray irradiation would reduce the respiration of wheat that was slightly damp (14.0 - 17.0 percent moisture wet basis) and not impair the milling and baking qualities.

A review of the literature disclosed that spoilage of wheat was due principally to mold growth under the right conditions of temperature, humidity, and aeration. The optimum conditions of mold growth were found to be about 86 degrees Fahrenheit at about 20 milliliters of air per gram of dry matter and at high humidities.

Three types of tests were conducted: respiration, thermos bottle, and comprehensive. The respiration tests were conducted at optimum conditions of temperature and aeration for mold growth and at the humidity corresponding to the equilibrium moisture content of the wheat. These were ten-day tests with the carbon dioxide produced on the tenth day and the free fatty acid at the end of the tests used as a measure of deterioration. The thermos bottle tests consisted of recording the temperatures within pint thermos bottles when held at 86 degrees Fahrenheit for 20

days. The free fatty acid determination at the end of the test was also used as a criterion of deterioration. The comprehensive tests were carried out for conditions of greatest mold growth and at the doses found most effective in controlling the production of carbon dioxide in the respiration tests. At the completion of the conditions for maximum mold growth a series of milling and baking tests was made according to standards of the American Association of Cereal Chemists.

Five runs of the respiration tests were completed at various levels of treatment. The 900,000 rep (see page 4 for definition of rep) treatment was found to be one of the most effective treatments in lowering the production of carbon dioxide, whereas the 700,000 rep treatment seemed to cause the least free fatty acid at the end of the tests.

The thermos bottle tests were not very effective as there was a significant difference in temperature in only one case. The free fatty acid determination was lowest at the 700,000 rep treatment.

The comprehensive tests were carried out at zero and 900,000 rep treatments. There was a burned odor and taste at the 900,000 rep level. While this was not great, it would be objectionable to the consumer. The other milling and baking tests showed only slight differences between no treatment and 900,000 rep treatment.

Cathode ray doses from 500,000 to 900,000 rep reduced the respiration rate of slightly damp wheat (15.0 - 16.0 percent), but the doses at 900,000 rep had a slightly burned odor and taste for both the milling and the baking tests.

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Wheat one or two percent above the moisture content level for safe storage causes heavy losses for the farmer and the elevator operator. The farmer without grain drying equipment must combine his wheat at a moisture content which will insure safe storage, and must protect it from the weather after it is binned. The elevator operator is plagued with migration of moisture within the bin caused by changes in air temperature. The migration of moisture may cause spoilage by raising the moisture content of the grain in parts of the bin.

Only recently have the processes of deterioration of damp wheat been understood. Formerly it was believed that respiration of the stored grain was alone responsible for heat production. It was believed that when respiration was sufficiently rapid, heat was produced more rapidly than it was dissipated.

It had been observed that the heating of damp grain was usually accompanied by mold growth. It is now known that microorganisms are always present on the surface of grains and within the seed coat and it is now generally agreed that molds cause the sharp increase in respiration when the moisture content of grain exceeds a certain value.

For some time it has been known that cathode ray irradiation can kill many forms of living organisms including

molds. A logical question then arises. Will cathode ray irradiation prevent spoilage of high moisture content grain by killing molds on the surface and within the seed coat? Wheat placed in bins immediately after irradiation will be difficult to reinfest.

Cathode ray irradiation is cheap because of the high efficiency of conversion of electrical energy into cathode ray energy. Overall efficiencies as high as 15 percent are possible for converting electrical energy into radiation energy absorbed by matter. At this rate if electrical energy were selling for one cent a kilowatt hour, then a 500,000 rep treatment would cost 7.8 cents per ton of wheat.

Purpose of the Experiments

The purpose of the experiments carried out in this project was to determine if cathode ray irradiation reduced respiration to a low level in slightly damp wheat and to see if the milling and baking qualities were impaired.

Points Investigated

Three types of tests were performed: respiration, thermos bottle, and comprehensive.

The points investigated of irradiated wheat under fixed storage conditions were the rate of respiration, the rate of rise in temperature, the change in fat acidity, and the changes in milling and baking qualities.

The rate of respiration was determined after ten days while held at 87 degrees Fahrenheit and fixed rates of air exchange.

The rise in temperature of wheat kept in thermos bottles for twenty days was measured by thermocouples.

At the conclusion of each run rancidity determinations were made of a sample of each test.

Milling and baking tests were performed by the International Milling Company according to Cereal Laboratory Methods of the American Association of Cereal Chemists. These tests were made for those dosages of irradiation that showed the greatest promise of reducing respiration.

Definition of Terms

In this thesis the letters "rep", which will appear many times, represent a word for roentgen equivalent physical. There is some confusion as to its definition, but it is best taken as the absorption of 93 ergs of radiation energy per gram of body tissue. This unit usually applies to ionization radiation not covered by the roentgen.[27] The roentgen shall be the quantity of X- or gamma-radiation such that the associated corpuscular emmission per 0.001293 grams of air produces, in air, ions carrying one electrostatic unit of quantity of either sign.[27]

The term cathode rays will appear often. It is generally understood to be energetic electrons produced by a man-made machine.

Frequently the two terms "radiation" and "irradiation" appear. "Radiation" in this thesis refers to an act or process of diffusion or emission of radiant energy. "Irradiation" is the act or process of receiving incident radiant energy.

The term "percent moisture" or "moisture" as used in this thesis refers to the ratio expressed in percent of the weight of moisture to the total wet weight.

REVIEW OF LITERATURE

Respiration

Respiration processes occur in almost every living cell, whether plant or animal, to carry on their metabolic functions.[1,33] Respiration carried on by the intake of atmospheric oxygen in the cell is known as aerobic respiration and is the usual type referred to when respiration is spoken of. On the other hand, anaerobic respiration occurs in the absence of atmospheric oxygen and is responsible for fermentations carried out by microorganisms to produce carbon dioxide and other organic compounds.

Below is a chemical equation for the cumbustion of a carbohydrate and a fat under aerobic conditions.[1]

D-glucose: a carbohydrate.

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + 677 \text{ calories}$$

180 grams + 134.4 liters → 134.4 liters + 108 grams

Tripalmitin: a fat.

$$(C_{15}H_{31}COO)_3\ C_3H_5 + 72\tfrac{1}{2}O_2 \rightarrow 51CO_2 + 49H_2O + 7617 \text{ calories}$$

806.8 grams + 1624 liters → 1142 liters + 833 grams

The quantity of heat given off in combustion by other carbohydrates is similar.[33]

In anaerobic respirations carbon dioxide also is given off and a number of organic compounds are formed. However,

the cells undergo internal oxidation and reduction. Under these conditions the energy released by a unit of substrate is less than for aerobic respiration.

It should be stated that neither the oxygen consumed nor the carbon dioxide produced should be considered as a complete index of respiration if external and internal conditions are not controlled. However, if the temperature is held constant and the oxygen supplied is constant, the amount of carbon dioxide produced can be used as an index of comparison of metabolic activity.

The total respiration of a grain can be accounted for by the viability of grain, or microorganisms, and of insects.[1] Insects are generally of a local nature and can be much better controlled than the activities of the other two. The respiration of microorganisms and of the grain itself is influenced by such factors as temperature, moisture content, supply of oxygen, prior history of the grain, etc.

In dry grain that is insect free, the respiratory rate is very low.[1] As the moisture content increases, the respiratory rate increases.[5] At some critical moisture content the respiratory rate accelerates rapidly until the grain begins to heat. This can be illustrated best by Figure 1. taken from Bailey and Gujar.[5] When the heat produced by respiration exceeds the rate at which the heat can be dissipated, the grain increases in temperature.

Recently, most investigations have shown that the sharp

increase in respiration is due to the growth of molds. A relative humidity of about 75 percent is a minimum for the growth of molds at room temperatures. According to the work of Coleman and Fellows[11], hard red spring wheat of 14.7 percent moisture (wet basis) would be in equilibrium with an atmospheric relative humidity of 75 percent. This is just at the moisture level in wheat where there is a sharp increase in respiration.

Respiration tests in which grain has been continuously aerated support the theory that the acceleration of respiration above a certain moisture level is due to mold growth. Milner, Christensen, and Geddes have made the following observation concerning mold growth:

> That molds are the primary cause of heating and deterioration of various kinds of stored seeds at moisture contents where molds can grow has been shown by a number of workers, including Gilman and Barrow (1930)[19], Milner and Geddes (1946, 1946a)[35,36], Nagel and Semeniuk (1947)[37]. In a comprehensive review of the literature on the deterioration of corn in storage, Semeniuk and Gilman (1944)[42] state that ". . . the conditions under which deterioration occurs and the changes which follow its initiation, indicate that it is primarily a biological decomposition. . .

To continue:

> Sound wheat stored at 30 degree centigrade and at moisture contents above 16.1 percent was rapidly overgrown by molds. The increase in respiration and decrease in viability of the seed with increasing moisture content was proportional to the increase in molds.[34]

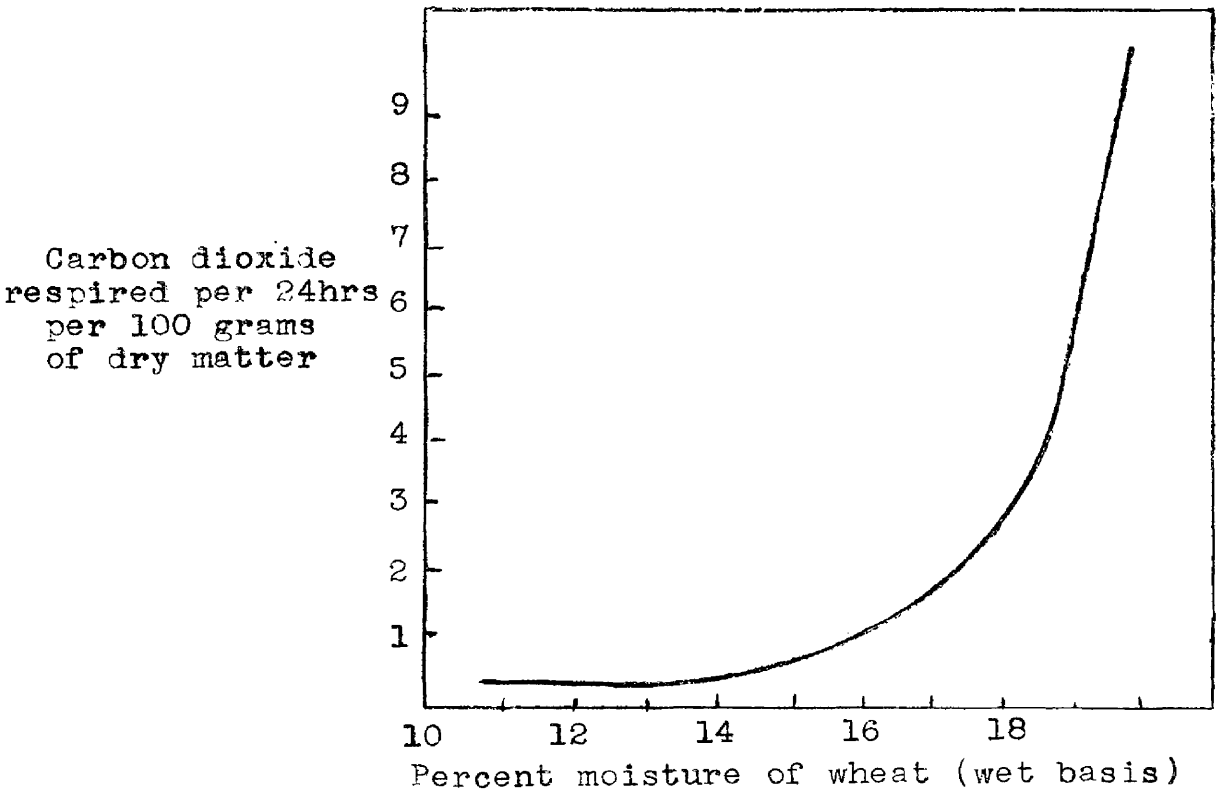


Fig. 1 Influence of moisture content on the respiration of wheat.[5]

In view of these results the author has concluded that mold growth is responsible for the initial spoilage and deterioration of wheat at moisture contents where mold will grow.

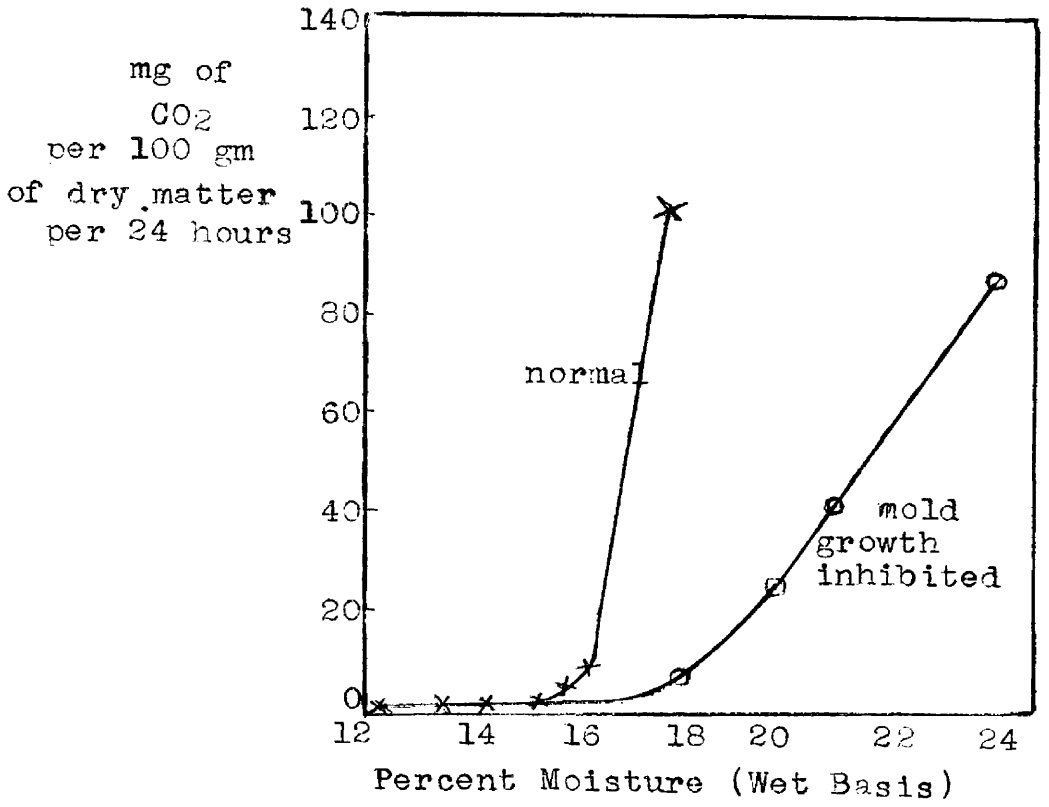


Fig. 2 Influence of moisture content on the respiration of normal wheat subject to mold growth and the same wheat with thiourea at a concentration of 1% to inhibit mold growth at 30°C. The respiration trials were conducted for 10 days and the rates plotted for the 10th day.[34]

Microflora

Microorganisms on grain. There are a wide variety of fungi and bacteria, including actinomycetes, on cereal grains.[1,15,21,24] Their activity results in spontaneous heating, the development of off-odors, tastes and various discoloration of the grains, although the exact nature of their activity is not always known.[1]

Bacteria, fungi, and actinomycetes are simple microorganisms. Fungi are classified as molds, yeasts, and yeast-like fungi. Most microorganisms found in grains

require organic materials for growth. Their diversity of type makes growth of one or another of them possible over a wide range of environmental conditions.

Microorganisms are found on both the inside and the outside of cereal grains. Semeniuk makes the following comment, "It is known that the parasitic fungi and bacteria listed in Table I may be carried internally. Their presence in kernels depends on their own parasitic aggressiveness, on the action of true parasites, on the stage of grain development when they are present in the air. . . , on grain suseptibility . . . , and on weather . . . ."[1]

However, Thompson[48] states that heating of damp wheat is due to a sub-epidermal fungi entering through the stem and that most of the heat of respiration is due to these internal fungi.

On the surface saprophytes are the main components while parasites may also be present. James, Wilson, and Stark[24] found on the surface of wheat those listed in Table II.

Table I.

PARASITIC FUNGI and BACTERIA FOUND INTERNALLY IN WHEAT*

Fungi
Calonectria graminicola
Fusarium spp.
G. zeae
H. sativum
S. nodorum
S. tritici
Bacteria
Xanthomonas Translucens

* Adapted from Dickson[15] and Greaney and Machacek[21]

Table II.

PARASITIC FUNGI and BACTERIA FOUND EXTERNALLY ON WHEAT*

Acrostalagmus cinnabarinus	*Hormodendrum pallidum*
Alternaria tenuis	*H. viride*
Aspergillus glaucus	*Mucor circinelloides*
Aspergillus candidus	*M. racemosus*
A. flavus	*Paecilomyces varioti*
A. fumigatus	*Penicillium chrysogenum*
A. niger	*P. flavi-dorsum*
A. oryzae	*P. frequentans*
A. versicolor	*P. purpurogenum*
Cephalosporium spp.	*P. rugulosum*
C. curtipes	*P. spinulosum*
Cephalothecium roseum	*P. terrestre*
Fusarium culmorum	*Rhizopus* spp.
F. poae	*Scopulariopsis* spp.
F. scirpi var.*acuminatum*	*S. brevicaule*
F. semitectum var. *major*	*Septoria nodorum*
Helminthosporium sativum	*Trichoderma lignorum*

* From James, Wilson and Stark[23]

The principal factors determining the activity of microorganisms in stored grains are temperature, moisture, and oxygen supply according to Semeniuk in Anderson and Alcock.[1]

Temperatures. Microorganisms have a maximum, a minimum, and an optimum temperature at which they grow. At temperatures outside the range of growth microorganisms die. They die quickly at temperatures above the limit for growth and slowly at temperatures below the limit for growth. Those at high moisture content die more rapidly than those at low moisture content when outside their range of growth.[1]

Microorganisms produce heat as a result of their metabolism , the amount of heat they produce depending upon such factors as temperature, moisture, oxygen concentration, nutrients, and the age of the cells.

Moisture. Water is required in the metabolism of microorganisms and is a part of their physical structure. Semeniuk classifies microorganisms according to their water requirements:

> Microorganisms are classified as hydrophytes, mesophytes, or xerophytes on the basis of their minimum moisture requirements for growth . . . . Hydrophytes grow when their minimum requirements are greater than 90% relative humidity, mesophytes when the minimum requirement is between 80 and 90%, and xerophytes when the minimum requirement is less than 80%. Hydrophytes grow best at 98 to 100% relative humidity, and xerophytes grow best at 95 to 100% relative humidity, or at some lower value . . . .[1]

Bacteria are hydrophytes, so far as is known. Therefore, bacteria grow faster on a solid or semisolid substrate in a

moist atmosphere than in a dry atmosphere.

