BROWNING OF WHEAT GERM IN RELATION TO SICK WHEAT

by

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INTRODUCTION

A type of deterioration of wheat in storage which affects principally the embryos of the grain is known in the grain trade as "sick" or germ-damaged wheat. This type of damage which if present, reduces the commercial grade, is recognized by the turning in color of the germ from normal light yellow to light tan, brown, and finally to a dark mahogany. "Sick" wheat has also been defined as that type of deteriorated wheat in which the seed loses its viability and the germ darkens in color and becomes rancid. This type of damage which occurs in both terminal and country elevators, has been recognized since 1921 (2), and appears to be most prevalent in years when damp harvest conditions are encountered.

Scientific investigation of the nature of this deterioration have been initiated only recently. At this time only about four papers in the literature have reference to this condition, in spite of the great economic losses in wheat storage and marketing which it leads to.

The present study is the second in a series of investigations of germ damaged wheat recently initiated at Kansas State College.

REVIEW OF THE LITERATURE

The initial causes of the germ damaged condition is not

clearly known, but a few workers have suggested that fungus growth is the principal cause. Thomas (22) believed that "sick" wheat was grain which had lost its viability due to toxic products given off by molds, principally the species Aspergillus flavus, which grow in damp grain. Germination tests were determined on the wheat after treating the seeds with filtrates of pure cultures of 13 species and strains of molds which are commonly found on grain. While all the filtrates lowered the germination, Aspergillus flavus had a very pronounced effect. Thomas apparently paid no attention to the characteristic discoloration of the embryos of "sick" wheat.

Milner, et al (14) found the kind of microflora and their distribution on samples of commercial "sick" wheat was 60 percent Aspergillus glaucus, 20 percent pencillium, and the remaining 20 percent as A. niger, A. flavus, A. candidus, Nigrospora, Rhizopus, Trichoderma and several unidentified bacteria. On sound samples they found 90 percent Alternaria, 5 percent Fusarium, and 5 percent Helminthosporium.

In recent unpublished studies Christensen[1] inocculated wheat heavily with storage molds and stored it for 10 to 15 days at moisture levels of 18 to 21 percent. He then dried the grain to 14 percent and stored it for two months during which a high percentage of "sick" wheat developed. Wheat free of storage

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[1]Clyde M. Christensen, "Studies on 'Sick' Wheat", Progress Report No. 2, Nov. 1, 1952.

molds, except for some Aspergillus glaucus, stored under similar conditions developed little "sick" wheat.

Swanson (20) found that "sick" wheat develops even in the absence of mold growth and that under such conditions serious damage to the baking quality of the flour results. Mold growth was inhibited by treatment of the seeds with ethyl mercury phosphate or by exclusion of the air from the grain.

Carter and Young (2) produced "sick" wheat in the laboratory by storing wheat in sealed containers at various moisture contents and temperatures over a time interval of 687 days. They eliminated all samples on which molds were visible and concluded that "sick" wheat formation is associated with anaerobic storage. In general an increase in "sick" wheat was accompanied by an increase in fat acidity and a decrease in germination.

Milner, et al (14) studied the influence of temperature, moisture, time, and storage atmosphere on the development of "sick" wheat in the laboratory. "Sick" wheat was produced by storing wheat of 18 percent moisture in sealed containers under atmospheres of carbon dioxide, nitrogen, and oxygen. Only under oxygen did molds proliferate while "sick" wheat developed under all atmospheres. These writers suggested that other factors such as oxidizing enzymes and non-enzymatic browning should be investigated as factors possibly involved in "sick" wheat formation.

Cole and Milner (4) in recent work have shown that the light absorption spectrum of extracts of normal wheat was characterized

by a peak at 270 mu and an inflection at 325 mu, both of which increased slightly when the grain was germ damaged. The damaged wheat extract also had a higher fluorescence. Fresh granular wheat germ was found to darken during storage as did the germs of wheat kernels. This increase in darkness was accompanied by increases in fat acidity and reducing sugars. Extracts of the brown germ gave increased light absorption at 245 and 325 mu and increased fluorescence over that of the sound germ extracts. Wheat germ dissected by hand from commercial sound and germ-damaged kernels gave extracts which showed the same changes in spectral and fluorescence characteristics as did the extracts of normal and discolored granular wheat germ. They suggested from these results that the brown pigment formed in granular wheat germ and in germs of wheat kernels is the product of a sugar-amino acid condensation known as the Maillard or non-enzymatic browning reaction.

Four theories of non-enzymatic browning now exist. The most popular is the Maillard or melanoidin reaction involving the condensation of amino acids with reducing sugars to form a dark colored substance. The ascorbic acid theory holds that an oxidation product of ascorbic acid is involved, but the ascorbic acid content of wheat is very low. Another theory is the "active-aldehyde" theory of Wolfrom, et al (25) which proposes that browning requires the preliminary decomposition of sugars and sugar acids to furfuraldehyde or similar compounds having an active carbonyl group, and that these products condense

with nitrogen compounds followed by polymerization to form brown resinous materials. The most recent theory by Hodge and Rist (10) suggests that non-enzymatic browning can occur by the following reactions.

1. Reducing sugars and amino compounds condense to form N-glycosides.

2. The N-glycosides rearrange spontaneously (Amadori rearrangement) to form desoxyaminoketoses.

3. These are dehydrated spontaneously to nitrogenous reductones.

4. Or the labile desoxyaminoketoses produce the Strecker degradation of amino acids forming aldehydes and carbon dioxide, and the aldehydes condense with themselves, amines, aldimines, amino acids, or proteins to produce brown polymers.

5. The nitrogenous reductones react slowly alone (in the presence of air) and rapidly with amino acids to produce brown pigments.