Molds can be hydrophytes, mesophytes, or xerophytes.[1] For this reason molds can be expected to be active at a much lower relative humidity than bacteria.

Oxygen. Microorganisms are subdivided, according to their oxygen requirements, into anaerobes and aerobes. Bacteria are in both these classes. Molds also grow at wide ranges of oxygen requirements.

Microflora in stored grain. As was pointed out before, molds grow at lower relative humidities than bacteria. It is usually the molds that cause heating and spoiling of grain in storage. Molds die slowly in stored grain when held below the minimum relative humidity for growth.

According to Semeniuk in Anderson and Alcock[1] molds grow faster and sporulate more abundantly as the relative humidity approaches 95 to 100 percent, the temperature approaches 28 to 32 degrees centigrade, and the oxygen concentration exceeds a level of about one percent.

Review of Conditions for a Respiration Run

To test the effectiveness of the cathode ray treatment in improving the storage quality of wheat, one must select conditions giving a critical difference that would be simple and direct to measure and capable of being measured over a reasonable length of time. Inasmuch as mold growth is the principal cause of the deterioration of wheat, it was

decided to select conditions for its maximum growth. Temperature and rate of aeration were selected on the basis of research done by others in determining maximum mold growth. The length of time to run a trial was chosen for a period that would be sufficient to give a definite indication of deterioration of the wheat. Finally, indexes of the condition of the wheat and degree of deterioration also had to be chosen.

Methods of measuring respiration. There are three general methods for measuring the respiration of dormant seeds. In one method the seeds are kept in a closed container for a sufficient length of time so that the carbon dioxide content will build up to a measurable amount. The air inside is then analyzed for carbon dioxide. This is called the closed system. In the second method seeds are subject to a continuous aeration and the air passing through the seeds is analyzed for carbon dioxide. Finally, microtechnics are employed to measure respiration of individual seeds.

Most workers in cereal grains, namely Bailey and Gujar[5], Coleman, Rothgeb and Fellows[12], Lamour, Clayton and Wrenshall[28], and Ramstead and Geddes[40], have used the closed system. Matz and Milner[32], and Milner, Christensen and Geddes[34] used the continuous aerated system.

Microtechnics were used by Stiles and William.[44] In this method the respiration of individual seeds was measured

by determining the electrical conductivity of a wire which is in the atmosphere of the seed being measured. The temperature of a metal wire changes depending upon the type of gases surrounding the wire, because gases present determine the rate at which heat is dissipated. In this manner the presence of carbon dioxide can be detected once the instrument is calibrated.

The Warburg-Barcroft manometeric apparatus also has been used in respiration studies with wheat.

Finally, there have been techniques developed for the measuring of respiration in grain bins.

Rate of air supply. The rate at which wheat and the microorganisms both within and on the surface of wheat respire is dependent upon the supply of oxygen.[1,5,34] However, it was not until recent times that respiration under fixed conditions of temperature, moisture content, and air exchange was used to determine the deterioration of cereals.

Perhaps the most careful work of determining the influence of various rates of aeration on wheat was that done by Milner, Christensen, and Geddes in 1947.[34] In this experiment studies were made of the respiration in relation to moisture content, mold growth, chemical deterioration, germination, and rate of aeration. The results are summarized in Table III. In rates above 12.5 milliliters per gram of dry matter per day, the increase in respiration is not great.

Matz and Milner (1951),[32] in order to determine the influence of chemicals on respiration, selected 20.0 milliliters of air per day per gram of dry matter as part of a standard test to determine rates of respiration.

For the work in this thesis an attempt was made to aerate the samples at about 20.0 milliliters per gram of dry matter. As a matter of record, the samples averaged between 16.0 - 20.0 milliliters per gram of dry matter per day. This variation in air exchange should make little difference in the results, as can be seen from Table III.

Table III.

INFLUENCE OF AERATION RATE ON INTERSEED CARBON DIOXIDE CONCENTRATION, RESPIRATORY RATE, RESPIRATION QUOTIENT, FINAL MOISTURE CONTENT, FAT ACIDITY, AND GERMINATION OF REGENT WHEAT
(From Milner, Christensen, and Geddes 1947)[34] p.190

Aeration Rate	Interseed CO_2 Concentration			Respiratory Rate		
	3rd day	5th day	9th day	3rd day	5th day	9th day
ml/gdm/day	%	%	%	mg.CO_2 per 100gdm per "		
Original Sample	---	---	---	---	---	---
0.16*(N_2)	5.60	8.99	12.41	6.4	10.1	13.0
3.2	7.28	15.47	18.69	40.3	85.3	101.1
6.4	4.78	11.47	16.14	52.8	126.2	178.2
12.5	3.02	6.67	12.44	65.2	143.3	269.3
18.9	2.02	4.39	8.86	65.6	142.7	289.7
25.1	1.64	3.49	7.32	70.5	150.2	316.5

Aeration Rate	Respiration Quotient			Moisture		Fat Acidity	Germination
	3rd day	5th day	9th d	Initial %	Final %	mg KOH 100 g	%
ml/gdm/day							
Original Sample	---	---	---	12.4	12.4	12.6	98
0.16*(N_2)	6.15	9.77	13.20	20.4	20.5	12.7	95
3.2	0.95	0.95	0.90	20.4	20.5	65.9	24
6.4	1.01	0.95	0.83	20.4	20.8	69.6	18
12.5	1.04	0.93	0.80	20.4	20.9	69.6	18
18.9	1.03	0.92	0.80	20.4	21.0	71.6	19
25.1	1.07	0.92	0.82	20.4	21.1	74.4	16

(All tests run at 30°Centigrade.)

* Volume of air (20.93% oxygen) equivalent to nitrogen containing 1.01 oxygen.

Temperature for the respiration tests. That molds are the principal cause of the deterioration of stored grains can be inferred from the discussion under microflora. In these tests it was decided to select a temperature that

would be maximum for the growth of molds. According to Semeniuk in Anderson and Alcock[1], molds grow fastest in the temperature range 28 to 32 degrees centigrade when the water-vapor pressure approaches 95 to 100 percent and the oxygen concentration exceeds a level of about one percent. A temperature of 30 degrees centigrade was therefore selected as a suitable temperature to test the respiration of wheat.

Milner, Christensen, and Geddes[34], Matz and Milner[32], and Bottomley, Christensen, and Geddes[8] used a temperature of 30 degrees centigrade to test the effect of mold growth on stored grains.

Length of time for a respiration run. After a temperature, humidity, and aeration rate were selected for maximum mold growth, it was necessary to choose a practical length of time to run the experiment so as to have comparable results. Milner, Christensen, and Geddes[34] have made a complete analysis of the rate of respiration of wheat at a number of moisture percentages from one to twenty days at 30 degrees centigrade. This is summarized in Table IV. It can be seen that at the ten-day level all samples up to 16.8 percent moisture (wet basis) show a definite trend in their respiration rates. Ten days for a respiration run at 30 degrees centigrade was selected by Milner, Christensen, and Geddes (1947)[34] and Matz and Milner (1951)[32].

A length of ten days is a practical limit that was long enough to show results, but not so long as to interfere with the number of runs possible with a limited time.

Table IV.

INFLUENCE OF MOISTURE CONTENT AND TIME ON THE RESPIRATION RATE OF WHEAT AT 30° CENTIGRADE
RESPIRATION RATE, MG.CO_2 PER 100 G. DRY MATTER PER 24 HOURS

(Extracted from Milner, Christensen, and Geddes[34])

Day	Moisture Content % (Wet Basis)						
	12.3	13.6	13.8	14.5	15.4	16.3	16.8
1	0.05	0.13	0.13	0.16	0.20	0.54	---
2	0.04	0.15	0.18	0.25	0.37	0.86	1.8
3	0.06	0.15	0.25	0.31	0.45	1.03	1.4
4	0.07	---	---	---	---	---	1.5
5	0.08	0.14	0.25	0.36	0.48	1.24	1.8
6	0.08	0.15	0.25	0.36	0.51	1.38	1.8
7	0.08	0.12	0.26	0.35	0.49	1.72	2.7
8	0.09	0.15	0.24	0.36	0.41	2.26	4.0
9	0.08	0.12	0.26	0.36	0.53	5.58	5.9
10	0.09	0.14	0.23	0.36	0.55	6.98	9.1
11	0.10	0.14	0.25	0.33	0.60	---	12.4
12	0.04	0.12	0.23	0.33	0.65	15.88	15.2
13	0.07	0.10	0.22	0.33	0.78	17.71	17.7
14	0.08	0.14	0.23	0.34	0.90	19.21	18.9
15	0.07	0.14	0.24	0.36	1.06	20.04	19.8
16	0.08	0.13	0.22	0.41	1.25	20.54	20.2
17	0.07	0.13	0.22	0.42	1.48	21.06	20.3
18	0.07	0.11	0.23	0.46	1.75	21.47	---
19	0.10	0.14	0.24	0.53	2.08	22.67	---
20	0.07	0.11	0.23	0.57	2.53	23.35	---

Indexes of deterioration. It has long been known that grain and mill products increase in acidity while in storage. As a result a series of tests have been developed to determine the quality of wheat in storage by measuring the titrable acidity of grain. Foremost of these are:

(1) The Besley and Baston Method.
(2) The Greek or Balland Method.
(3) Schalerud's Method.
(4) The Former A.O.A.C. Tentative Method.
(5) Methods Based on Determination of Free Fatty Acids.

None of these methods yielded correlated results. Zeleny and Coleman[50] made a critical study of this problem. Under conditions in which wheat was deteriorating in storage, a series of acid tests was made on samples collected at periodic intervals. The results of these tests are shown in Figure 3 below.

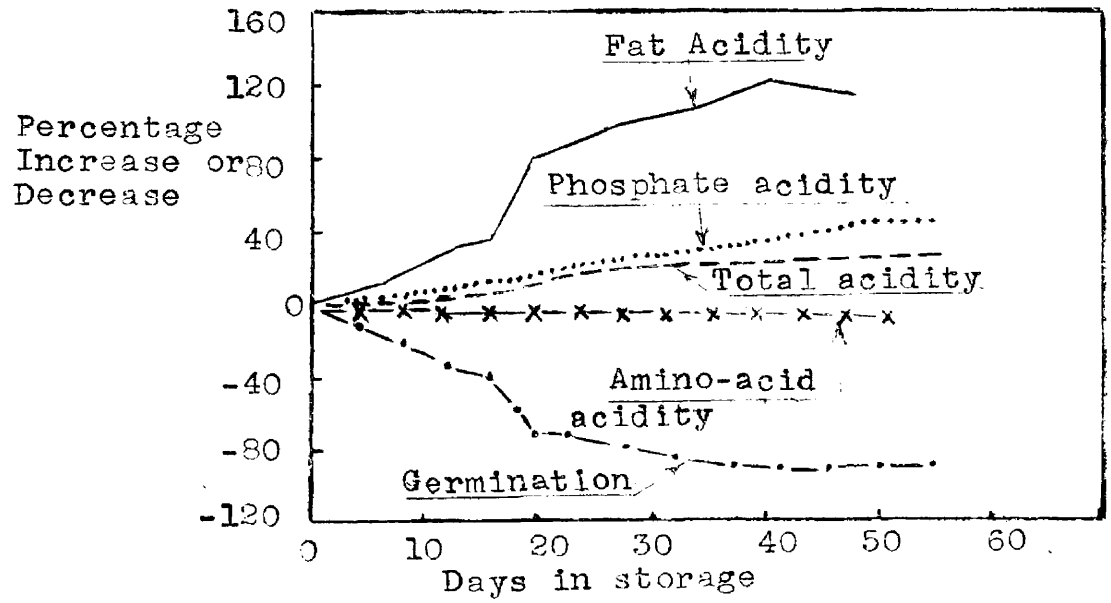


Fig.3 Percentage change in acidity values and germination of hard red winter wheat stored at 15.35% moisture at Hays, Kansas (Zeleny and Coleman).[50]

They concluded from these tests that a method based on determination of free fatty acids was the best test of the group to determine the deterioration of grain in storage. For this reason the determination of fat acidity was used as an index of deterioration in a series of experiments for this thesis.

Under the section, Respiration, it was shown that carbon dioxide is an index of the metabolic activity of living cells. While the complete combustion of a carbohydrate is not the same for every carbohydrate, their differences are not great. The complete combustion of a grain is determined by the grain itself, microorganisms, and insects. Insect activity can be controlled, and therefore the total metabolic activity of the grain itself and of microorganisms can be fairly well determined by the carbon dioxide respired. The rate of carbon dioxide given off is thus a critical index of the rate at which a grain is deteriorating.

Biological Effects of Irradiation

An atom consists of a charged heavy nucleus surrounded by electrons traveling in discrete orbits. An atom may become ionized by losing an electron, usually in the outer shell. Ionization can be caused by another electron striking the electron of the atom in the case of cathode rays, or by electromagnetic waves in the case of X-rays. In some instances, an electron may not be knocked entirely out of the shell, but only to another orbit of lower energy. In this

circumstance we say the atom is excited.[27] The ionization and excitation account for most of the dissipation of energy from irradiation except at very high energies of radiation.[31]

The exact process whereby biological changes due to irradiation take place is not fully known. It is theorized that some biological effects may take place owing to the loss of an electron, but of greater importance is the fact that the ionized atom can take part in a chemical change. Chemical combinations are set up which in turn can cause dissociation of the molecules. This, of course, can lead to drastic changes in the original material, since most organic materials consist of large molecular structures built of many atoms. The dose required to produce chemical change by direct action is inversely proportional to the molecular weight.[31] Another theory frequently advanced is that since the energy loss or gain is confined to a very small portion of the atom, this point could be at a very high temperature. This is the basis of the "point-heat" theory.[31]

A number of investigators have been interested in the effects of cathode and X-ray irradiation on cereals and microflora and insects found on cereals. The effect of cathode rays on germination and early growth of wheat was studied by Soderholm and Walker.[46] They found that dosages between 10,000 - 200,000 rep did not limit germination, but the high dosages had pronounced effect on further growth. They also found that dosages between 10,000 - 30,000 rep temporarily

limited the vigor of wheat. Dosages between 40,000 - 200,000 rep not only limited vigor, but completely checked continued growth.

Baker, Taboada, and Wiant[6] studied the effect of cathode rays on cereal insects. They found that 10,000 rep would sterilize flour beetle and granary weevil eggs and that this same dose would prevent the adults from reproducing. A dose of 5×10^5 rep was lethal to 100 percent of adult flour beetles immediately after treatment, whereas a dose of 2.5×10^5 rep was lethal to 100 percent of adult granary weevils immediately after treatment.

Smith[45] studied the influence of X-rays and heat on cereals. He noted the effect of chromosomal aberrations, percent of germination, and the height of seedlings after treatment with X-rays and heat. He found that severe heat delayed or prevented germination, but that if the seeds germinated, they grew more vigorously. X-rays did not delay or reduce germination so much, but after the more severe treatments all the seedlings did not germinate at once.

Dunn, Campbell, Fram, and Hutchins[16] made a series of experiments on the biological effects of irradiations. Some of the conclusions they reached are as follows:

1. Dosages required to destroy bacteria with cathode rays appear to be similar to those necessary with X-rays.

2. It took extremely high dosages to destroy enzymes. In many cases doses of 8,000,000 roentgens and higher were required.

3. For vitamins 500,000 roentgens resulted in high losses in the reduced ascorbic acid.

4. Non-spore forming bacteria were destroyed by dosages of less than 500,000 roentgens and from 64.5 to 99.9 percent of them were destroyed by 35,000 roentgens. Most spore forming bacteria were destroyed by application of less than 1,000,000 roentgens and 15 - 96 percent were destroyed by 25,000 roentgens.

5. The effect of X-rays on molds is summarized by the table below.

Table V.

LETHAL EFFECTS OF X-RAYS ON ASPERGILLUS NIGER[16]

Dose in roentgens	Average percentage destruction of molds	Range of percentage destruction of molds
25,000	95.96	91.29 to 98.25
50,000	99.63	98.93 99.96
100,000	99.98	99.999 99.999
250,000	99.999	99.999 100.00
500,000	100.00	100.00 100.00

In the case of the genus Mucor a dose of 1,000,000 roentgens destroyed all of the individual molds, whereas 500,000 roentgens destroyed 99 percent of them.

Lambou, et al,[26] used cathode rays produced by a Van de Graaff generator at three million volts on cottonseed to determine the effect on molds, bacteria, and germination. These are summarized in the table that follows.

Table VI.

EFFECT OF HIGH-VOLTAGE CATHODE RAYS ON INTERNAL INFECTION OF STONEVILLE 2B COTTONSEED[26]

Dose of cathode rays(rep)	Seeds infected with*			
	Molds %	Bacteria %	Molds and bacteria %	Total infected %
None	58	0	36	94
500,000	92	4	4	100
1,000,000	36	0	4	40
1,500,000	0	0	0	0
2,000,000	0	0	0	0
2,500,000	4t	0	0	4t
3,000,000	4t	0	0	4t

* - Samples of 50 seeds each were used for the analyses.
t - Probably a plate contaminant--molds were not of the same genera as appeared on the other plates.

Lambou[26] also found that high-voltage cathode rays reduced the number of microorganisms on and in the seed. It was done without bringing about any changes in the moisture and free fatty acid content, but there was a reduction in germination and an inhibition of growth.

Cathode Rays

Cathode rays, accelerated electrons, and beta rays all refer to high-energy electrons. Usually cathode rays and accelerated electrons refer to machine accelerated electrons, while beta rays refer to electrons emitted by radioactive decay.

Electrons are relatively light, small particles

containing a fixed charge.[27] In passing through matter an electron might give up a large part of its energy in a single inelastic collision with an atomic electron, or it may pass nearly through the material before colliding inelastically with an electron. An electron is light. It can rebound in many directions when it hits a heavy particle or another electron; as a result, there is a great difference of range of electrons of the same energy.

Electrons also lose energy by radiative collision. When an electron is accelerated or deaccelerated in the electric field of the nucleus, X-rays are produced. This phenomenon is known as "bremsstrahlung", a German word meaning "braking radiation."[27]

The specific ionization (total number of ions formed per centimeter of path corrected to one atmosphere) for the mean electron at various energies can be seen in Figure 4.