Hodge and Rist found that the desoxyaminoketose-amino acid system which they reproduced in the laboratory showed the same phenomena found in natural non-enzymatic browning systems as follows:

1. Substances reducing methylene blue and dichlorophenolindophenol rapidly at pH less then 8.5 were formed.

2. Fluorescent substances were produced.

3. Carbon dioxide was liberated from the amino acid *via* the Strecker degradation.

4. The browning reactions were greatly accelerated with increasing pH and with increasing temperature.

5. The browning reactions were delayed in the presence of relatively small amounts of bisulfite and were practically stopped in solutions containing high concentrations of bisulfite.

6. The browning reactions were stopped by hydrogenation of the reaction mixture.

Previous studies by several writers including Tarassuk and Simonsen (21), Jenness and Coulter (11), Haney (9), and Olcott and Dutton (18) have shown that natural browning as well as model browning systems of purified proteins or amino acids reacting with reducing substances such as sugars also exhibit characteristic increases in optical density of their extracts in the ultraviolet region of the spectrum as well as marked increases in fluorescence.

This review indicates that the literature dealing with non-enzymatic browning has become sufficiently definitive to permit the postulation of fairly precise chemical and physical criteria which naturally occurring reactions must fulfill in order to qualify as browning reactions.

STATEMENT OF THE PROBLEM

Granular wheat germ was used as an experimental material in these studies which were aimed at further clarification of the nature and cause of germ-damaged wheat.

Preliminary storage studies in sealed containers were carried out to determine if the browning in wheat germ is enzymatic and could be inhibited by autoclaving of the germ samples. Phenol oxidase activity of wheat germ was also determined.

Attention was next turned toward non-enzymatic browning because the phenol oxidase activity of wheat germ was found to be very low. Also during this interval, work by Cole (3) showed that the characteristic properties of non-enzymatic browning including increased optical densities and fluorescence occurred in extracts of browned wheat germ samples.

A method for studying the browning of wheat germ by which the relationship of mold growth to the browning effect during storage could be precisely determined was needed. Molds growing on granular wheat germ and on germs of intact wheat kernels should be of the same species. A difficulty encountered with the use of closed containers in storage studies is that exhaustion of oxygen greatly inhibits the growth of molds which may appear normally in germ-damaged wheat. Accordingly a storage method was adopted by which the temperature, aeration rate, and humidity were controlled, while mold growth in the germ samples could be determined from the carbon dioxide production and other gas exchange characteristics.

Since the storage studies required control of the atmospheric humidities surrounding the wheat germ, the equilibrium hygroscopic moisture of germ at various humidity values had to be determined, as this information does not appear in the literature.

The usual method for determing hygroscopic characteristics of a material is to expose it to an atmosphere of constant relative humidity until moisture equilibrium is reached. The main disadvantage of this technique is that at humidities above 75 percent molds can grow, and their metabolic activity may increase the moisture content of the material and change the hygroscopic equilibrium.

The fluorescence and optical density of acid extracts of germ were determined in order to follow the browning effects produced during the controlled storage experiments. In addition changes in the solubility characteristics of the wheat germ protein were noticed and this led to another means, devised in the course of these studies, to relate chemical changes to the browning effect.

MATERIALS AND METHODS

Materials

Samples of unprocessed granular wheat germ used in this work was obtained from General Mills Inc., Minneapolis, Minnesota, and unprocessed flat germ was obtained from Commander-Larabee Milling Company, Minneapolis, Minnesota.

Method of Determining Hygroscopicity

The technique used for determining hygroscopic moisture equilibrium of wheat germ was the graphical interpolation method of Landrock and Proctor (12), which was based on the principal that at any given initial moisture content of a material under any given atmospheric humidity the rate of gain or loss in the moisture content of the material increased with the increasing difference between atmospheric and equilibrium humidity of the material. If the only variable is the relative humidity of the test atmosphere the gains or losses in moisture content of the product is almost linear when plotted against the relative humidities of the atmospheres in which the samples were stored. The relative humidities corresponding to zero gain or loss of weight on the curves of the graph are the equilibrium humidities of the sample at the corresponding moisture contents.

Ten humidity dishes were constructed to provide for relative humidities from 50 percent to 95 percent at 5 percent intervals. Each humidity unit consisted of a 150 mm crystallizing dish, a glass tray standing on four legs and fitted in the humidity dish, a glass cover plate well greased to make an air-tight fit, and about 250 grams of a sulfuric acid solution to maintain the desired humidity (24). The test samples were placed on the glass tray well about the sulfuric acid solutions.

The commercial granular wheat germ to be used for the hygroscopic studies first had to be purified. This germ contained at

least 5-10 percent impurities mostly as particles of bran and endosperm. Samples of the wheat germ were sieved for three minutes on a Ro-Tap sifter using number 12, 14, 16, and 20 sieves. Wheat germ mostly free of particles of endosperm, but not free of bran, was obtained on the number 16 sieve. Portions of this germ were then allowed to fall in a fine stream through a controlled blast of air. Half of the resulting pile of germ farthest from the air blast contained a greater percentage of bran and was discarded. The remaining germ was retained and further purified by picking out the remaining foreign particles by hand.