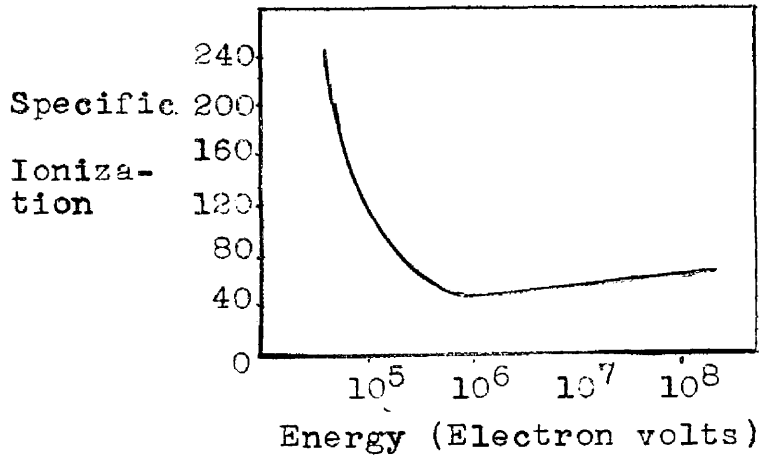


Fig.4 The Bragg ionization curve[27]

The shape of this curve can be explained as follows. At low energies or speeds an electron can interact with other electrons of an atom and therefore can transfer some of its energy to them easily. In some instances enough energy may be transferred to other electrons to produce delta rays. Delta rays are electron ejected from the shell of an atom by interaction with electrons coming from outside the atom.

In the case of faster electrons the speed is great enough so that the field of the electron does not have time to interact with the field of other electrons. This is true at about one million volts, as shown in Figure 4.

As the energy or speed of the electron increases beyond one million electron volts, relativistic considerations must be taken into account. Thus the electric field of the electron is condensed in a plane normal to its path. This increases its strength in this space, and consequently the electron reaches out further to interact with more electrons.

The range energy relationships for electrons of one million electron volts and below can be seen on Figure 5. This curve was obtained empirically by Glendenin.[20]

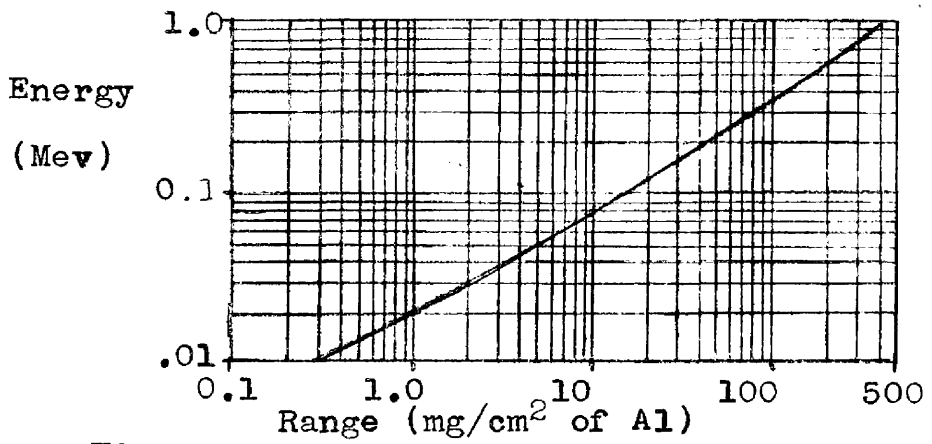


Fig.5 Range of low-energy beta particles

From Glendenin, Nucleonics 2,1 (1948)[20]

EQUIPMENT

Description of the Resonant Transformer Electron Accelerator

The essential features of the accelerator are the tube, the transformer, the tank, and the motor-generator set. These are all combined into two large units with appropriate controls and protection. The transformer is enclosed in a tank made of three-eighths inch steel. Within are the primary windings and the secondary windings. At the center of the transformer is the tube. The output is a sine-wave energy curve delivered during the rectifying half-cycle. It delivers 1,000,000 volts at the peak.

The transformer. The primary windings are excited by the motor-generator set. Three-phase, 60-cycle, 220-volt current is converted to 180-cycle, single-phase current. The number of secondary turns is so chosen as to make its natural period of oscillation match that of the supply circuit. This transformer has no metal core, and the magnetic flux does not interfere with the operation of the tube. The advantage of this system is reduction in weight, no hysteresis losses, and small space requirements. The secondary coil is placed just within the primary windings. To protect the secondary coil from the high voltage, sulfur hexafluoride gas under pressure of 60 pounds per square inch in the steel tank is used to increase the dielectric strength between the

turns of the secondary. Also the potential is graded from one end of the coil to the other so as to prevent creepage over the solid dielectric between coils. The secondary windings are connected at intervals to the 12 sections of the tube.

Narrow strips of silicon steel electrically separated from one another are placed on the outside of the primary windings to shield the steel tank from the magnetic field of the transformer.

The tube. The tube consists of 12 sections of pyrex glass tubing sealed to fernico rings which carry the accelerating electrodes. These electrodes or plates inside the tube are of stainless steel and provide acceleration to the electrons. At the top of the tube is the cathode, which consists of 6.5 convolutions of 8.5 millimeters tungsten wire mounted in an electrostatic cup. The whole tube is evacuated to about 28 inches of mercury. A focusing coil near the bottom of the tube determines the spread of the beam below. Electrons shoot out through a stainless steel window at the bottom of the tube.

The electrical controls. There are two principal controls, tube voltage and filament current. The generator field controls the voltage in the primary of the transformer, and the primary voltage controls the voltage in the secondary coil. The secondary coil determines the tube voltage.

Beneath the steel tank a reversible motor rotates a glass tube which controls the filament current. The glass tube extends up through the tank to a variable inductance in the filament circuit. Change in the inductance controls the filament current.

Power for the transformer is obtained from a synchronous motor-generator set. Three-phase, 60-cycle, 230-volt power is converted into 180-cycle, single-phase power. There are the usual motor controls and safeties.

A blast of air beneath the window of the tube cools the window. Whenever the electron beam is passing through the window, this blast operates.

Beneath the accelerator is a conveyor belt that can run at a number of precise speeds. This is not a part of the accelerator, but is one of the controls to determine dose rate of the material being irradiated.

Efficiency. The efficiency of the machine can be estimated as follows: at 1,000,000 volt peak and nine milliamperes in the tube there will be a beam-out current of six milliamperes. Fourteen kilowatts of input power will be required at this setting. There will be (.707)(.006)(1,000,000) or about four kilowatts of beam-out power which will make an efficiency roughly of about 30 percent. If the beam is used to 50 percent capacity on the material being irradiated, 15 percent of the energy will be applied to useful work.

The Respiration Chamber

The continuous aerated method was selected to measure respiration in this study because it represents a set of conditions that would be apt to occur in grain during storage. The microtechnic method was not selected since individual seeds tend to vary too greatly from the average. The interrupted method, in which carbon dioxide is allowed to accumulate between the seeds, was rejected because respiration is suppressed when carbon dioxide accumulates and therefore tends to inhibit maximum respiration.

The respiration chamber consisted of the box, the train of Erlenmeyer flasks, the heating system, and the timer, the purpose of the chamber being to maintain wheat at a constant temperature and to supply a constant source of air at a given relative humidity free of carbon dioxide.

The box was constructed of one-quarter inch plywood covered with one-half inch celotex insulation and reinforced with one-by-two-inch wood at the edges. It was 24 inches high, 30 inches wide and 42 inches long. A shelf approximately 30 by 30 inches divided the box horizontally through the middle. This permitted a 6 by 30 inch space on each end of the shelf which provided a path for the air to circulate inside the box. Heat was provided by one 100-watt electric light bulb. A thermostat inside controlled the light bulb.

The box was tested for variation in temperature. A hygrothermograph placed inside the box showed a variation of plus

or minus one degree at 86 degree Fahrenheit, with a cyclic period averaging 24 minutes. This hygrothermograph was calibrated with a mercury thermometer calibrated in turn with a certified thermometer.

In the respiration method adopted, air was passed over a thin layer of wheat in a gallon bottle and analyzed at intervals for carbon dioxide. Air was sucked through the system by the continuous drop in level of a saturated solution of calcium chloride in a gallon bottle (see Figure 6). The solution level was lowered by a synchronous motor of a time clock F. A rubber tube 'b' connected from the gallon bottle D determined the height of liquid level in this gallon bottle.

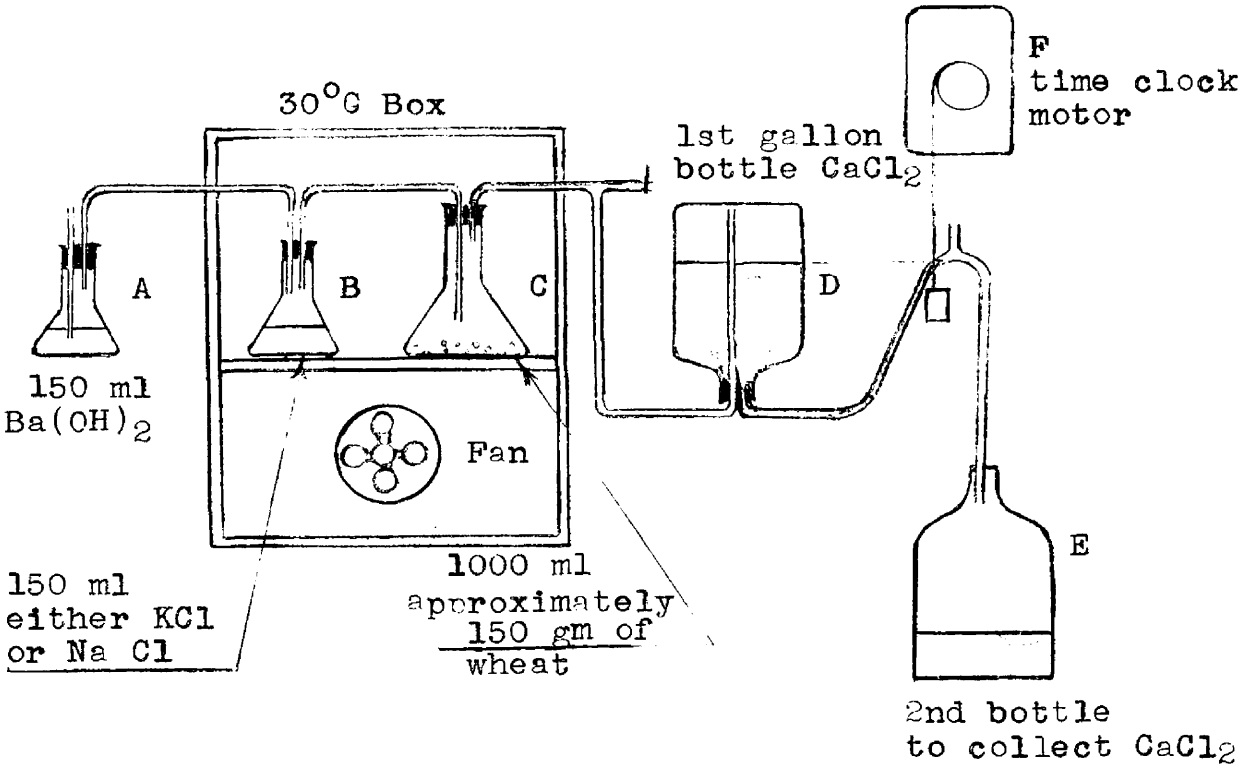


Fig. 6 Schematic diagram of respiration chamber

As the time clock lowered the rubber tube, the liquid level in both the gallon bottle and the tube dropped. The general scheme can be seen in Figure 6.

The liquid that ran out of bottle D was collected in bottle E. If the air inside the system was at atmospheric pressure, then the amount of air passing through the system could be determined by the amount of liquid collected in the second bottle E. Actually there was only a slight difference between the inside pressure and atmospheric pressure as measured by a water manometer. Usually the variation of pressure between inside and outside was one-eighth to one-quarter inch of water.

Respiration Chamber for the Comprehensive Samples

A grain sample for comprehensive testing had to be at least twelve hundred grams in size owing to the number of tests and quantity of grain required per test. This larger sample necessitated a change in apparatus from that used for the respiration determinations. It was desired that conditions for maximum rate of mold growth be duplicated, which were 86 degrees Fahrenheit and 20 milliliters of air per gram of dry matter in 24 hours. A large room was used to maintain a temperature of 87 degrees Fahrenheit by electric heat and a suitable thermostat. Eighty-seven degrees Fahrenheit was one degree higher than was desired, but was difficult to change.

The air exchange system depended on the displacement

of one gallon of water on an average of every four hours and 48 minutes. The average air exchange was about 16.0 milliliters of air per gram of dry matter per 24 hours which is a little lower than the 19.0 milliliters averaged in the respiration tests. Even so there should be insignificant differences in respiration according to Table III.

The apparatus used for making the 10-day conditions for ideal mold growth consisted of three one-gallon bottles and two 150-milliliter Erhlenmeyer flasks connected in a train by rubber tubing. The gallon bottle A (Figure 7) contained a screen cylinder 3 3/4 inches in diameter and approximately 10 inches in height. Wheat was placed between this cylinder and the walls of the glass bottle, forming a thickness of wheat approximately one inch through.

A gallon of air was exchanged in the following manner. Gallon bottle B was filled with water. When pinch cock d was opened, water ran down to bottle C. This in turn drew air through the train of Erlenmeyer flasks and the wheat sample to replace the water which had run out (see Figure 7). Every four hours and 48 minutes this process was repeated by exchanging bottles B and C, and exchanging the tube connections, the procedure being continued for 10 days. Although this method did not provide continuous aeration, it was a close approximation to such conditions.

Erlenmeyer flasks E and F were used for the same purpose as those in the respiration tests. Flask E contained an

excess of barium hydroxide to remove any excess carbon dioxide in the atmosphere. Flask F was used to keep the air passing through the wheat sample at the desired relative humidity by use of suitable salt solutions.

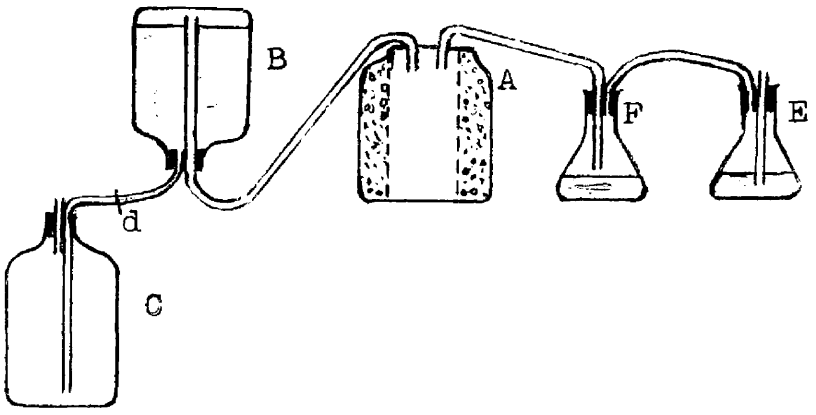


Fig.7 Apparatus for comprehensive tests

List of Other Equipment

Other equipment used is listed below.

Hygro-Thermograph (10° - 100°F) (0 - 100 percent RH), The Instrument Corporation

Tag-Heppenstall Moisture Tester, Model 8004

Analytical Balance (accurate to 0.1 mg.), Central Scientific Company

Boerner Divider, Seedburo Equipment Company

Potentiometer (Direct temperature reading) (1°F), Leeds and Northrup

Various mercury-in-glass thermometers

Graduated cylinders

Burettes

Drying oven

EXPERIMENTAL METHODS

Three types of tests were made in this experiment: respiration tests, thermos-bottle tests, and comprehensive tests. The respiration tests were conducted under conditions suitable for maximum mold growth. The thermos bottle tests attempted to duplicate conditions in a grain bin.

The comprehensive tests were a series of tests to determine the effectiveness of irradiation in the reduction of mold growth and to find what deteriorating effect irradiation had on the quality of wheat for food. These tests were more complete than simple carbon dioxide measurements and fat acidity determinations. Also tests actually required in the milling industry were performed.

Respiration Tests

Five respiration runs were made, each run consisting of eight samples that were kept in a respiration chamber at 86 degrees Fahrenheit for a period of 10 days. These runs were made with samples of approximately 150 grams of wheat per sample. At the end of 10 days the wheat samples were analyzed for the amount of carbon dioxide given off in 24 hours (test for respiration) and tested for moisture by drying a small sample in an oven for 72 hours at 212 degrees Fahrenheit. Then the remainders of the samples were placed in storage at 40 degrees Fahrenheit until shipped to the laboratory for

fat acidity determinations.

The first two runs were exploratory in nature to find approximately what irradiation dosages were necessary to reduce total respiration to a level comparable with that of very dry wheat (10.0 - 12.0 percent wet basis).

The irradiation doses for the first run were 0 rep, 130,000 rep, 261,000 rep, and 392,000 rep. It was found after 10 days that these doses had little effect upon respiration. On a second run at 0 rep, 1,000,000 rep, 2,000,000 rep, and 3,000,000 rep respiration was affected to a considerable extent in all but 0 rep samples. Runs three, four, and five were carried out at treatments of 0 rep, 500,000 rep, 700,000 rep, and 900,000 rep to find out how effective these doses were at 14.0 - 15.0 percent, 15.0 - 16.0 percent, and 16.0 - 17.0 percent moisture.

Of the eight samples in a run, four of the samples were of low moisture content (approximately 10.0 - 12.0 percent moisture content wet basis) and were used as a control. The other four were of high moisture content (14.0 - 17.0 percent). The high moisture samples were in one of three ranges 14.0 - 15.0 percent, 15.0 - 16.0 percent, or 16.0 - 17.0 percent. Moisture contents above 14.0 percent wet basis are not safe for storage, whereas those from 10.0 - 12.0 percent moisture are safe. For each run of eight there were three levels of irradiation. (See Table VII.) The samples were arranged in a series of four pairs: one pair with no treatment, one at

a low level of treatment, another at a medium level of treatment, and a final pair at a high level of treatment. Each pair consisted of a sample at low moisture content and one at high moisture content. (See Table VII.)

Table VII.

SAMPLES OF WHEAT FOR A RESPIRATION TEST

Moisture Content	Doses			
	no	low	medium	high
High	Sample 1	Sample 3	Sample 5	Sample 7
Low	Sample 2	Sample 4	Sample 6	Sample 8

The arrangement shown in Table VII. is known in statistics as two-way classification.

By analysis of variance it is possible to determine if there is a significant difference between sample 2 (low moisture content, no dose) and sample 7 (high moisture, high dose) at a certain level of significance, or in this instance whether the effect of irradiation on high moisture wheat was comparable to keeping wheat at a low moisture content.

Method of selecting samples. Two lots of wheat from the 1955 crop of hard red spring wheat were furnished by the International Milling Company. Lot No. 1 was used on the first run and lot No. 2 for the remainder of the runs. For the respiration runs a sample about two gallons in size, sufficient for the run, was selected at random for the lot. This large

sample was split equally by a Boerner Divider (see Figure 8). One of these samples was split in half in the Boerner Divider, and then each of these halves was again split in half. This would make four samples of about one quart each and one sample of about one gallon. Each one quart sample was poured into a gallon bottle, stoppered, and placed in cold storage at about 40 degrees Fahrenheit. The remaining one gallon sample was sprayed with distilled water, as explained under the section "Conditioning grain to the proper moisture" page 42. This gallon was placed in storage at 40 degrees Fahrenheit, tested for moisture after two days, and sprayed again if necessary until the proper moisture levels was reached. Then this sample was split in the Boerner Divider and each half sample split again. making four quart samples. Next, each of these samples was placed in a four gallon bottle. The bottles were stoppered and placed in storage at 40 degrees Fahrenheit.