A special tempering apparatus was constructed for the tempering of 20 gram samples of wheat germ for the hygroscopic studies. Wheat germ cannot be tempered simply by the addition of a calculated amount of water for the germ must be moving and the water must be applied as a fine spray. If this is not done, large lumps will be formed in the granular germ. This apparatus consists of a 250 ml filtering flask, a small Hersberg stirrer, and an atomizing tube. The atomizing tube delivers and atomizes the water at a point just above the germ which was whirled at a rapid rate by the Hersberg stirrer. The atomizing tube was fitted to the filtering flask by means of a rubber stopper, and consisted of a glass capillary tube surrounded concentrically by a loosely fitting glass tube fire polished on its lower atomizing end until only a pin hole remains. The top end of the capillary tube was flared for receiving water while tempering wheat germ. The stirrer was fitted loosely through a glass

sleeve in the stopper, and was sealed air-tight by means of a small piece of rubber tubing. When a vacuum was applied to the filtering flask, air moved through the outer jacket of the atomizing tube, and water pipetted slowly into the top flanged end of the capillary tube was sucked to the atomizing end where it was mixed with the moving air to form a fine spray. The tip of the atomizing end was treated with a silicone grease to prevent water from wetting and dripping from it. With this apparatus wheat germ samples for hygroscopic determinations were tempered to various moisture levels.

For the hygroscopic determination aluminum flour moisture dishes with inverted slip-in lids were used. The lids and dishes were weighed separately on an analytical balance to the nearest tenth of a milligram. The following procedure was used for determining the hygroscopic equilibrium at each moisture level.

Very nearly two grams of sample were weighed into the moisture dish. Immediately the lid was placed on the dish to prevent evaporation, and the exact weight was determined. Then the lid of the moisture dish was removed, and the dish was placed in one of the constant humidity units for exactly one hour. The dish was then removed, the lid immediately placed on it, and the exact weight of the dish and germ was determined. The above procedure was repeated on other portions of wheat germ of the same moisture content in other constant humidity units. The gains and losses of weights of the germ samples at each moisture level were plotted against the relative humidities at which they were stored.

Technique for Storage of Wheat Germ Under Controlled Conditions of Temperature, Humidity, and Air Supply with Simultaneous Determination of Gas Exchange Characteristics

A method for studying the browning of wheat germ by which the relationship of mold growth to the browning effect could be precisely determined was needed. In experiments with closed containers the lack of oxygen greatly inhibits the growth of molds. Accordingly a method was adopted in which the temperature, aeration rate, and humidity were controlled while mold growth in germ samples could be determined by the characteristics of the carbon dioxide production. With this method originated by Milner and Geddes (15), a slow continuous stream of air was drawn through the sample, and the resulting gas mixture was collected and analyzed daily for carbon dioxide and oxygen.

Samples of fresh granular wheat germ (300 g.) were tempered to various moisture contents in a special tempering apparatus. This apparatus consisted of a can 9" in diameter and 11" high, a Hersberg stirrer, and an atomizer. The wheat germ samples were placed in the can and whirled at a rapid rate by the stirrer while water was added to the germ with the atomizer through a small hole in the lower side of the can. Wheat germ samples of various moistures were prepared and were stored in a refrigerator at about 0°C. Humidity flasks containing sulfuric acid solutions (24) which maintained humidities corresponding to the equilibrium humidities of the germ samples were constructed and fitted to the air lines of the respirometers to humidify the air going into the sample flasks.

Within less than 24 hours after tempering the samples were fitted into the respiration apparatus, the apparatus was started, and every 24 hours a sample of the gas collected from each germ sample over the previous 24 hour period was obtained and analyzed for carbon dioxide and oxygen with a Haldane-Henderson gas analyzer (15).

Methods for Determining Fluorescence, Optical Density and pH of Wheat Germ

Changes in fluorescence, optical density, and pH were used to follow the browning effect produced during storage of wheat germ. Fluorescence and optical density measurements were determined according to the procedure used by Cole (3). Duplicate one gram samples of wheat germ which had been ground in a small Wiley mill through a number 30 screen were placed in 250 ml Erlenmeyer flasks to which was added 50 ml of .2N HCl. The wheat germ samples were extracted for one hour with swirling of the contents of the flasks every 15 minutes. Then the solutions were filtered through a number 4 Whatman filter paper, and the filtrates were used for fluorometric and colorimetric measurements. Fluorescence was determined with a Coleman fluorimeter using a B_1-S filter and a standard of .1 p.p.m. fluorescein with which the dial was set at 60. One ml of each filtrate was diluted to 50 ml with .2N HCl for the determination. Five ml of each filtrate was diluted to 25 ml with .2N HCl and optical densities were

determined in the ultra violet region with a Beckman spectrophotometer, model DU.

Since the pH of a material greatly influences the rate of browning and the pH has been reported to decrease with increasing browning (10) (26), the pH of wheat germ samples during storage was determined by the following procedure. In a mortar .50 grams of wheat germ was ground with 5 ml of distilled water, and the resulting mixture was allowed to stand in a test tube for 45 - 60 minutes. The pH of the resulting slurry was determined on a Beckman model G pH meter.

EXPERIMENTAL RESULTS

Catechol Oxidase Activity of Wheat Germ

In a preliminary study to determine whether the browning of wheat germ may be due to enzymatic causes, the catechol oxidase activity of wheat germ was determined by the method of Milner (13). Samples of sound ground granular wheat germ were agitated with catechol and excess ascorbic acid in the presence of oxygen in such a manner that the o-benzoquinone produced was continually reduced by the ascorbic acid to catechol. The extent of catechol oxidation was determined by titration of the residual ascorbic acid with 2,6- dichlorophenolindophenol. Preliminary determinations showed catechol oxidase activity of germ to be only 2.0 mg ascorbic acid destroyed per gram of germ per hour. This compares

with values of about 30 for wheat and 120 for wheat bran (17). Close examination of the granular wheat germ showed that it was contaminated with small amounts of bran which is very high in catechol oxidase activity. Thus the low oxidase activity of the germ samples was probably due to bran impurities.