A definite numbering system was used. The first four samples that were not conditioned with water were numbered 2, 4, 6, and 8. The samples that were conditioned with water were numbered 1, 3, 5, and 7. Samples 1 and 2 were not irradiated. Samples 3 and 4 received the lowest doses; samples 5 and 6, the next; samples 7 and 8, the highest doses.

Each of these samples was handled in the following manner after irradiation. About 50 grams of wheat was used for a moisture determination; 150 grams for a respiration run;

100 grams for a fat acidity determination; one pint for the thermos bottle test. At the conclusion of the respiration run the 150-gram sample was used to make a 50-gram moisture determination and a 100-gram fat-acidity determination. Moisture content was determined by finding the loss in weight after drying 72 hours at 212 degrees Fahrenheit.

All samples were placed in storage at 40 degrees Fahrenheit when not actually undergoing a respiration run, moisture determination, or fat acidity determination.

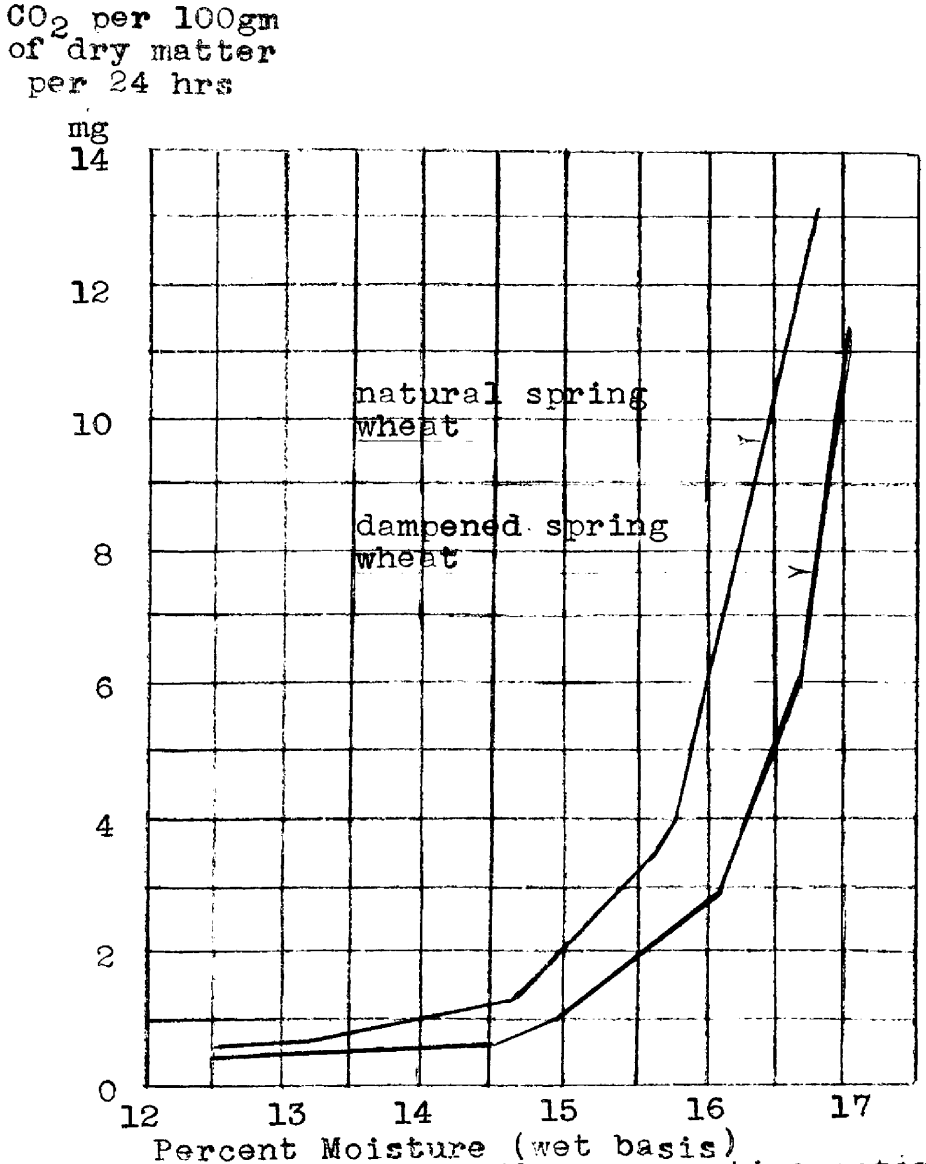
Figure 8. A Boerner Divider which was used to make an equal and random division.

Conditioning grain to the proper moisture. It was difficult to find wheat direct from the field with the desired moisture content for a given experiment. Other investigators (5, 32, 34) have resorted to sprinkling the grain with distilled water and waiting a few days for the grain to reach equilibrium before testing in a respiration run. Bailey and Gujar[5] compared wheat that had been sprinkled with distilled water with grain that was naturally damp. The results of this investigation are shown in Figure 9. In this experiment they had waited three days after wetting the grain before testing in a respiration run. There appeared to be some difference in the rate of respiration, but it was not great.

In view of this work by Bailey and Gujar[5], it was decided to sprinkle the grain to the desired moisture level and then wait for the grain to come to equilibrium. A small fly sprayer was used for sprinkling. Usually it took a week for the grain to reach the desired level.

The average sample was conditioned in the following manner: it was sprayed with distilled water, poured into a sealed container, and then placed in a cold storage room at 40 degrees Fahrenheit. The following day a sample was tested for moisture on the Tag-Heppenstahl moisture meter. The moisture deficit from the selected moisture level was noted and more water was sprayed on; then the sample was returned to the sealed container and storage room. This procedure

was repeated until the desired moisture level was reached, after which the sample was kept in the sealed container in cold storage at 40 degrees Fahrenheit until tested for respiration. The final moisture content was determined by drying in an oven for 72 hours at 212 degrees Fahrenheit and finding the loss in weight.



Respiration of hard spring wheat incubated at 38.8°C. for four days.

Fig.9 Graph showing the comparative activity of naturally damp wheat and wheat dampened in the laboratory three days before being incubated.[5]

Figure 10. Spraying wheat with distilled water to condition it to the proper moisture level.

Irradiating wheat to the required dosage. All irradiation was performed by the electron accelerator described on pages 29 - 32. The irradiation dosages were determined by the distance from the window of the electron accelerator, by the speed at which the samples passed by this window, and by the beam-out current. A conveyor beneath the electron accelerator window could be raised and lowered to appropriate levels. Also the speed of the conveyor could be changed by a simple adjustment on the variable-speed gear box. Doses had been worked out in advance on a series of mathematical curves which could be converted into a series of speeds, beam-out currents, and distances from the window. The actual adjustment of the electron accelerator and conveyor was made by Richard Nicholas, the operator.

The wheat to be irradiated was spread out one wheat kernel in thickness on an aluminum tray six inches wide and

40 inches long. This tray held about one-half pint of wheat (see Figure 11). The tray of wheat was placed on the conveyor ; all persons left the room, and the conveyor was started electrically after the machine reached the level of 1,000 kilovolts peak. At the conclusion of the run the conveyor was stopped; the electron accelerator was shut off; the tray was removed, and its contents poured into the appropriate sample bottle. Another tray had been prepared meanwhile and it was placed on the conveyor. This procedure was repeated until all samples had been treated.

Figure 11. The wheat sample on the conveyor ready to be irradiated.

Excluding carbon dioxide from the incoming air. The first flask A in Figure 6 was used to remove carbon dioxide that might be in the atmosphere. Air from the room was drawn into the train of flasks by the displacement of calcium chloride from the first gallon bottle D. The barium ion of barium hydroxide has a strong affinity for the carbonate ion and forms a very insoluble barium carbonate. Carbon dioxide entering a solution of barium hydroxide first forms carbonic acid and then ionizes to form hydrogen and carbonate ions. The carbonate ion combines with the barium ion to form the precipitate barium carbonate. This reaction will take place to eliminate completely carbon dioxide in the incoming air provided that there is an intimate mixture between the air and the barium hydroxide. It is easy to provide an excess of barium hydroxide, but difficult to make an intimate mixture between the incoming air and the barium hydroxide solution. To a large extent the intimacy of the mixture depended upon the size of bubble passing through the barium hydroxide solution. This in turn depended upon the rate at which air passed through the system. In this particular case the rate of air passage averaged 2.4 liters per 24 hours or about 1 2/3 milliliters per minute. The bubbles coming through seemed small.

No test was made of the efficiency of this method in excluding carbon dioxide, but it is estimated that carbon dioxide in the air had little influence on the results.

The content of carbon dioxide in air varies from place to place and time to time, but averages about 0.03 milliliters per liter. This should not decrease the accuracy excessively, as measurements of carbon dioxide to approximately plus or minus one milliliter per liter were made in these tests. The solution in the first bottle A removed any excessive carbon dioxide.

Maintaining the wheat samples in the respiration chamber at a constant moisture level. The moisture content of atmospheric air varies considerably from day to day and from one part of the year to another. In addition, as the air is heated from about 77 to 86 degrees Fahrenheit (as it generally was in this experiment) the relative humidity became lower. In this experiment it was desired that wheat be kept at four ranges of moisture content: between 14.0 - 15.0 percent, 15.0 - 16.0 percent, 16.0 - 17.0 percent, and 10.0 - 12.0 percent.

It was necessary to condition air that was drawn over the samples in order to prevent the air from changing the moisture content of the wheat. Coleman and Fellows[11] tested a number of wheats to determine the equilibrium conditions between the moisture content of wheat and the corresponding relative humidity. Their work on hard red spring wheat is listed in Table VIII.

Table VIII.

MOISTURE CONTENT OF HARD RED SPRING WHEAT IN EQUILIBRIUM WITH VARIOUS RELATIVE HUMIDITIES[11]

Moisture Content of Hard Red Spring Wheat		Relative Humidity of Atmosphere
Dry Basis	Wet Basis	
%	%	%
7.27	6.71	15
9.25	8.47	30
11.21	10.08	45
13.40	11.8	60
17.32	14.7	75
24.61	19.7	90
33.42	25.0	100

In order to maintain a moisture content of a wheat sample throughout a respiration test run it was necessary to maintain the relative humidity in accordance with the results shown in Table VIII. To have done this mechanically would have been costly. A salt solution will maintain a certain vapor pressure depending upon the chemical, its concentration, and its temperature. The easiest method of attaining a constant concentration was to maintain saturated salt solutions by placing an excess of salt in the solution. The difficulty with this method was that the saturated salt solution that came nearest to maintaining the desired relative humidity would not have been close enough. This can be seen in Table IX.

Table IX.

RELATIVE HUMIDITY OF A FEW SATURATED SALT SOLUTIONS HELD AT 30°C
(From International Critical Tables, Volume 3, pp.351-85)

Saturated Salt	Vapor Pressure mm. of Hg.	Relative Humidity at 30°C %
$LiCl \cdot H_2O$	3.56	10.8
$MgCl_2 \cdot 6H_2O$	10.3	32.4
$MnCl_2 \cdot 4H_2O$	17.0	51.8
$CuCl_2$	21.52	65.5
KCl	26.9	81.8
NaCl	23.9	72.7

In order to make use of the material in Table VIII it was plotted on a graph as shown on Figure 12. From this figure it can be seen that to maintain a moisture content of 16.0 - 17.0 percent a relative humidity between 80 - 84 percent must be maintained; for wheat of 15.0 - 16.0 percent relative humidities between 75 - 80 percent; for wheat of 14.0 - 15.0 percent relative humidities between 70 - 75 percent; and for 10.0 - 12.0 percent wheat, relative humidities between 47 - 62 percent. Table IX revealed that a saturated solution of potassium chloride was in equilibrium at 81.8 percent relative humidity and therefore could be used for maintaining wheat between 16.0 - 17.0 percent moisture. In a similar way sodium chloride could be used for maintaining wheat between 14.0 - 15.0 percent. Inasmuch as potassium

chloride solution could maintain a relative humidity of 81.8 percent and this was very close to the 15.0 - 16.0 percent moisture level, it was used for maintaining this level in the experiment.

The average inside air in the room surrounding the respiration chamber averaged 70 degrees Fahrenheit at 50 percent relative humidity. If this air was heated to 86 degrees Fahrenheit it would have a relative humidity of about 29.7 percent. This is about right for 8.5 - 9.0 percent wheat which is close to 10.0 - 12.0 percent moisture. Therefore, the air for the 10.0 - 12.0 percent wheat was passed over water containing a dilute solution of sodium chloride.

In summary, the following solutions were used to maintain moisture of wheat at the required levels:

16.0 - 17.0	percent	Saturated KCl
15.0 - 16.0	"	" KCl
14.0 - 15.0	"	" NaCl
10.0 - 12.0	"	Dilute NaCl

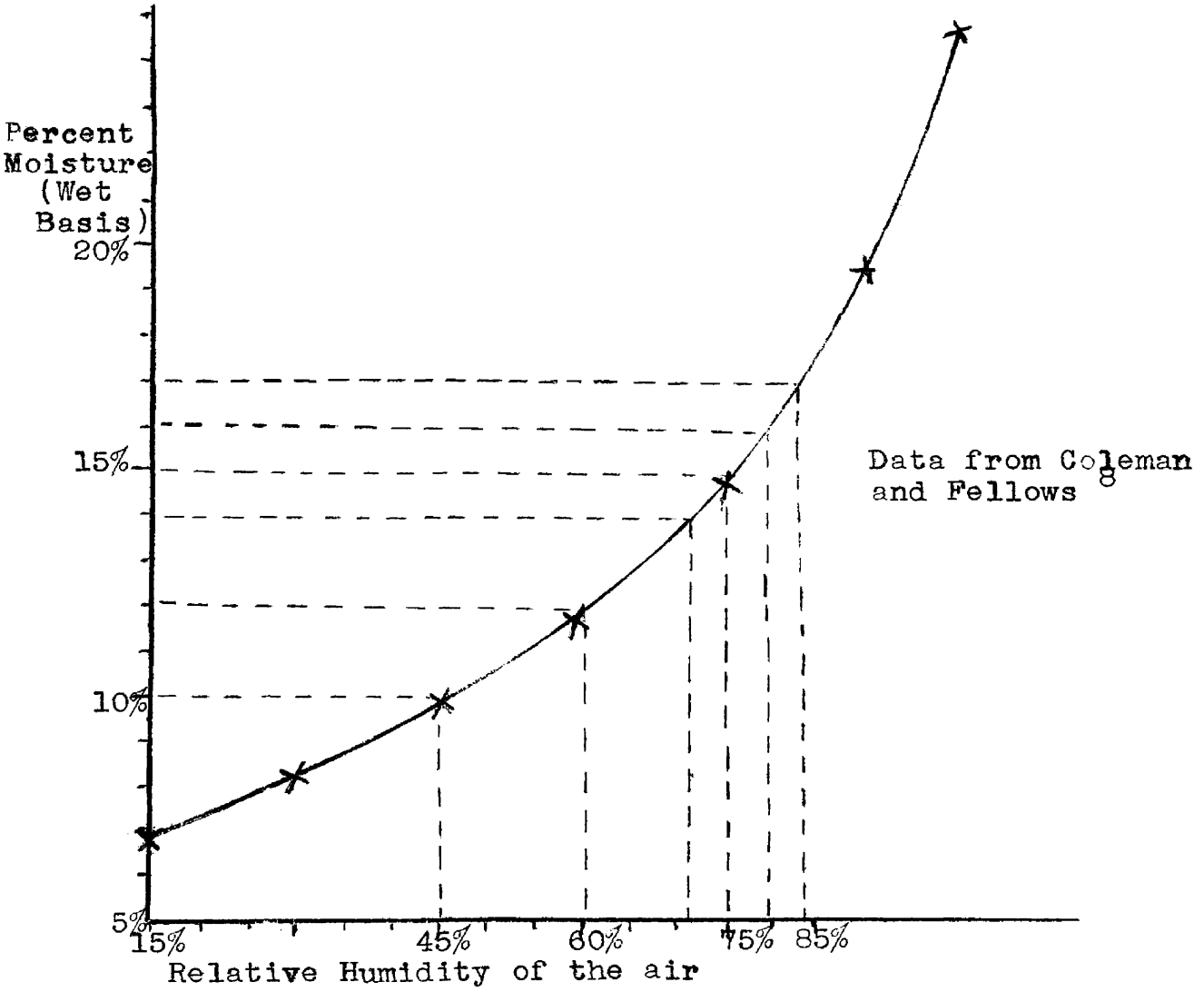


Fig. 12 Equilibrium humidities of hard red spring wheat at various moisture levels.

Maintaining a constant air supply. According to the section entitled 'Rate of air supply' (page 15), it could be expected that the maximum rate of mold growth would be at about 20.0 milliliters of air per day per gram of dry matter. If the samples were approximately 125 grams of dry matter each, then there should have been (20.0) (125) or 2,500 milliliters of air supplied per day per sample.

A constant supply of air was obtained by allowing a concentrated solution of calcium chloride to drain from a gallon bottle at a constant rate. The displacement of the solution sucked in air, which passed through the train of bottles, consisting of the Erlenmeyer flask that removed the carbon dioxide from the incoming air, the Erlenmeyer flask that maintained a constant relative humidity, and the Erlenmeyer flask that contained the wheat sample.

In Figure 6 the operation of the air supply system can be seen. Time clock F allowed the tube b to drop at a fixed rate. The level of tube b determined the level in gallon bottle D. As tube b was lowered, the excess solution of calcium chloride solution drained over into bottle E. The rate at which b fell determined the rate at which air passed through the system. The time clock determined the speed at which the tube fell, and therefore it determined the rate at which air passed through the wheat sample. As a result it was necessary only to use the right size of pulley on the time clock to control the rate at which the air passed

through the system.

The total quantity of air that passed over the wheat could be measured if the solution of calcium chloride were measured and if the air passing into bottle D were at atmospheric pressure. The solution passing into bottle E could be measured to the nearest milliliter by means of volumetric flasks and graduated cylinders, and by calibrated dip sticks to the nearest 20 milliliters. In gallon bottle D the pressure could be determined by observation of the tube level above the solution level in Erlenmeyer flask A. It was observed to be never more than one-quarter inch different from the solution level. As the atmospheric pressure was fairly close to 29.0 cm of mercury, one-quarter inch of water was approximately (29.0) (13.6) (2.54) (4) or one part in 4,000, a negligible amount.

Figure 13. Filling gallon bottle D of respiration chamber.

It was necessary to refill bottle D each day (Figure 11) to repeat the experiment for another 24 hours. This refilling was accomplished by closing pinch cocks at J and K and opening the pinch cock at L. New solution was then admitted at H. This operation can be seen in Figures 13 and 14.

Calcium chloride solution was used, as carbon dioxide is only slightly soluble in calcium chloride solution. Inasmuch as carbon dioxide was the gas to be measured to determine the rate of respiration, it was essential that very little be lost. A test was made of the set-up in the experiment, disclosing that approximately 0.7 milligrams of carbon dioxide was absorbed into the system each 24 hours. This amount was for new solution; for solution that would be in contact with carbon dioxide every day, as it was in this experiment, the amount absorbed would be even less.

Determination of carbon dioxide content. As was explained previously in the section 'Respiration' (page 5), the rate at which wheat respires can be determined by the quantity of carbon dioxide given off. In this experiment the quantity of carbon dioxide given up was measured for a 24-hour interval and was used as an index of deterioration and condition of the wheat.

When carbon dioxide is passed through a solution of barium hydroxide, the carbon dioxide first goes into solution forming carbonic acid, and then the carbonate radical combines with the barium ion to form the very insoluble

barium carbonate. The completeness of this reaction is determined by the intimacy the carbon dioxide reaches with the solution. The total quantity of carbon dioxide that passed into the solution could be determined in these experiments by measuring the remaining hydroxyl-ion concentration of a known original quantity and normality of barium hydroxide. If the carbon dioxide is sufficiently mixed with the barium hydroxide solution, all of the carbon dioxide will go into solution and form insoluble barium carbonate. That is,

$$CO_2 + H_2O \longrightarrow H_2CO_3$$
$$H_2CO_3 + Ba(OH)_2 \longrightarrow BaCO_3 + 2H_2O$$

The remaining barium hydroxide will be that which is not precipitated by the carbon dioxide. The difference between the original quantity of barium hydroxide and that remaining which did not combine with the carbon dioxide is the amount that had combined chemically with the carbon dioxide. With the chemical equation for combination of carbonic acid and barium hydroxide known, the total quantity of carbon dioxide combined can be found.

The method of using barium hydroxide to precipitate carbon dioxide and titrating the residual barium hydroxide against an acid of known normality was the method used by Bailey and Gujar[5] in their classical experiments to determine the respiration of wheat.

In order to determine the normality of the barium hydroxide accurately, it was titrated against a carefully

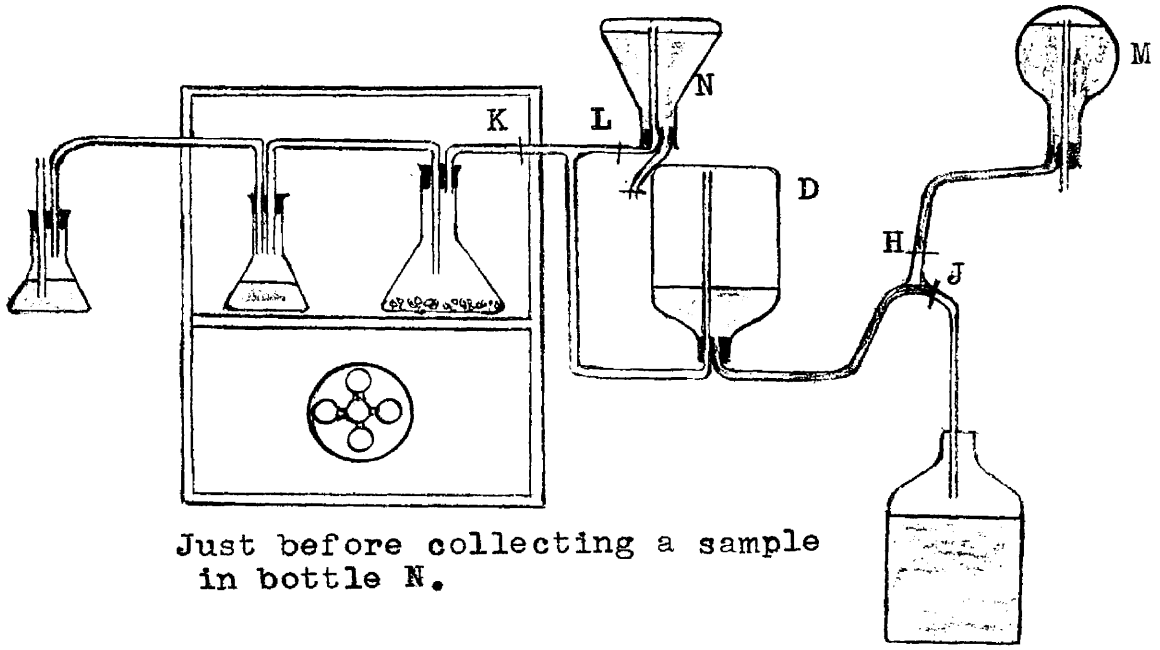
prepared solution of potassium acid phthalate each time a respiration test was made. Also the barium hydroxide was titrated against the hydrochloric acid in the experiment to standardize the hydrochloric acid. Both of these titrations were triplicated to ensure accuracy.

Potassium acid phthalate was used as a standard, first because it does not change greatly with time, and secondly because owing to its large atomic weight (204.0), it is relatively easy to use in making an accurate normal solution.

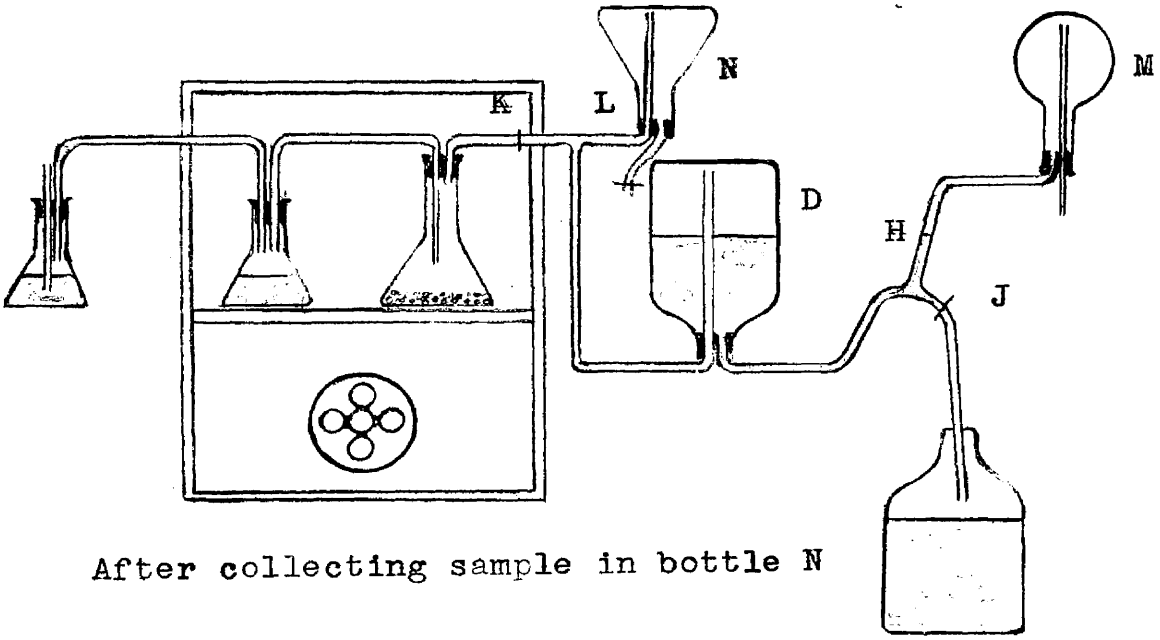
Dilute solutions of both barium hydroxide (usually about .05 normal) and hydrochloric acid (usually about .10 normal) were used to increase the accuracy of reading the burette during titration.

Method of obtaining a respiration sample. Generally a one-liter sample of air at atmospheric pressure was the size of sample for respiration determination that was drawn from the air that had passed over the wheat. As was explained in the section "Maintaining a constant air supply" (page 53), the total air passed over the sample in 24 hours was collected in bottle D (Figure 13). The air that had collected in this bottle was at atmospheric pressure or slightly below (perhaps one-quarter inch of water below atmospheric pressure). A sample of one liter was drawn by closing pinch cocks J and K, filling one liter of calcium chloride solution through H and drawing out one liter of the air at point

L (see Figure 14). Previously, the one liter Erlenmeyer flask N had been filled with one liter of distilled water.



Just before collecting a sample in bottle N.



After collecting sample in bottle N

Fig. 14 Method of obtaining a respiration sample

The calcium chloride solution in volumetric flask M forced the air out of bottle D at the same time the distilled water drained out below sample bottle N and helped draw in the sample. If exactly one liter of distilled water was placed in sample bottle N and one liter of calcium chloride solution was placed in volumetric flask M, then a liter of air would be drawn out of the bottle D into sample bottle N. Allowance was made for the volume of tubing.

This method of drawing air over wheat and collecting a sample of air was similar to the methods used by Milner, Christensen, and Geddes (1947)[34], Matz and Milner (1951)[32], and Bottomley, Christensen, and Geddes (1952)[8].

Measuring the respiration of a sample of wheat. After drawing the sample as explained in the section "Method of obtaining a respiration sample" (page 56), thirty milliliters of barium hydroxide solution of about 0.05 normality was added to the sample of air drawn. This was done by filling a 50-milliliter burette exactly full, placing the tip end in one of the rubber tubes, opening the pinch cock on the rubber tube, opening the valve on the burette, and allowing exactly 30 milliliters to flow into the flask. This method can be seen best by observing Figure 15. When the flask had been filled to 30 milliliters, the pinch cock on the rubber tube was closed and the burette withdrawn. There were now 30 milliliters of barium hydroxide in contact with the sample of

air containing the carbon dioxide. The sample bottle was slightly above atmospheric pressure owing to the addition of the 30 milliliters of barium hydroxide. The increased pressure helped precipitate the barium carbonate. Then the sample bottle was vigorously shaken up and down two hundred times to ensure that all carbon dioxide would be taken out of the air sample. It can be seen that there is an advantage to this method as compared to collecting carbon dioxide by passing it once over a solution as is sometimes done. The method described here ensures precipitation of almost all carbon dioxide as a carbonate.

Figure 15. Adding 30 milliliters of $Ba(OH)_2$ to the sample.

The stopper on the sample bottle was now opened, and distilled water was used to wash down the inside of the sample bottle to ensure that all of the solution would be titrated. Two drops of phenolphthalein were added and the solution titrated against hydrochloric acid of about 0.1 normality.

The total quantity of carbon dioxide was calculated as shown in the section "Determination of carbon dioxide content" (page 54). The total carbon dioxide respired was:

$$\text{Total carbon dioxide respired by sample of wheat in 24 hours} = \frac{\left(\begin{array}{c}\text{Total air passed}\\ \text{over wheat in 24}\\ \text{hours in ml.}\end{array}\right)\left(\begin{array}{c}\text{Carbon dioxide}\\ \text{present in}\\ \text{1,000 ml.}\end{array}\right)}{1{,}000}$$

Usually, the respiration rate of wheat is given in milligrams of carbon dioxide per 24 hours per 100 grams of dry matter. Since there was on an average 125 grams of dry matter, a correction had to be made for this difference.

Errors in determining the rate of respiration. The method of determining the rate of respiration or the quantity of carbon dioxide in a sample of air is subject to many possible errors. The greatest error would be that not all the air was thoroughly mixed with the solution of barium hydroxide, and consequently some of the carbon dioxide would escape and not be measured. A second possible source of error was that some of the carbon dioxide would be absorbed into the solution of calcium chloride in which it was in contact.

There were several other possible sources of error, such as inaccurate measurement of solutions and volumes, and losses in tubing.

It was decided to check the method against a known quantity of carbon dioxide. This was done with the apparatus shown in Figure 16. A quantity of dry ice was weighed carefully inside a small insulated box on an analytical balance. As soon as it was weighed it was quickly dropped in the gallon bottle A, of Figure 16. Bottle A was stoppered quickly in order to maintain atmospheric pressure,and test tube B was lowered until the level of solution in the test tube was the same as that in the graduated cylinder. Then calcium chloride solution was allowed to enter gallon bottle A from gallon bottle C by opening the two pinch cocks d and e. Two samples of air were then removed from bottle C by the method explained in "Method of obtaining a respiration sample" (page 56). These were immediately mixed with barium hydroxide according to the method given in "Measuring the respiration of a sample of wheat" (page 61), and the quantity of carbon dioxide determined for two samples. Again after the sample had been in contact with the solution for 24 hours, two more samples were taken. A comparison was made between the quantity of carbon dioxide obtained by titration and by weighing the dry ice. A second comparison was made between the carbon dioxide found by titration immediately after placing the dry ice in the bottle and the amount found by titration 24 hours later.

These results are shown below:

13.25 milligram proportion of dry ice per 900 milliliters of air

First sample	15.2 milligrams	Determination of CO_2 per 900 milliliters by titration immediately after transferring in bottle C.
Second "	15.5 "	
First sample	14.5 milligrams	Determination of CO_2 per 900 milliliters by titration 24 hours later.
Second "	14.7 "	

It can be seen there was not a great difference in any of the two. Actually, the titration shows slightly more than the actual method by weight. There was slightly less carbon dioxide after 24 hours than before.

A second test was performed with the results shown below:

19.6 milligram proportion of dry ice per 900 milliliters of air

First sample	16.3 milligrams	Determination of CO_2 per 900 milliliters by titration.
Second "	15.6 "	
First sample	13.8 milligrams	Determination of CO_2 per 900 milliliters by titration 24 hours later.
Second "	a failure	

From the results it can be seen that the determinations by titrations were more consistent than those by weight. It is believed that some water condensed on the dry ice in some cases and led to errors in the determinations by weights. To avoid this, samples were cut from the interior of a piece of dry ice.

From these results an idea of the error in reporting carbon dioxide respired can be obtained.

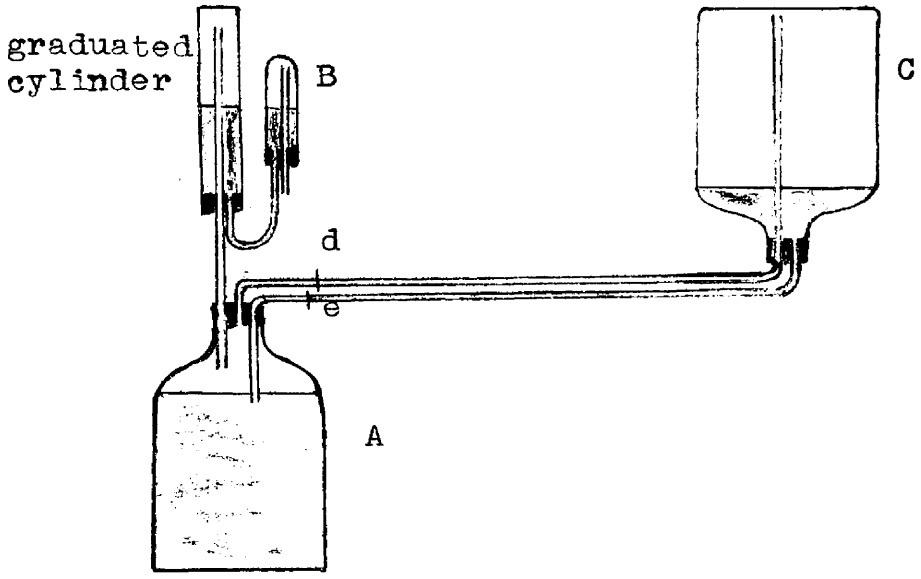


Fig. 16 Apparatus for determining total errors for rate of respiration.

Thermos Bottle Tests

A series of tests was made with pint thermos bottles. The purpose of these tests was to duplicate as nearly as possible conditions in a wheat bin. As far as heat transfer is concerned, a perfectly insulated surface has the same effect mathematically as an infinite quantity of wheat. In other words, from a heat transfer standpoint the wheat in the thermos bottle should be in conditions similar to a sample in the top and center of a large wheat bin. The greatest difference between a true wheat bin and the thermos bottle is that eration is possible only from the top of a

thermos bottle. The sides and bottom contribute nothing to air flow. Under these conditions molds and anaerobic microorganisms will develop that are not characteristic of conditions in grain bins. A second difference occurs because the vacuum bottle is not a perfect insulator. It was hoped, nevertheless, that these tests would approximate the conditions in a wheat bin.

The samples for these tests were all obtained from a division of those used in the respiration run. Therefore, the method of selecting the samples, conditioning them, and testing for moisture were the same. Also, they were irradiated in the electron accelerator before they were divided. After irradiation a division was made for those undergoing a respiration test and those used to form a thermos bottle test.

The thermos bottle tests were performed by filling eight pint-size thermos bottles with wheat of the same test classification as those for the respiration run. For example, each respiration run consisted of eight samples which up to and including irradiation were the same as the thermos bottle samples. At this point the samples were divided and for each respiration sample there was a thermos bottle sample. The thermos bottles each had a copper-constantan thermocouple placed in the bottom of the bottle before filling with wheat. The eight thermos bottles were then placed in a room kept at 87 degrees Fahrenheit and were kept at this temperature for

20 days. Thermocouple temperatures were read on the second day and every other day until the conclusion of the run. At the end of the run each sample was poured into a separate paper bag and kept at 40 degrees Fahrenheit or below until shipped for fat acidity determinations.

Each thermocouple was calibrated in the following manner. A pint thermos bottle was filled with water and adjusted to a temperature of 87 degrees Fahrenheit on a mercury thermometer which had been calibrated with a certified thermometer. The thermocouple was placed in the solution, the water was constantly stirred for about three minutes, and a reading on the potentiometer was compared with a reading on the calibrated thermometer. Usually, there was not more than a half degree Fahrenheit temperature difference in reading the thermocouples.

Comprehensive Tests

Respiration run for comprehensive tests. During the respiration tests it was found that at doses of 900,000 rep the respirations in high moisture content wheat, when subjected to a ten-day test at 86 degrees Fahrenheit and 16.0 - 20.0 milliliters of air per gram of dry matter, were nearly the same as those in very low moisture content wheat. This seemed to indicate that mold activity was at a low level and hence spoilage should be low. For this reason it was decided to compare a series of moisture levels at no irradiation and

irradiation at 900,000 rep. The moisture levels decided upon were 10 - 12 percent, 14 - 15 percent, 15 - 16 percent, and 16 - 17 percent on a wet basis. These were the same moisture levels of wheat used in the regular respiration run. Moisture determinations were made by drying 72 hours at 212 degrees Fahrenheit and finding the change in weight. The general set up of the samples is given in Table X.