Influence of Autoclaving Damp Germ on Subsequent Darkening in Storage

On the theory that the elimination of enzymes, molds, and other micro-organisms from damp grain might lead to information on factors influencing the darkening of germ during storage, an experiment was undertaken in which samples of flat germ containing 9.6 percent and 17.6 percent moisture, and portions of granular germ with 12.8 percent and 18.8 percent moisture were autoclaved for periods of 15, 30, and 45 minutes, and then transferred to screw cap bottles for storage at 10°C and 42°C. Unautoclaved controls of corresponding moistures were also stored at 10° and 45° C. Some darkening occurred during autoclaving, but the greatest degree of darkening occurred during the period of 30 to 45 minutes. Samples of high moisture content darkened more than samples of low moisture content. Apparently mold contamination occurred while transferring the germ samples after autoclaving as some samples stored at 42°C showed mold growth accompanied by darkening. Other samples not developing mold growth did not darken. The experiment was repeated using granular germ containing 13.2 and 16.7 percent moisture with precautions

to prevent mold contamination after autoclaving. After 13 weeks of storage at 42° C. none of these autoclaved samples had darkened while the corresponding non-autoclaved controls became brown. However after 14 weeks these sterile autoclaved samples also began to darken slightly.

These results appear inconclusive, but they do suggest that autoclaving inhibits darkening of sterile stored damp germ, although not completely. Mold growth appeared to hasten darkening when it occurred on autoclaved germ. Another possible conclusion is that autoclaving prevents darkening of germ by inhibiting some mechanism other than that of mold growth or enzyme activity, such as that leading to non-enzymatic browning.

Influence of Extraction on Color Changes and Other Properties of Stored Wheat Germ

Wheat germ is known to contain as its three major chemical constituents sugar (principally sucrose and raffinose), protein, and oil. To determine which of these major constituents might be involved in the darkening of germ on storage, an experiment was carried out with germ samples which were previously extracted repeatedly with cool petroleum ether and with water. Presumably the ether treatment removed most of the oil while the water treatment removed most of the sugars.

Samples of ether-extracted, water extracted and ether-water extracted germ samples were stored in constant humidity jars at

60 and 90 percent at temperatures of 10° and 42° C. These humidities were maintained by sulfuric acid solutions of various concentrations (24).

After eleven weeks of storage at 42°C. and 60 percent humidity none of the three extracted germ samples had darkened. After the eleventh week of storage the ether extracted sample darkened slightly while the other two extracted samples did not darken during eighteen weeks of storage. During eight months of storage the ether-water extracted sample did not darken while the water and ether extracted germ samples had darkened slightly.

The water extracted and ether extracted samples stored at 42°C. and at 90 percent humidity began to darken after only two weeks of storage. The water extracted sample turned from a light tan to a definite salmon color while the fat extracted sample turned from a very light tan to a brown color. Mold growth could be clearly seen in the water-ether extracted sample, but in spite of this mold the sample did not darken any until the fourth week of storage. During thirteen weeks of storage the water extracted sample had changed to a dark salmon color, the ether extracted sample had changed to a dark mahogany brown, while the water-ether extracted sample had darkened only slightly. Little additional change in color appeared during seven months of storage.

Colorimetric and fluorometric measurements were made on the extracted samples after seven months of storage of the 90

percent humidity samples and eight months of storage of the 60 percent humidity samples. These procedures had been developed by Cole (3) during this storage period. The 90 percent humidity samples were first dried over calcium chloride for 24 hours before making these determinations. Light absorption spectra of extracts of these samples were determined between the wave length of 220 mu and 350 mu.

The fluorescence data obtained from extracts of these samples are shown in Table 1. An extremely high fluorescence value was obtained with the ether extracted sample stored at 90 percent humidity and 42°C. The removal of the sugars and other water soluble substances by the water extraction greatly inhibited the high increase of fluorescence in the other two samples.

Table 1. Influence of extraction on development of fluorescence in stored wheat germ.

Storage conditions	Fluorescence after extraction		
	Ether	Water	Ether and water
60% R.H., 8 mo., 10°C	3.1	3.3	4.5
60% R.H., 8 mo., 42°C	16.6	11.2	10.4
90% R.H., 7 mo., 10°C	30.7	12.4	4.3
90% R.H., 7 mo., 42°C	1170.0	27.1	36.7

With the 60 percent humidity samples only very slight differences in absorption spectra were obtained between corresponding

samples stored at 10°C. and 42°C.

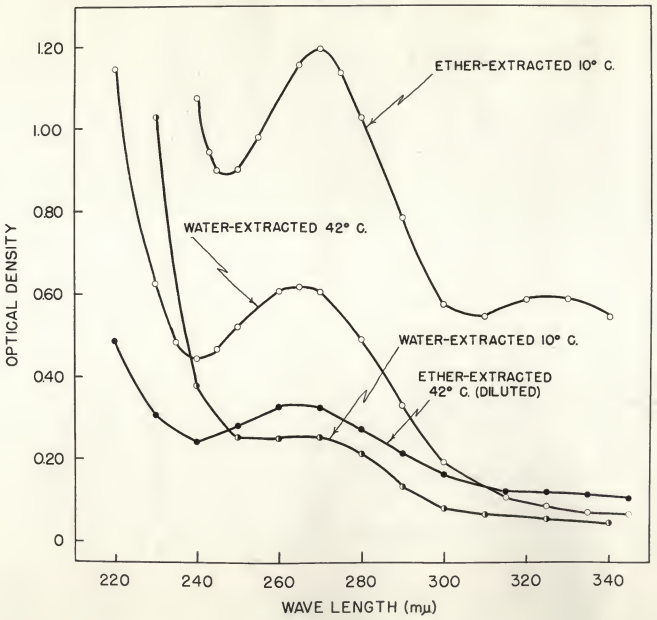
A much greater difference in absorption spectra as shown in Plate I, was obtained between corresponding samples stored at 90 percent humidity. The optical density of the extract of the ether extracted sample stored at 42°C. was so high it was off the scale of the spectrophotometer. The extract was diluted one hundred-fold before an absorption curve could be determined. The relative optical densities for the ether extracted sample stored at 10°C were much lower than those obtained for the corresponding sample stored at 42°C. With the water extracted sample stored at 42°C. a small absorption peak was obtained at 265 mu while no peak was obtained with the corresponding sample stored at 10°C. Only a very slight difference in absorption spectra was obtained between corresponding ether-water extracted samples.