Table X.

NUMBER OF SAMPLES FOR COMPREHENSIVE TESTS

Doses rep	Moisture Percentage of Wheat (Wet Basis)			
	10 - 12%	14.0 - 14.9%	15.0 - 15.9%	16.0 - 16.9%
0	one	one	one	one
900,000	one	one	one	one

The samples selected for the comprehensive test were from the same lot of wheat as those for repiration runs Nos. 2, 3, 4, and 5. These samples were selected by choosing a sample of wheat of about 20 pounds. This was subsequently divided in the Boerner Divider into halves; then each half again was split and so on until there were eight samples of about 1,200 grams each.

The samples were conditioned to bring them to the desired moisture level. This was done in the same manner as those for the respiration run as explained on pages 42 and 43.

All samples were subjected to a 10-day test at 87

degrees Fahrenheit and an aeration rate of about 16.0 milliliters per gram of dry matter to check thoroughly the preceding respiration and fat acidity determinations in order to see if 900,000 rep were effective in reducing the spoilage of wheat by molds.

Milling and baking tests. All tests were performed by the International Milling Company of Detroit, Michigan. They followed the procedures of Cereal Laboratory Methods, 5th edition, of the American Association of Cereal Chemists.

The baking method was an intermediate sponge and dough method, employed routinely in the International Milling Company's laboratory.

The wheat was ground in a Buhler mill. A five-pound sample was weighed, tempered to 16 percent moisture, and allowed to stand for approximately 18 hours, at which time it was run through a laboratory Buhler mill at the rate of about five pounds in 12 minutes. The flour was collected and weighed, and the percentage yield calculated on a wheat input basis. Likewise, the bran and shorts were separated and similarly calculated.

ANALYSIS AND RESULTS

Three distinct types of tests were analyzed: the respiration tests, the thermos bottle tests, and the comprehensive tests. The respiration tests were an accelerated experiment conducted at optimum conditions for rapid mold growth. The thermos bottle tests attempted to reproduce conditions in a grain bin. The comprehensive tests reproduced conditions for rapid deterioration of wheat and tested conditions of no treatment against the treatment that had been most effective in reducing respiration. Finally, the comprehensive tests included most of the standard milling tests such as dry matter, percent bran, percent shorts, milling time, baking tests, odor, taste, etc.

Analysis of Respiration Tests

The respiration tests consisted of five runs of ten days duration at 86 degrees Fahrenheit. From 16.0 - 20.0 milliliters of air per gram of dry matter were passed over the wheat per day. The amount of carbon dioxide respired on the tenth day was used as a criterion to determine the rate of deterioration of wheat. Fat acidity determinations were made of these samples of wheat both before and after each run for the third, fourth, and fifth runs. These data were likewise analyzed.

The first, second, and third respiration runs were

exploratory in nature. It was known that dosages up to 1,000,000 rep were required to kill certain molds completely. Since doses of 100,000 rep cause a 99.98 percent mortality of certain molds, it was believed that doses in these lower ranges would be effective in reducing the activity of molds to a low level.

The fourth and fifth runs were to determine how effective treatments were in the ranges of 500,000 to 900,000 rep. The experiments revealed that these ranges of doses reduced production of carbon dioxide.

Analysis of the first respiration run. The first respiration run was conducted at 0, 130,000 rep, 261,000 rep, and 392,000 rep. Two levels of moisture in wheat were tested, one between 11.9 - 12.2 percent and the other between 16.6 - 16.9 percent. The results of this test can be seen in Table XI and Figure 17. This was purely an exploratory run.

Table XI.

RESULTS OF THE FIRST RESPIRATION RUN

	0 rep		130,000		261,000		392,000	
Moisture content at start (Wet basis)	16.6	12.2	16.9	11.9	16.7	12.05	16.85	12.1
Average air in ml. per day/100grD.M.	14.95	13.65	14.95	15.1	14.41	13.2	15.1	14.3
CO_2mg/100gr D.M.10th day	35.3	13.1	35.5	14.0	35.3	11.6	35.1	14.0

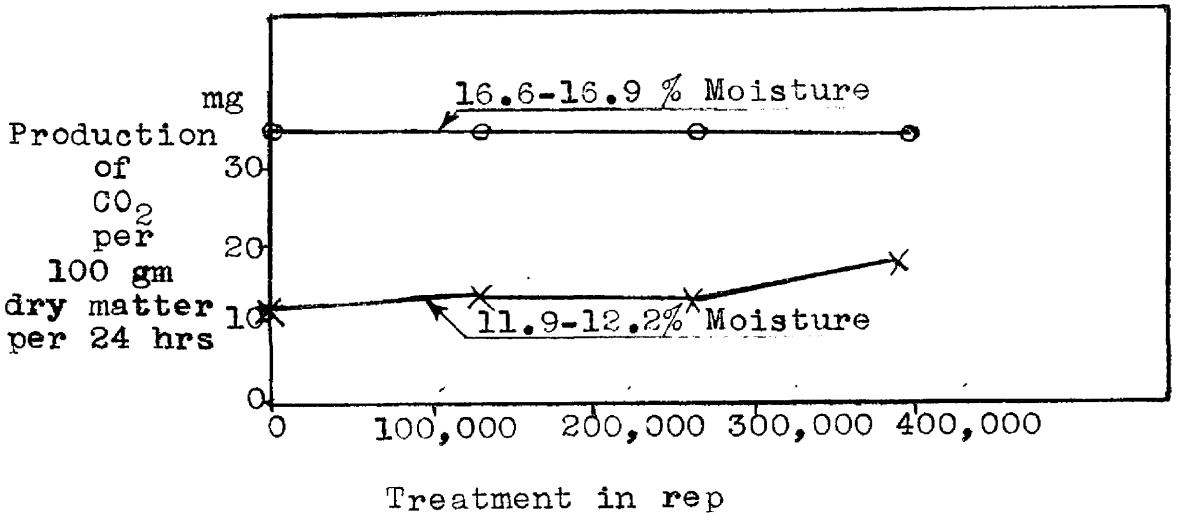


Fig. 17 Production of CO_2 vs. Treatment--Run One

The method of analysis of variance was used to analyze these data (see Table XII).

Table XII.

ANALYSIS OF VARIANCE CO_2 PRODUCED 10th DAY--FIRST RUN

	Sum of Squares	d.f.	Mean Square	F	$F_{.95}$
Moisture Means	974.4	1	974.4	1047.7	10.1
Treatment Means	1.4	3	.47	.505	9.28
Residual	2.8	3	.93		
Total	978.6	7			

It can be seen from Table XII that there is a definite difference in respiration rate between the moisture means of the wheat at 12.1 - 12.2 percent and 16.6 - 16.9 percent, but practically no difference in the rate between treatment means. This can be seen best from Figure 17. It was concluded for this set of conditions that the doses applied had no significant effect upon reducing the production of carbon

dioxide. Higher doses were necessary.

Analysis of the second respiration run. Inasmuch as doses up to nearly 400,000 rep were not successful in reducing the production of carbon dioxide, it was believed necessary that a much wider range of doses should be given. This was done in the hope that a pair of doses would bracket the position where carbon dioxide production was reduced. Wheat samples at nearly the same range of moisture levels at the previous run were given doses of 0, 1,000,000, 2,000,000, and 3,000,000 rep. This wheat was hard red spring wheat from the fall harvest of 1955, but of a different lot from run number one. All the remaining runs were carried out with this second lot of wheat.

The results of the second respiration run are shown on Table XIII and Figure 18.

Table XIII.

RESULTS OF THE SECOND RESPIRATION RUN

	0 rep		1,000,000		2,000,000		3,000,000	
Moisture content at start (Wet basis)	16.7	12.8	16.6	12.8	16.5	12.8	16.8	11.9
Average air in ml. per day/100grD.M.	14.71	13.9	14.1	14.45	15.55	15.05	15.65	15.15
CO_2mg/100gr D.M.10th day	16.2	3.9	4.4	3.9	5.0	1.72	0	0

Respiration is zero at the 3,000,000 rep level for both

the high moisture content wheat and the low moisture content wheat. Evidently at this point both the molds and enzymes were neutralized. At the 1,000,000 rep level there was not a great deal of difference in carbon dioxide production of the wet wheat and the dry wheat of no treatment. An analysis of variance was made to determine at what level of significance there was a difference in the treatment means at 0 rep and 1,000,000 rep. A moisture content between 11.9 - 12.8 percent was used as one level and 16.5 - 16.8 percent moisture as the other level. The results of the analysis of variance are shown below.

Table XIV.

ANALYSIS OF VARIANCE FOR CO_2 PRODUCED ON 10th DAY--SECOND RUN

	Sum of Squares	d.f.	Mean Square	F	$F_{.95}$
Moisture Means	32.31	1	32.31	1.98	10.1
Treatment Means	104.85	3	34.95	2.15	9.28
Residual	48.84	3	16.28		
Total	186.00	7			

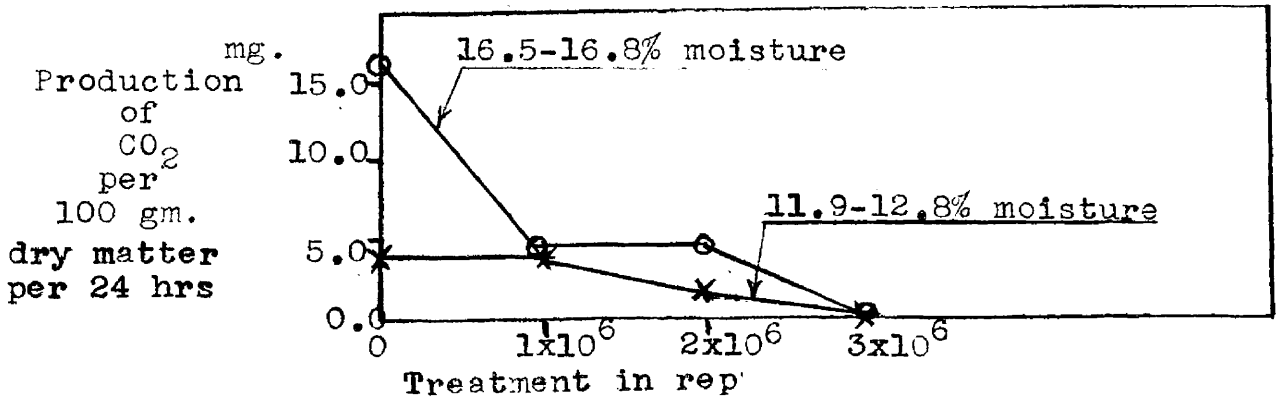


Fig.18 Production of CO_2 vs. Treatment--Run Two

There was no difference in moisture means or treatment means at the five percent level of significance. However, there was a difference in wheat not treated and wheat treated at 1,000,000 rep at high moisture according to the t test. There may be some criticism of making a t test when the treatment means show no significant difference; however, according to Goulden[22], "It is valid to make any t test provided that the test is preconceived at the time the experiment is designed." A comparison of this type had been contemplated before making the experiment.

$\sqrt{\frac{2(16.28)}{1}} = 5.68$ The standard error of a mean difference.

We shall compare the treatment means at 0 and 10^6 rep.

16.2 mg. CO_2 at 0 rep
4.4 mg. CO_2 at 1,000,000 rep

$$t = \frac{16.2 - 4.4}{5.68} = 2.08$$

$t = 1.64$ for three degrees of freedom at the 20 percent level of significance.

From the results of runs one and two it would appear profitable to explore the possibilities between 400,000 rep where there was no effect and 1,000,000 rep where there was an effect.

Analysis of the third respiration run. Since doses of 1,000,000 rep had an effect on reducing the respiration

and doses of 392,000 rep had no effect, it was decided to test doses of 500,000, 700,000, and 900,000 rep, respectively. The results of the third respiration run can be seen in Table XV. Two air samples were taken and tested for CO_2.

Table XV.

RESULTS OF THE THIRD RESPIRATION RUN

		0 rep		500,000		700,000		900,000	
Moisture content at start (Wet basis)		15.65	11.3	15.5	11.3	15.5	11.7	15.9	11.3
CO_2mg sample /100gr D.M.	1st	15.9	1.9	4.1	3.8	1.7	0.2	1.3	0.7
10thday	2nd	13.1	3.3	6.5	3.8	4.0	3.0	3.9	3.2
Free fatty acid 10th day mg.KOH /100gm D.M.		16.0	13.7	14.2	15.5	15.4	13.0	16.0	14.2

An analysis of variance for the carbon dioxide test on the 10th day is given in Table XVIa and the same is shown in Table XVII for the free fatty acid test on the 10th day.

Table XVIa.

ANALYSIS OF VARIANCE OF CO_2 PRODUCED ON 10th DAY--THIRD RUN

	d.f.	S.S.	M.S.	F*	$F_{.95}$
Replications	1	7.84	7.84	0.26	5.59
Treatments	3	105.96	35.32	1.17	4.35
Moisture	1	58.52	58.52	1.93	5.59
Interaction	3	92.95	30.18		
Error	7	7.40	1.06		
Total	15	272.67			

* Interaction mean square used as the denominator for the F ratio.

Since the interaction mean square was significantly larger than the error mean square, the interaction mean square was used for making tests in Table XVIa as to whether treatment mean square and moisture mean square were significantly different from interaction mean square. According to the F test it can be seen that there is no significant differences in moisture means nor treatment means.

In order to exclude the effect of the large interaction, analyses of variance were made for both high moisture wheat and low moisture wheat. These analyses are shown in Tables XVIb and XVIc.

Table XVIb.

ANALYSIS OF VARIANCE OF CO_2 PRODUCED ON 10th DAY--THIRD RUN HIGH MOISTURE ONLY

	d.f.	S.S.	M.S.	F	$F_{.95}$
Replications	1	3.3	3.3	.10	10.13
Treatments	3	187.7	62.3	19.47	9.28
Error	3	9.6	3.2		
Total	7	200.6			

Table XVIc.

ANALYSIS OF VARIANCE OF CO_2 PRODUCED ON 10th DAY--THIRD RUN LOW MOISTURE ONLY

	d.f.	S.S.	M.S.	F	$F_{.95}$
Replications	1	5.5	5.6		
Treatments	3	5.6	1.9	3.8	9.28
Error	3	1.5	0.5		
Total	7	13.7			

According to the F test there is no significant difference between treatments at low moisture.

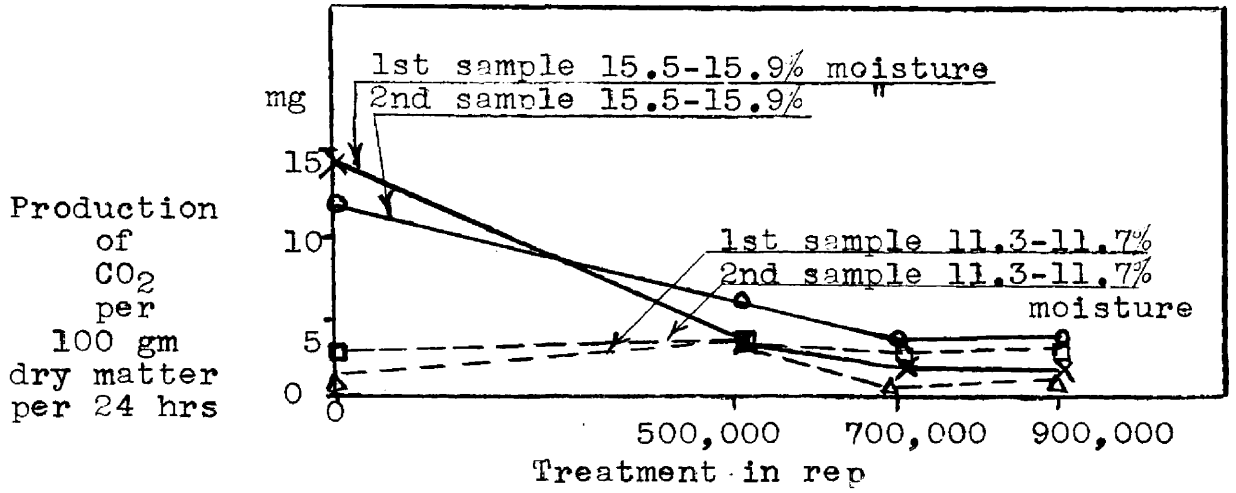


Fig.19 Production of CO_2 vs.Treatment-- Run Three

According to the F test there was a significant difference between treatment means at the five percent level of significance for high moisture.

							Carbon dioxide respired per 24hrs
Average	of	0	rep	treatment	at	high moisture	14.5
"	"	500,000	"	"	"	"	5.3
"	"	700.000	"	"	"	"	2.9
"	"	900,000	"	"	"	"	2.6

$\sqrt{\frac{2(3.2)}{2}} = 1.79$ The standard error of a mean difference.

Comparing 0 rep treatment with 500,000 rep treatment.

$$t = \frac{14.5 - 5.3}{1.79} = 5.13$$

Comparing 500,000 rep treatment with 900,000 rep treatment.

$$t = \frac{5.3 - 2.6}{1.79} = 1.51$$

$t = 2.36$ for seven degrees of freedom at the five percent level of significance.

From these comparisons there was at the five percent level of significance a difference between 0 rep treatment and the 500,000 rep, 700,000 rep, and 900,000 rep treatments, but none between the 500,000 rep, 700,000 rep, and 900,000 rep treatments. (A significant difference was shown between the two extremes at 500,000 and 900,000 rep treatments. Consequently, there must be no significant differences at the 700,000 rep treatment and the 500,000 and 900,000 rep treatments.)

Analysis of variance was made for the free fatty acid test at the end of the run, the results appearing in Table XVIII.

Table XVII.

FREE FATTY ACID (mg.KOH/100gm D.M.)--END OF THIRD RUN

Moisture% Wet Basis	Treatment in reps			
	0	500,000	700,000	900,000
Low moisture 11.3 - 11.7%	13.7	15.5	13.0	14.2
High moisture 15.5 - 15.9%	16.0	14.2	15.4	16.0

Table XVIII.

ANALYSIS OF VARIANCE OF FREE FATTY ACID -- END OF THIRD RUN

	Sum of Squares	d.f.	Mean Square	F	$F_{.95}$
Moisture Means	3.38	1	3.38	.120	10.1
Treatment Means	.89	3	.30	.011	9.28
Residual	84.53	3	28.17		
Total	88.8	7			

Clearly there is no difference in treatment means and moisture means. The free fatty acid determinations evidently are not sensitive enough to show any distinct differences. There seem to be no more profitable comparisons.

Analysis of the fourth respiration run. Because the treatments at 500,000, 700,000, and 900,000 rep had shown excellent results so far as respiration was concerned, it was decided to test these treatments at a slightly lower level of moisture. The results of this test appear in Table XIX and Figure 20.

Table XIX.

RESULTS OF THE FOURTH RESPIRATION RUN

		0 rep		500,000		700,000		900,000	
Moisture content % at start (Wet basis)		14.3	10.8	14.1	10.9	14.2	10.9	14.3	10.8
CO_2mg/ 100grD.M. 10th day	Sample 1st	1.9	1.05	1.0	1.0	2.0	2.4	0.0	2.6
	2nd	2.6	1.05	3.0	1.4	2.1	1.9	1.8	3.1
Free fatty acid 10th day mgKOH/ 100gm.D.M.		14.8	13.1	13.0	13.0	13.7	11.9	14.2	11.9

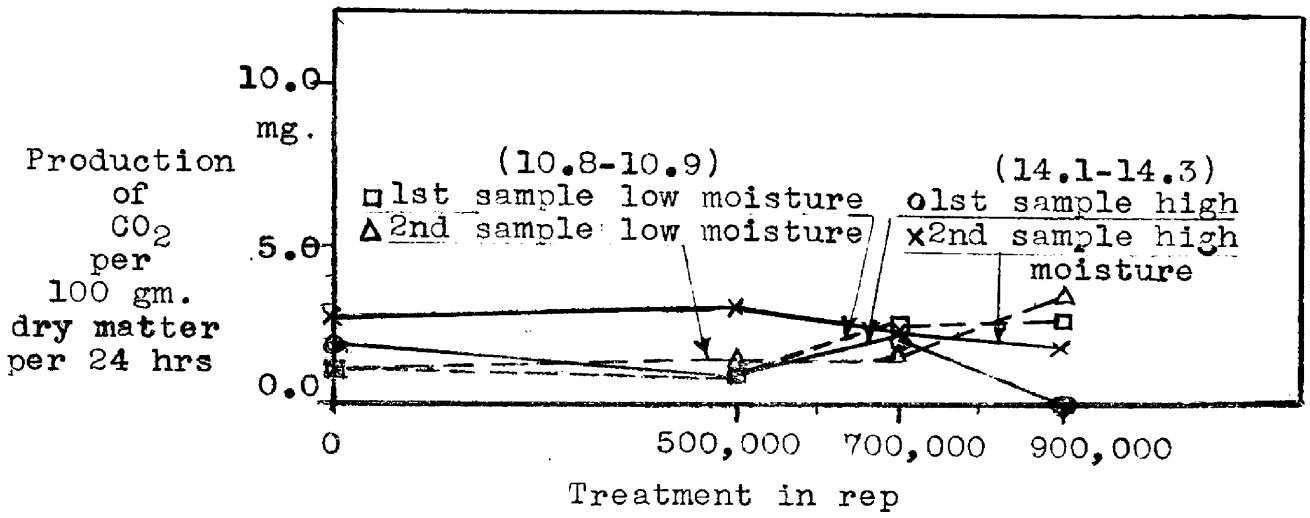


Fig. 20 Production of CO_2 vs. Treatment--Run Four

The amount of carbon dioxide produced in the fourth run was analyzed by the method of analysis variance in Table XX.

Table XX.

ANALYSIS OF VARIANCE FOR CO_2 PRODUCED ON 10th DAY--FOURTH RUN

	Sum of Squares	d.f.	Mean Square	F	$F_{.95}$
Replications	1.56	1	1.56	4.45	5.59
Treatment Means	0.63	3	.21	.60	4.35
Moisture Means	0.00	1	0.00	.00	5.59
Interaction	5.89	3	1.96	5.60	4.35
Error	2.48	7	.35		
Total	10.56	15			

Only the interaction was significant at the five percent level for the F test. Evidently, the low moisture content did not produce any significant change. Apparently, below 15.0 percent moisture wheat will keep safely without treatment, a fact proved true in practice.

Analysis of variance was applied to the free fatty acid

tests made at the end of the run, the results appearing in Table XXII.

Table XXI

FREE FATTY ACID (mgKOH/100gr.D.M.)--END OF FOURTH RUN

	Treatment in reps			
	0	500,000	700,000	900,000
Low Moisture Content 10.8 - 10.9%	13.1	13.0	11.9	11.9
High Moisture Content 14.1 - 14.3%	14.8	13.0	13.7	14.2

Table XXII.

ANALYSIS OF VARIANCE OF FREE FATTY ACID--END OF FOURTH RUN

	Sum of Squares	d.f.	Mean Square	F	$F_{.95}$
Moisture Means	4.20	1	4.20	8.34	10.1
Treatment Means	1.57	3	.523	1.04	9.28
Residual	1.51	3	.503		
Total	7.28	7			

It is evident that the F test does not show any difference in treatment means or moisture means at the five percent level of significance for the free fatty acid tests. Here again there seem to be no more profitable comparisons.

Analysis of the fifth respiration run. The fifth respiration run was with two levels of moisture between 12.0 - 12.3 percent and 15.7 - 16.1 percent and treatments of 0,

500,000, 700,000, and 900,000 rep. The results should compare with those of run three. These data for this run are shown in Table XXIII and Figure 21.

Table XXIII.

RESULTS OF THE FIFTH RESPIRATION RUN

		0 rep		500,000		700,000		900,000	
Moisture cont. at start (Wet basis)		16.1	12.1	16.0	12.0	16.0	12.3	15.7	12.1
CO_2mg/100 Sample gr D.M.	1st	11.0	3.6	4.7	2.7	2.9	2.9	1.7	1.1
	2nd	11.7	2.3	3.7	2.1	2.1	1.7	1.7	1.9
Fr.fat.acid 10th day mg KOH/100gm D.M.		18.4	15.5	15.5	14.2	15.4	13.7	16.7	16.0

Table XXIVa.

ANALYSIS OF VARIANCE FOR CO_2 PRODUCED ON 10th DAY--FIFTH RUN

	Sum of Squares	d.f.	Mean Square	F*	F.95
Replication means	0.72	1	.72	0.05	5.59
Treatment means	72.53	3	24.18	1.55	4.35
Moisture means	28.09	1	28.09	1.80	5.59
Interaction	45.79	3	15.63		
Error	2.41	7	.344		
Total	149.54	15			

* Interaction mean square used as the denominator to determine the F ratio.

Since the interaction mean square was so much larger than the error mean square, the interaction mean square was used in the denominator to determine the F ratios. Here again

there were no significant differences in treatment means nor moisture means.

Analyses of variance were made for both the high moisture treatments and the low moisture treatments to avoid the effect of the large interaction.

Table XXIVb.

ANALYSIS OF VARIANCE FOR CO_2 PRODUCED ON 10th DAY--FIFTH RUN

HIGH MOISTURE ONLY

	d.f.	S.S.	M.S.	F	$F_{.95}$
Replications	1	.15			
Treatments	3	116.16	38.7	117.27	9.28
Error	3	1.08	.33		
Total	7	117.24			

According to the F test there was a significant difference between treatment means at the five percent level of significance.

			Carbon dioxide respired per 24hrs
Average of	0	rep treatment at high moisture	11.4
" "	500,000	" " " " "	2.2
" "	700,000	" " " " "	2.5
" "	900.000	" " " " "	1.7

$\sqrt{\frac{2(.33)}{2}} = .57$ Standard Error of a Mean Difference.

Comparing 0 rep treatment with 700,000 rep treatment.

$$t = \frac{11.4 - 2.5}{.57} = 15.61$$

Comparing 700,000 rep treatment with the 900,000 rep which is the largest comparison that can be made above 0 treatment.

$$t = \frac{2.5 - 1.7}{.57} = 1.40$$

t = 2.36 for seven degrees of freedom at the five percent level of significance.

According to these comparisons there was at the five percent level of significance a difference between 0 rep treatment and the 500,000, 700,000, and 900,000 rep treatments, but none between the 500,000, 700,000, and 900,000 rep treatments. (The first comparison was made between those that had the least difference and the second comparison was made between the 500,000, 700,000, and 900,000 rep treatment that showed the greatest difference.)

Table XXIVc.

ANALYSIS OF VARIANCE FOR CO_2 PRODUCED ON 10th DAY--FIFTH RUN

LOW MOISTURE ONLY

	d.f.	S.S.	M.S.	F	F.95
Replications	1	0.66	0.66	1.40	
Treatments	3	2.14	0.71	1.51	9.28
Error	3	1.41	0.47		
Total	7	4.21	0.60		

According to the F test at the five percent level there is no significant difference between treatments at low moisture.

Analysis of variance was carried out for the free fatty acid tests.

Table XXV.

FREE FATTY ACID (mgKOH/100grD.M.)--END OF FIFTH RUN

	Sum of Squares	d.f.	Mean Square	F	$F_{.95}$
Moisture Means	5.45	1	5.45	12.67	10.1
Treatment Means	8.06	3	2.69	6.25	9.28
Residual	1.29	3	.430		
Total	14.80	7			

There is clearly a difference in moisture means at the five percent level of significance and nearly a difference in treatment means. Examination of Table XXV reveals that at 500,000 and 700,000 rep there is a low point in the fat acidity. This can be observed also in Table XVII for the third respiration run for high moisture wheat. At the higher doses (900,000 rep) the free fat acidity is increased. A

t test to determine the difference between treatment means of 0 treatment and 500,000 rep yields:

$$\sqrt{\frac{2\ (.430)}{2}} = .635$$ The standard error of a mean difference.

$$\frac{(14.2 + 15.5)}{2} = 14.8 \quad \text{at 500,000 rep}$$

$$\frac{(15.5 + 18.5)}{2} = 17.0 \quad \text{at 0 rep}$$

$$t = \frac{17.0 - 14.8}{.635} = 3.31$$

$t = 3.18$ at the 5.0 percent level of significance for 3 degrees of freedom.

This shows a strong significant difference between 0 and 500,000 rep at high moisture content.

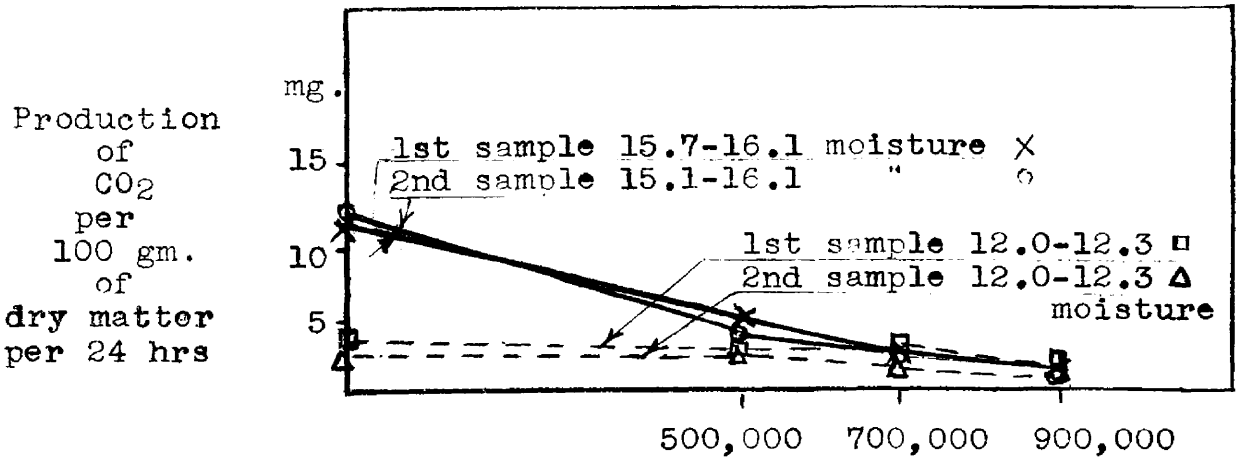


Fig. 21 Production of CO_2 vs. Treatment--Run Five

Summary of the Respiration Tests

1. At moisture levels above 15.0 percent there was a significant difference between treatments at 0 and treatments above 500,000 rep for the respiration tests.

2. There was not a great difference in effect upon respiration at treatments below 500,000 rep.

3. The free fatty acid test was not a satisfactory method of testing for changes due to treatment.

4. In some cases there was an actual increase in free fatty acid with treatment, especially at the 900,000 rep level of treatment.

Analysis of the Thermos Bottle Tests

The thermos bottle tests consisted of five runs of 20 days duration with an average room temperature of about 87 degrees Fahrenheit. The thermos bottles were one pint in size and were filled to the top and not stoppered. A calibrated thermocouple was placed in the bottom of each bottle and the temperatures read every other day. The samples placed in the bottles were a division of the same sample made for the respiration run. An exception to this was run five, in which an attempt was made to duplicate the moisture percentages of the respiration run. Actually the high moisture level averaged 16.8 percent against about 16.0 percent for the respiration run. The treatments were on the same level as the corresponding respiration run.

Analysis of the thermos bottle test--run one. Table XXVII lists the temperatures recorded for the thermos bottles of run one. The analysis of variance for the temperatures is shown on Table XXVIII. The analysis of variance was made for two variables of classification of repeated measurements.

Table XXVII.

THERMOS BOTTLE TEMPERATURES DEGREES FAHRENHEIT--RUN ONE

Treatment rep	Moisture% (Wet basis) start	Day of Reading 1	3	5	7	9	11	13	15	17	19	20	Average Temperature
0	16.6	78	87	85	87.5	87.25	87	88	87	89	88	87	86.4
0	12.2	76.5	87	85	87.5	86	86	87	86	88	87	87	85.7
130,000	16.9	77.5	87	85	87.5	87	87	88	87	89.5	88	88	86.5
130,000	11.9	76	87	84.5	87	86	86	86.5	86	88	87	86	85.4
261,000	16.7	78	87	84.5	87	87	87	88	87	90	89	88	86.59
261,000	12.05	86	87	84.5	87	87	86	87	86.5	89	87	86	86.6
392,000	16.85	75	86.5	84.5	87	87	87	88	87.5	90	89	87	86.2
392,000	12.1	79	86.5	84.5	86.5	87	86.5	87	86	88	87	86	85.8
Room Temperature		88	88	85	87	87.5	87	87	87	90	87	87	87.3

Table XXVIII.

ANALYSIS OF VARIANCE THERMOS BOTTLE TEMPERATURES--RUN ONE

	Sum of Squares	d.f.	Mean Square	F *	$F_{.95}$
Treatments	5.80	3	1.93	.177	4.07
Moistures	1.04	1	1.04	.095	4.00
Interaction	8.64	3	2.88		
Subtotal	15.48	7	2.21		
Within groups	643.74	80	8.05		
Total	659.22	87	4.70		

* Denominator is the pooled sum of interaction and within group mean squares, as the interaction is not significant.

According to the F test at the five percent level of significance, there is no differcence in treatment means or moisture means. However, there does appear to be a small difference between the low moisture average temperatures and the high moisture average temperatures, except for the 261,000 rep treatment when both average temperatures were about the same.

The treatment at this level had little effect, which was similar to that of run one of the respiration tests.

Since the zero treatment, high-moisture average temperature is not the highest average temperature, the thermos bottles evidently were too small and aeration too inadequate to produce the high temperatures necessary for a significant difference.

The reason the room temperatures appear higher than the thermocouple temperatures in the thermos bottles was that the mercury thermometer was located at a higher elevation in the room than the thermos bottles. The room in which the thermos bottles were kept tended to have a slightly higher temperature near the ceiling. This would account for the higher average temperature of the room thermometer in some instances.

Analysis of the thermos bottle test--run two. In run two the treatments were increased to 1×10^6, 2×10^6, and 3×10^6 rep corresponding with those in the respiration

tests. Table XXIX lists the temperatures for this run. As in run one an analysis of variance was made for two variables of classification of repeated measurements. This is shown in Table XXX.

Table XXIX.

THERMOS BOTTLE TEMPERATURES DEGREES FAHRENHEIT--RUN TWO

Treatment rep	Moisture% (Wet basis) start	Day of Reading 1	3	5	7	9	11	13	15	19	20	Average Temperature
0	16.7	83	86	86	88	88	87	87	87	87	87.5	88.6
0	12.8	83	86.5	86	87.5	87	86	86	86	86	86.5	86.
1 x 10^6	16.7	84	86	86	87	87	86	86	86	86	86.5	86.1
1 x 10^6	12.8	85	87	86	88	87	86	86	86	86	87	86.4
2 x 10^6	16.5	84	86	87.5	86.5	86.5	86	86	86	86	86.5	86
2 x 10^6	12.8	83.5	86.5	86	87.5	87	86	86	86	86	86.5	86.1
3 x 10^6	16.8	84	86.5	86	87.5	87	86	86	86	86	86.5	86.1
3 x 10^6	11.9	84	86	86	88	86.5	86	86	86	86	86.5	86.1
Room Temperature		87	87	87	90	87	87	86.5	86.5	86	87	87.1

Table XXX.

ANALYSIS OF VARIANCE THERMOS BOTTLE TEMPERATURES--RUN TWO

	Sum of Squares	d.f.	Mean Square	F* ratio	$F_{.95}$
Treatments	0.9	3	0.03	.0165	2.74
Moistures	0.1	1	0.10	.055	3.98
Interaction	2.2	3	.73		
Subtotal	3.2	7	.46		
Within group	78.1	72	1.09		
Total	81.3	79	1.03		

* Denominator is the pooled sum of interaction and within group sum of squares as the interaction is not significant.

According to the F test there is no significant difference in treatment means or moisture means at the five percent level of significance.

In this experiment it was expected that there would be a rise in temperature of the no treatment, high moisture sample as there was a large production of carbon dioxide in the respiration tests. For this reason a t test was made to determine the difference between no treatment, high moisture and 1 x 10^6 rep treatment, high moisture.

$$\sqrt{\frac{2\ (1.071)}{10}} = .463$$ The standard error of a mean difference.

$$t = \frac{88.6 - 86.1}{.463} = 5.4$$

$t = 2.90$ for the 0.5 percent level of significance for 72 degrees of freedom.

This very significant difference indicates that treatments of 1,000,000 rep and above had a very strong effect on temperature. This result was similar to that of the respiration run.

Analysis of thermos bottle test--run three. In test-run three the treatments were lowered to 500,000, 700,000, and 900,000 rep. In run two the treatment at 1,000,000 rep was very effective, while the treatment at 392,000 rep in run one had no effect. Between these two treatments a turning point should be found.

The results of run three are shown in Table XXXI. No analysis of variance for temperatures was made (see Table XXXI), since there is practically no difference in average temperatures. Evidently at moistures below 16 percent very little heat is produced. Run two had been at higher moistures and therefore a treatment of 10^6 rep had some effect. In run three, shown below, all moistures were below 16.0 percent and there was little difference between samples.

Free fatty acid determinations were made of all samples, the results of which are given in Table XXXII. Analysis of variance was made of the free fatty acids in Table XXXIII.

Table XXXI.