The results indicate clearly that removal of oil from germ had little influence on the appearance of the characteristic browning, fluorescence, and colorimetric changes during storage which had been noted in whole germ or in intact wheat (4). On the other hand extraction with water, which presumably removed the sugars, resulted in marked changes in the normal color development and in the optical density and fluorescence properties. This evidence suggests that sugars are involved in the characteristic browning reaction whereas the oils are not.

EXPLANATION OF PLATE I

Light absorption spectra of extracted wheat germ samples stored at 90 percent humidity and at temperatures of 10° and 42° C. for seven months.

PLATE I


ETHER-EXTRACTED 10° C.
WATER-EXTRACTED 42° C.
WATER-EXTRACTED 10° C.
ETHER-EXTRACTED
42° C. (DILUTED)
OPTICAL DENSITY
1.20
1.00
0.80
0.60
0.40
0.20
0
220
240
260
280
300
320
340
WAVE LENGTH (mμ)

Hygroscopicity of Wheat and Wheat Germ

Hygroscopic equilibriums were determined by the procedure of Landrock and Proctor (12) on ground flat wheat germ, whole granular wheat germ, and whole kernels of 1952 Kiowa wheat of 11.2 percent protein content. Plate II shows the gains and losses in weights of samples of granular wheat germ.

The rate of loss or gain of water during the one hour storage period, as indicated by the greater slope of the weight-change curves, was found to be greater with granular wheat germ than with intact Kiowa wheat seeds. The critical moisture, that is the moisture value in equilibrium with a humidity of 75 percent, which is shown in Plate III, was found to be 14.0 percent for ground flat wheat germ, 13.2 percent for whole granular wheat germ, and 13.5 percent for whole Kiowa wheat seeds. This value for wheat is somewhat lower than those obtained by previous writers working with equilibrium methods for hygroscopic moisture determinations (5) (8) (19).

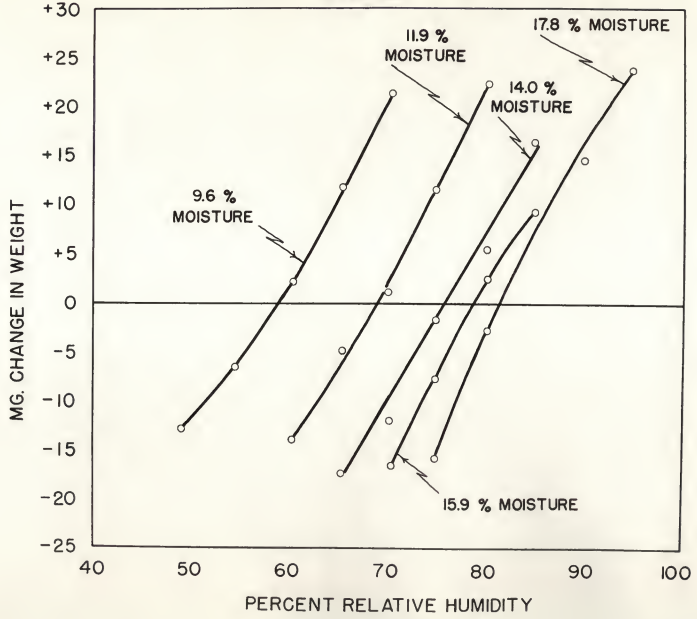
Development of Turbidity Method for Determining Peptizable Protein

The storage experiment with water and ether extracted wheat germ samples indicated that the browning was greatly inhibited by elimination of sugar. The characteristic browning of normal sugar-containing wheat germ was accompanied by large increases in fluorescence and optical density of the germ extract. Since

EXPLANATION OF PLATE II

Gain or loss in weight of granular wheat germ at various moistures contents in atmospheres of various humidities.

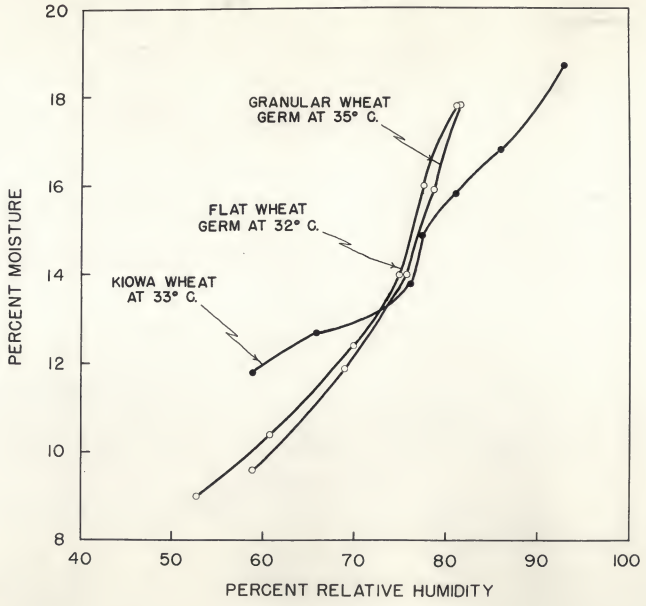
PLATE II


+30
+25
+20
+15
+10
+5
0
-5
-10
-15
-20
-25
MG. CHANGE IN WEIGHT
40
50
60
70
80
90
100
PERCENT RELATIVE HUMIDITY
9.6 % MOISTURE
11.9 % MOISTURE
14.0 % MOISTURE
17.8 % MOISTURE
15.9 % MOISTURE

EXPLANATION OF PLATE III

Equilibrium hygroscopic moisture content of wheat and germ at various relative humidities.