THERMOS BOTTLE TEMPERATURES DEGREES FAHRENHEIT--RUN THREE

Treatment rep	Moisture% (Wet basis) start	Day of Reading 2	4	6	8	10	12	14	16	18	20	Average Temperature
0	15.65	83	86	87	86	86	85.5	86	86	87	85.5	85.8
0	11.3	83	86	86.5	86	86	85.5	86	86	87	85	85.7
500,000	15.5	84	87.5	86.5	86	86	85.5	86	86	87	85	85.9
500,000	11.3	83	86	86	86	86	85.5	86	86	87	86	85.7
700,000	15.5	83	86	86	86	86	85.5	86	86	87	85	85.6
700,000	11.7	83	86	86	86	86	85.5	86	86	87	85	85.6
900,000	15.9	83	86	85.5	86	86	85.5	86	86	87	85.5	85.6
900,000	11.3	83	86	86	86	86	85.5	86	86	87	85.5	85.7
Room Temperature		84	87.5	86	86	87	87	87	87	87	85	86.3

Table XXXII.

FREE FATTY ACID(mgKOH/100grD.M.)TEST FOR THERMOS BOTTLE--RUN THREE

Moisture% Wet Basis at start	Treatment reps			
	0	500,000	700,000	900.000
11.3 - 11.7	13.0	13.0	20.2	13.1
15.5 - 15.9	17.8	16.0	14.2	15.5

Table XXXIII.

ANALYSIS OF VARIANCE FREE FATTY ACID(Mg.KOH/100gmD.M.)--RUN THREE

	Sum of the Squares	d.f.	Mean Square	F ratio	$F_{.95}$
Moisture Means	2.20	1	2.20	.190	10.1
Treatment Means	10.50	3	3.50	.302	9.28
Residual	34.70	3	11.57		
Total	47.40	7			

According to the F test there is no difference in treatment means or moisture means at the five percent level of significance. The results of this test were almost predictable except for the sample at the treatment 700,000 rep, low moisture. The high value of 20.2 introduces the possibility of a large error either in sampling, measuring, or testing. Except for this value the low moisture content wheat has low values, and for high moisture content wheat the values are lower for the treated samples than for the untreated.

Evidently there is a large error or errors somewhere that will not permit us to find a meaningful difference.

Analysis of the thermos bottle test--run four. The results of the thermocouple temperatures in the thermos bottles can be seen in Table XXXIV. This run was made at an even lower moisture percentage than run three, which was done to obtain comparable runs with the respiration tests. No analysis of variances was made of the mean temperatures as, one can see, there is practically no difference in mean temperatures.

The results of the free fatty acid tests appear in Table XXXV and the analysis of variance in Table XXXVI.

Table XXXIV.

THERMOS BOTTLE TEMPERATURES DEGREES FAHRENHEIT--RUN FOUR

Treatment rep	Moisture% (Wet basis) start	Day of Reading 2	4	6	8	10	12	14	16	18	Average Temperature
0	14.3	84	85	85.5	85.5	86	86	86.5	87.5	88.5	86.0
0	10.8	84	85	85.5	85.5	86	86	86	87.5	88.5	86.0
500,000	14.1	84	85.5	85.5	85.5	86	86	85.5	87.5	87.5	85.9
500,000	10.9	84	86	85.5	85.5	86	86	85.5	86	88	85.8
700,000	14.2	85	86	85.5	85.5	86	86	85.5	86	87.5	86.9
700,000	10.9	85	86.5	85.5	85.5	86	86	86	86	87.5	86.0
900,000	14.3	85	86.5	85.5	85.5	86	86	87	86	87.5	86.1
900,000	10.8	86	86.5	85.5	85.5	86	86	87	87	87.5	86.3
Room Temperature		87	87.5	87	87	87	87	86.5	87	87	87

Table XXXV.

FREE FATTY ACID(mgKOH/100grD.M.) FOR THERMOS BOTTLE--RUN FOUR

Moisture% Wet basis at start	Treatment reps			
	0	500,000	700,000	900,000
10.8 - 10.9	17.7	17.2	16.0	16.7
14.1 - 14.3	17.7	17.7	16.5	17.2

Table XXXVI.

ANALYSIS OF VARIANCE FREE FATTY ACID(MgKOH/100gmD.M.)--RUN FOUR

	Sum of Squares	d.f.	Mean Square	F ratio	$F_{.95}$
Moisture Means	.28	1	.28	8.48	10.1
Treatment Means	2.45	3	.816	24.72	9.28
Residual	.10	3	.033		
Total	2.83	7			

According to the F test at the five percent level of significance there is a difference in treatment means. A t test was made between the high moisture treatment at 500,000 rep and 700,000 rep.

The standard error of a mean difference is:

$$\sqrt{\frac{2(.0333)}{1}} = .257$$

$$t = \frac{17.7 - 16.5}{.257} = 4.67$$

$t = 4.18$ at the 2.5 percent level of significance for three degrees of freedom.

This shows a strong significant difference.

Analysis of the thermos bottle test--run five. Run five was carried out at higher moisture percentages than any previous run. Also the wheat was tested for moisture on a Tag-Heppenstall moisture meter. This sample had to be dried slightly to bring it below 17 percent during preparation of the sample, and it is believed most of this drying occurred on the surface. A Tag-Heppenstall moisture meter tends to 'read' the surface moisture, and therefore this sample may have been even slightly higher in moisture than indicated.

The results of the thermos bottle temperatures are shown in Table XXXVII.

Table XXXVII.

THERMOS BOTTLE TEMPERATURES DEGREES FAHRENHEIT--RUN FIVE

Treatment rep	Moisture% (Wet basis) start	Day of Reading 2	4	6	8	10	12	14	16	18	Average Temperature
0	16.8*	85	86.5	86	85.5	87	88	87.5	87.5	88.5	86.8
0	10.8*	85	86.5	86	85.5	86	87	87	86	87.5	86.3
500,000	16.8*	85	86.5	86	85.5	86	87	87.5	86.5	87.5	86.4
500,000	10.9*	86	86.5	86.5	85.5	86	87	87	86.5	87	86.4
700,000	16.8*	86	87	86.5	85.5	86	87	87	86.5	88	86.5
700,000	10.9*	86	86.5	86.5	85.5	86	87.5	87	86.5	87	86.5
900,000	16.8*	86	87	87	86.5	86	87.5	87.5	87.5	88.5	86.9
900,000	10.8*	86	87	87	85.5	86	86	87.5	87	87.5	86.6
Room Temperature		87	87.5	87		87	87	86.5	87	87	87

* Taken by Tag-Heppenstall Moisture Meter.

No analysis of variance was made of the mean temperatures as practically no difference in temperatures occurred.

The results of the free fatty acid determinations are given in Table XXXVIII and the analysis variance on Table XXXIX.

Table XXXVIII.

FREE FATTY ACID(mgKOH/100grD.M.)FOR THERMOS BOTTLE--RUN FIVE

Moisture% (Wet basis) at start	Treatment Rep			
	0	500,000	700,000	900,000
12.0 - 12.3	16.0	16.6	16.0	16.0
15.7 - 16.1	26.0	24.2	19.6	21.9

Table XXXIX.

ANALYSIS OF VARIANCE FREE FATTY ACID(mgKOH/100gmD.M.)--RUN FIVE

	Sum of Squares	d.f.	Mean Square	F ratio	$F_{.95}$
Moisture Means	91.80	1	91.8	25.64	10.1
Treatment Means	12.70	3	4.23	1.18	9.28
Residual	10.76	3	3.58		
Total	115.26	7			

According to the F test there is a significant difference between the moisture means. This might be expected as it had the highest moisture average of any run so far.

Let us compare high moisture, 0 treatment with high moisture, 700,000 rep treatment.

The standard error of a mean difference is:

$$\sqrt{\frac{2(3.58)}{1}} = 2.68$$

$$t = \frac{26 - 19.6}{2.68} = 2.39$$

$t = 2.35$ at the 10.0 percent level of significance for three degrees of freedom.

This shows again that there is a low point in the free fatty acid determination at 700,000 rep, high moisture.

Summary of the Thermos Bottle Tests

1. A pint thermos bottle sample apparently is too small to effect a rise in temperature. In only one run was there a significant difference between a treatment and no treatment and this was not very large. Evidently to achieve results similar to a grain bin, air should be piped down to the bottom of the bottle. No doubt an aerobic action was taking place which was not conducive to heat production.

2. In all tests made, the free fatty acid test in the high moisture level showed lowest at 700,000 rep treatment with an increase in the free fatty acid test at 900,000 rep treatment. The general trend was a decrease in fat acidity from no treatment to the 700,000 rep treatment and then an increase in fat acidity. This increase in fat acidity at the 900,000 rep level was also noticeable in the respiration tests.

Comprehensive Tests

The purpose of these tests was to find out how samples of wheat treated at zero and 900,000 rep reacted to milling and baking tests. There were eight samples, four of which had no treatment and four the 900,000 rep treatment. The 900,000 rep treatment was selected as it was the one that was most successful in reducing the respiration to the lowest level. The moisture levels in the test were 12.2 - 12.2 percent, 14.0 - 14.1 percent, 14.6 - 14.7 percent, and 15.6 - 15.7 percent. It had been intended that moisture percentages at the 14.6 - 14.7 percent and 15.6 - 15.7 percent levels should be one percent higher.

The respiration part of the test was run for ten days with an average passage of air over the samples of 16.0 milliliters per gram of dry matter. This rate of air passage was slightly lower than that of the respiration tests.

The air exchange was obtained by making five air changes of 3.8 liters at equal intervals of time every 24 hours. The average ambient temperature was 87 degrees Fahrenheit.

A complete set of milling and baking tests was made for each of these samples. The results of these tests are shown on Tables XL and XLI. The milling and baking tests were made by the International Milling Company.

Table XL.

MILLING DATA

%Moisture at start of test	12.2	12.2	14.1	14.0	14.7	14.6	15.7	15.6
Treatment rep	0	$9x10^5$	0	$9x10^5$	0	$9x10^5$	0	$9x10^5$
%DWMoisture**	10.4	10.5	11.6	11.9	12.1	12.0	13.5	13.2
%WTR Moisture	14.8	14.9	14.8	15.6	14.7	15.0	14.8	14.9
% Extraction	70.4	71.7	70.5	70.7	71.5	70.8	70.8	70.3
% Bran	21.2	21.3	32.0	21.5	21.7	21.7	21.6	21.9
% Shorts	6.0	6.5	5.9	6.0	6.3	6.3	6.4	6.4
Milling time*	5',01"	10,13	4,38	4,51	5,00	4,51	4,47	4,39
% Flour moisture	13.1	12.9	13.0	13.0	13.0	13.0	13.1	13.3
%Flour ash at 14	.415	.471	.458	.472	.452	.432	.458	.434
%Flour prot. at 14	13.55	13.72	13.70	13.45	13.85	13.85	13.75	13.75
Mgs.maltose at 14	157	188	160	192	160	181	157	178
FFA (mg.KOH/ 100gm at0%mt)	23.9	23.8	24.5	25.1	22.6	21.9	21.3	22.6
Odor of flour-water paste	Norm.	V-Bad	Norm.	V-Bad	Norm.	Bad	Norm.	Bad

* 1P, 2P, and 3P/1000 gram samples, 4P, 5P, 6P, 7P, and 8P/1100 grams.

** Before milling.

D.W.Moisture means percent moisture of the dry weight of the the wheat prior to tempering before milling.

%WTR Moisture means percent of moisture of the dry weight after tempering and just prior to milling.

% Extraction means percent of total flour obtained from total wheat.

Mgs. Maltose at 14 means milligrams of maltose at 14.0% moisture of 100 grams of dry matter.

FFA (mg.KOH/100gm at 0%mt) means free fatty acid of 100 grams of dry matter.

Table XLI.

BAKING DATA

%Moisture at start of test	12.2	12.2	14.1	14.0	14.7	14.6	15.7	15.6
Treatment rep	0	$9x10^5$	0	$9x10^5$	0	$9x10^5$	0	$9x10^5$
Absorption	61%	60%	61%	62%	60%	60%	61%	62%
Mixing time	6'	7'	7'	6½'	7½'	6'	9'	8'
Dough handling	Good	Sl.soft	Good	Sl.soft	Good	Good	Good	Good
Avg.loaf ol.	3040	3000	3075	2890	3025	3975	3115	2925
Total score (max.41)	38.0	37.0	38.0	34.5	38.0	37.0	38.0	37.0
Odor	OK	Mild	Mild	Strong	OK	Mild	OK	Mild
Taste	OK	Mild	OK	VStrong	OK	Mild	OK	Strong
Baking quality	VG-	Good	VG-	Fair	VG-	Good	VG-	Good

Notes made by those conducting the bake test and the scoring of the baked loaves were as follows:

"The odor might not be strong enough to be objectionable to a customer, but the taste, designated as mild, would be objectionable. The odor and taste were like burned feathers."

"When the flour was checked for odor by making a flour-hot water paste, the odor of those samples designated as very bad was described as similar to that of burned hair."

"Absorption in the baking test means percent of water needed at 14.0 percent moisture (dry basis) to obtain a dough of satisfactory and optimum consistency."

Analysis of Milling and Baking Tests. Table XL shows that there is not a great difference in percentage of moisture of the samples before the milling tests are made as all samples were purposely not stoppered or sealed after going through the ten-day respiration run. However, it can be seen that there is a slightly higher moisture for those samples that were higher in moisture at the start of the test. Evidently, they had not reached equilibrium.

Table XLI discloses that there is an unusually long milling time for the 12.0 percent, 900,000 rep treatment. This time is more than twice the average, and there seems no reason why this should be so.

The test for milligrams of maltose at 14 percent moisture seems to indicate a difference in treatments. This is indicated in an analysis of variance test in Table XLIII.

Table XLII.

TEST FOR MILLIGRAMS OF MALTOSE AT 14% MOISTURE

	Moisture (Wet basis) at start of test			
Treatment	12.2 - 12.2%	14.0-14.1%	14.6-14.7%	15.6-15.7%
0	157	160	160	157
900,000	188	192	181	178

Table XLIII.

ANALYSIS OF VARIANCE FOR MGS. OF MALTOSE AT 14% MOISTURE

	Sum of Squares	d.f.	Mean Square	F	$F_{.95}$
Moisture Means	.78	3	.26	.257	9.28
Treatment Means	11.29	1	11.29	11.18	10.1
Residual	3.04	3	1.01		
Total	15.11	7			

According to the F test at the five percent level of significance there is a difference in treatment means for milligrams of maltose at 14 percent moisture. This treatment effect is not great, but significant.

The free fatty acid test showed a difference in moisture means, as shown in Tables XLIV and XLV.

Table XLIV.

FREE FATTY ACID (mgKOH/gmD.M.) FOR COMPREHENSIVE TESTS

Treatment	Moisture (Wet basis) at start of test			
rep	12.2 - 12.2%	14.0 -14.1%	14.6 -14.7%	15.6 -15.7%
0	23.9	24.5	22.6	21.3
900,000	23.8	25.1	21.9	22.6

Table XLV.

ANALYSIS OF VARIANCE, FREE FATTY ACID(mgKOH/100gmD.M.)
COMPREHENSIVE TEST

	Sum of Squares	d.f.	Mean Square	F	$F_{.95}$
Moisture Means	10.89	3	3.63	9.55	9.28
Treatment Means	.15	1	.15	.0395	10.1
Residual	1.14	3	.38		
Total	12.18	7			

The F test reveals a difference in moisture means at the five percent level of significance.

The average loaf volume was about the same except for the 14.6 - 14.7 percent moisture, 900,000 rep treatment. There is no accounting for this unusually high loaf volume since the other treatments averaged slightly lower than the non-treated samples.

The mixing time increased a small amount as the moisture increased. Treatment seemed to have no definite effect. This can be seen best in Table XLVI.

Table XLVI.

MIXING TIME

Treatment rep	Moisture (Wet basis) at start of test			
	12.2 -12.3%	14.0 -14.1%	14.6 -14.7%	15.6 -15.7%
0	6'	7'	7½'	8'
900,000	7'	6½'	6'	8'

The total score averaged 37.0 for the treated samples and 38.0 for the non-treated samples with the exception of 34.5 for the low moisture (12.2 percent), 900,000 rep treatment. For some reason this scored very low.

Baking quality was very good minus on the non-treated samples and good on the treated samples with the exception of the treated 14.0 percent moisture sample which was graded fair.

Summary of the milling and baking tests. The milligram of maltose at 14 percent moisture seemed to be influenced by the 900,000 rep treatment. Other effects were noticed such as total score, average loaf volume, and dough handling, but none were significant.

A review of all the results of the milling and baking tests does not show a great deal of difference in milling and baking quality, except for the odor, for both the milling and baking tests and the taste for the baking test. The taste was designated as mild; however, it would be objectionable to a consumer.

SUMMARY

Treatments of 500,000 rep and above reduced the respiration rate of wheat between 15.0 - 16.8 percent moisture (wet basis) in the ten-day respiration test at 86 degrees Fahrenheit. In most cases it was found that for treatments of 500,000 rep there was little difference in respiration between high-moisture wheat (15.0 - 16.8 percent) and low-moisture wheat (10.8 - 12.8 percent). This indicated that treatments of 500,000 rep reduced the activity of molds to a low level.

Treatments of 392,000 rep and below had no effect upon the respiration rate.

The free fatty acid test was not a satisfactory index of deterioration. As a rule, for the respiration tests for high-moisture content wheat (15.0 - 16.8 percent) there was a reduction in free fatty acid at the 500,000 and 700,000 rep treatment and an actual increase again at the 900,000 rep treatment.

A pint thermos bottle was too small a sample to effect a rise in temperature due to the heat production of wheat. In only one run was there a significant difference in temperature between a treatment and no treatment, and this difference was not very large. Evidently, to achieve results similar to a grain bin, air should be piped

down to the bottom of the bottle. No doubt aerobic action was taking place which was not conducive to heat production.

In the thermos bottle tests there was a decrease in free fatty acid at the high moisture level 500,000 rep and 700,000 rep treatments with an increase again at the 900,000 rep treatment. This was similar to the results in the respiration tests.

The most significant result of the comprehensive milling and baking tests was the burned odor and taste. While this was not great, it would be definitely noticed by a consumer. In the work of Wiant[49] it was found that dosages of 50,000 rep and above had a burned taste and odor. To avoid this burned taste and odor dosages must be lower than 50,000 rep, at least through the bread-making part of the wheat. According to the work of Thompson,[48] the heating of stored wheat is due to a fungi next to and inside the epidermis of the wheat. If this is true, then it is not necessary to give such penetrating dosages to wheat nor at such high dosages throughout. Perhaps a pass on either side may be adequate if of the right dosage and penetration. Also, some way may be found to roll wheat while under the beam to give an even penetration through the epidermis.

Finally, there were some minor milling and baking effects. The milligrams of maltose at 14.0 percent moisture seemed to be increased at the 900,000 rep treatment. Other effects noticed were the slight decrease in total score and

average loaf volume at higher levels of treatments. None were significant.

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