PLATE III


20
18
16
14
12
10
8
PERCENT MOISTURE
GRANULAR WHEAT
GERM AT 35° C.
FLAT WHEAT
GERM AT 32° C.
KIOWA WHEAT
AT 33° C.
40
50
60
70
80
90
100
PERCENT RELATIVE HUMIDITY

these properties are characteristic of non-enzymatic browning and current theories regarding this type of browning postulate that the protein of the material is involved chemically in this reaction, a method for determining the changes in the germ protein which might accompany browning was developed.

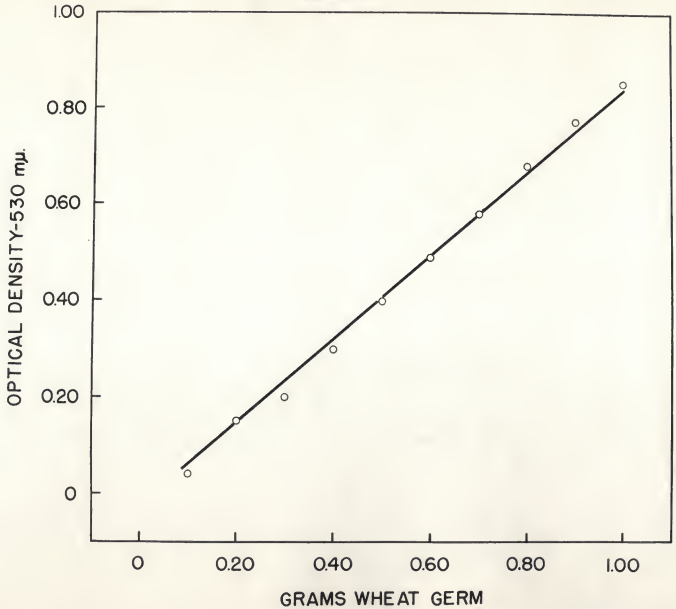
The peptizable protein procedure developed is based on the Zeleny photometric method (27) for determining the protein content of wheat flour and involves first the extraction of the non-gluten protein of germ in dilute potassium sulfate. By adding small aliquots of the peptized protein to buffers of various pH's (HCl-sodium citrate, pH 2.0-3.0), a colloidal suspension of optimum turbidity and stability was found to be formed at pH 2.4.

The data of Plate IV shows that the light absorption of the suspensions formed by this procedure followed Beers law in that it was a linear function of the size of the wheat germ sample. Further confirmation of the linearity of the light absorption of the suspensions from browned wheat germ samples was shown by the fact that the amount of protein peptized from germ samples of various degrees of browning was also found to be a linear function of the light absorption of the suspensions, as shown in Plate V. The amount of protein peptized was determined by the Kjeldahl nitrogen method as the difference between the total nitrogen peptized by the 5 percent potassium sulfate solution and the nitrogen soluble in .3N trichloracetic acid. This linearity with browned germ samples is a clear indication that the germ

EXPLANATION OF PLATE III

Equilibrium hygroscopic moisture content of wheat and germ at various relative humidities.

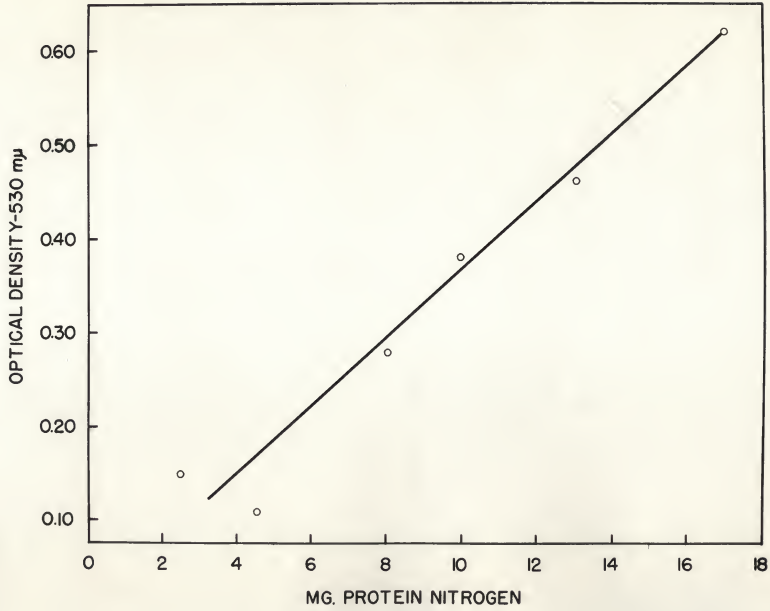
PLATE IV


1.00
0.80
0.60
0.40
0.20
0
OPTICAL DENSITY-530 mμ.
0
0.20
0.40
0.60
0.80
1.00
GRAMS WHEAT GERM

EXPLANATION OF PLATE V

Relation of protein nitrogen content of extracts of browned granular wheat germ to optical density of its peptized suspensions.

PLATE V


OPTICAL DENSITY-530 mμ
0.60
0.50
0.40
0.30
0.20
0.10
0
2
4
6
8
10
12
14
16
18
MG. PROTEIN NITROGEN

protein is directly involved in the browning characteristic of the germ.

The detailed procedure developed and used for determing peptizable protein was as follows: A .70 gram sample of wheat germ which had been ground through a number 30 screen was shaken intermittently for fifteen minutes with 50 ml. of 5 percent potassium sulfate in a 250 ml glass stoppered Erlenmeyer flask. The mixture was filtered through a number 4 Whatman filter paper and 3 ml of the filtrate was pipetted into a Coleman spectrophotometer tube containing 10 ml of hydrochloric acid-sodium citrate buffer (pH 2.4). The resulting turbid solution was allowed to stand for 20 to 45 minutes. Then the tubes were fitted with rubber stoppers and the contents were mixed by three to four inversions of each tube. The light absorption was then read on a Coleman spectrophotometer at a wave length of 530 mu.

Storage of Wheat Germ in Respirometers at 30°C.

To ascertain the relationship of browning to mold growth at 30°C. under controlled conditions, duplicate sample pairs of germs at three moisture levels were prepared as described, and were stored in the respiration apparatus at 30°C. for fourteen days. In the course of the experiment aliquots from one sample of each moisture level were removed at various time intervals for fluorescence, colorimetric, pH, and peptizable protein measurements. Gas exchange data was determined daily on the remaining

three samples.

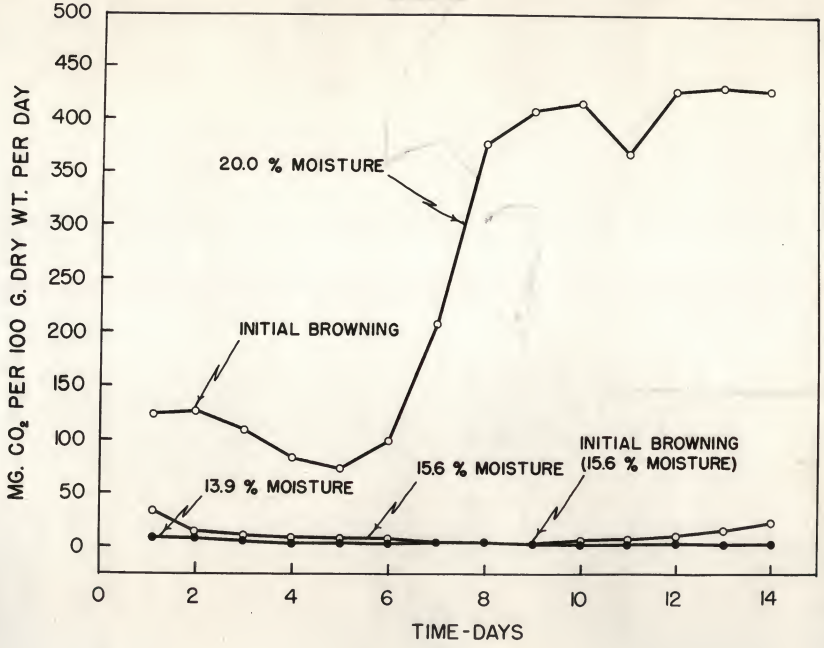
The rate of carbon dioxide evolution as shown in Plate VI, was high on the first day for the sample with 20.0 percent moisture and was low for the samples with 13.9 and 15.6 percent moistures. During the next day, the rate of carbon dioxide evolution of the highest moisture sample rose slightly and then declined for three days before rising strongly again on the fifth day because of fungal respiration (16). Mold growth was visible on the sample on the sixth day. The initial high rate and subsequent decline in carbon dioxide evolution in freshly wetted seeds has been previously reported for peas (7), wheat (23), and soybeans (16). The rate of oxygen absorption during the experiment followed an almost identical pattern to that of carbon dioxide evolution. The respiratory quotient values of the 13.9 and 15.6 percent moisture samples as shown in Plate VII, were 8 and 5.7, on the first day, dropped sharply on the second day, and then gradually declined for a week before leveling off at about 1, and 0.5. The R. Q. values of the 20.0 percent moisture sample rose slightly to about 1.7, and then dropped before leveling off on the seventh day at about 1.0.

Milner and Geddes (16) in their work with soybeans also found that with the onset of mold growth the carbon dioxide evolution rose sharply and the R. Q. approached unity. They concluded that the sharp upward break in the respiration curve could be used to detect the first onset of mold growth before it was visible to the eye. In this experiment mold growth on the

EXPLANATION OF PLATE VI

Carbon dioxide production from granular wheat germ of various moisture contents at 30°C.

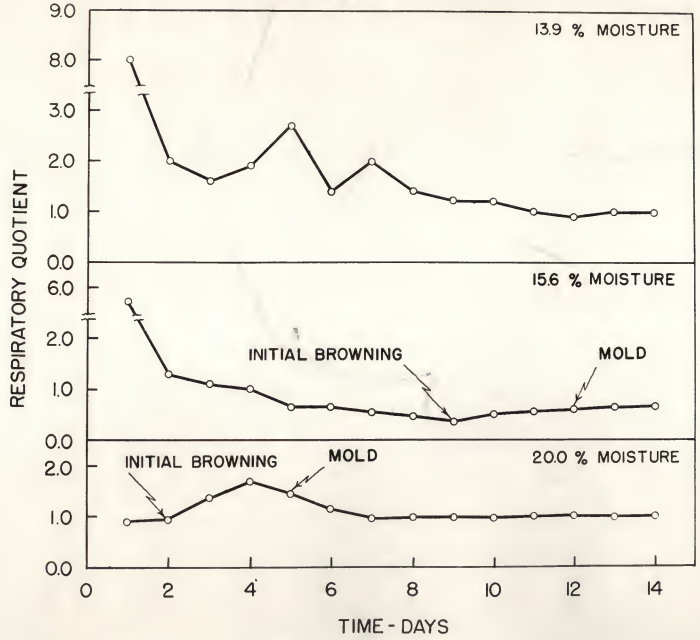
PLATE VI


MG. CO_2 PER 100 G. DRY WT. PER DAY
500
450
400
350
300
250
200
150
100
50
0
0
2
4
6
8
10
12
14
TIME-DAYS
20.0 % MOISTURE
INITIAL BROWNING
13.9 % MOISTURE
15.6 % MOISTURE
INITIAL BROWNING
(15.6 % MOISTURE)

EXPLANATION OF PLATE VII

Respiratory quotient values of granular wheat germ at various moistures contents at 30 C.

PLATE VII


13.9 % MOISTURE
15.6 % MOISTURE
20.0 % MOISTURE
INITIAL BROWNING
MOLD
INITIAL BROWNING
MOLD
RESPIRATORY QUOTIENT
TIME - DAYS
9.0
8.0
3.0
2.0
1.0
0.0
6.0
2.0
1.0
0.0
2.0
1.0
0.0
0
2
4
6
8
10
12
14

20.0 percent moisture sample was indicated by the break in the respiration curve one day before molds could be seen on the sample. Grey, white, and green molds were visible on this germ sample at the end of the experiment.

The fluorescence and light absorption of extracts, and peptizable protein of the samples of 13.9 percent and 15.6 percent moisture as shown by Plates VIII, IX, and X, stayed relatively constant during the fourteen day experiment. After the second day, when the germ of 20.0 percent moisture had lost its yellow color and had begun to brown, the fluorescence and optical density increased gradually while the peptizable protein decreased in an analogous manner to the increase in fluorescence and optical density.

The pH of all three wheat germ samples stored at 30°C. decreased gradually during the storage period as shown by Plate XI. The rate of decrease was slightly greater on the samples of higher moisture content. The 20.0 percent moisture sample decreased about 0.6 pH unit during the fourteen days of storage. Several pH titration curves done on water extracts of sound and browned wheat germ showed that wheat germ is a well buffered system.

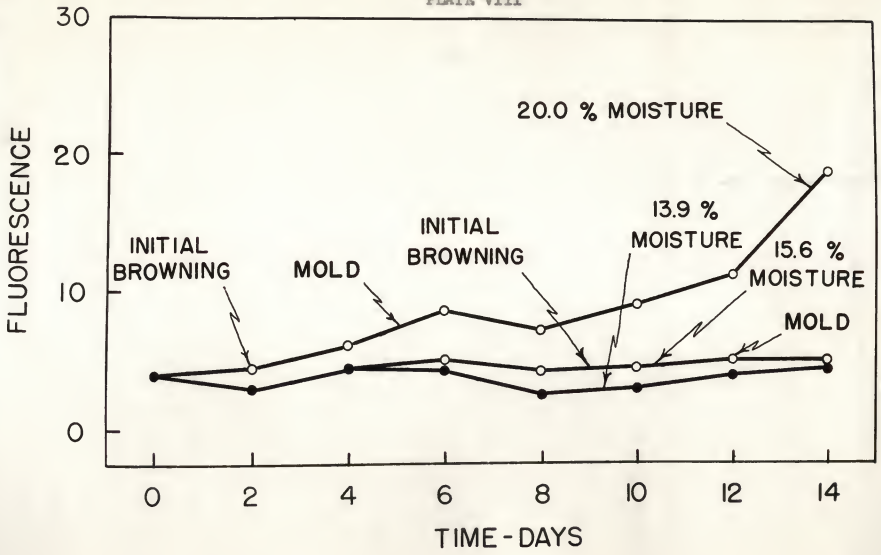
Storage of Wheat Germ in Respirometers at 40°C.

Duplicate sample pairs of granular wheat germ of three moisture levels were prepared and were stored in the respiration apparatus at 40°C. for twenty-one days. As in the previous

EXPLANATION OF PLATE VIII

Change in fluorescence of extracts of germ stored at various moisture contents at 30°C.

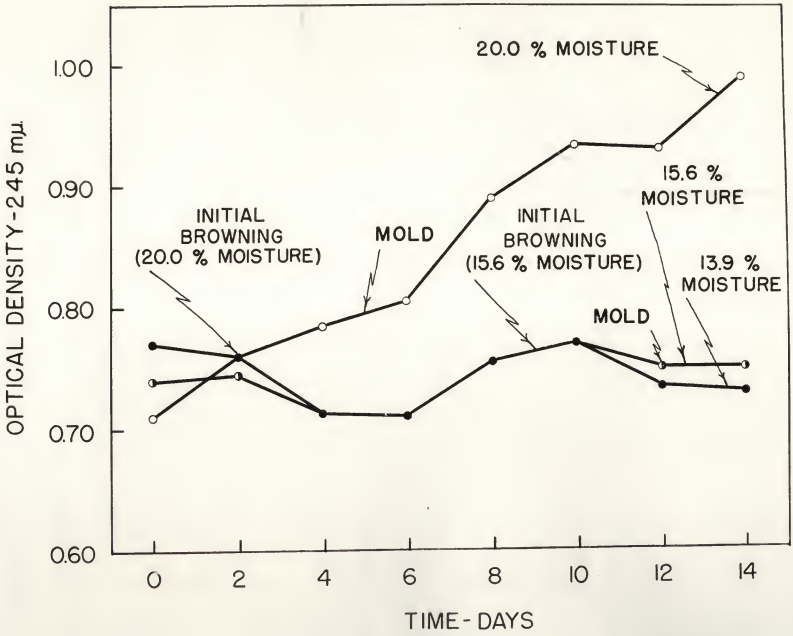
PLATE VIII


30
20
10
0
FLUORESCENCE
20.0 % MOISTURE
INITIAL BROWNING
MOLD
INITIAL BROWNING
13.9 % MOISTURE
15.6 % MOISTURE
MOLD
0
2
4
6
8
10
12
14
TIME - DAYS

EXPLANATION OF PLATE IX

Change in optical density of extracts of germ stored at various moisture contents at 30°C.

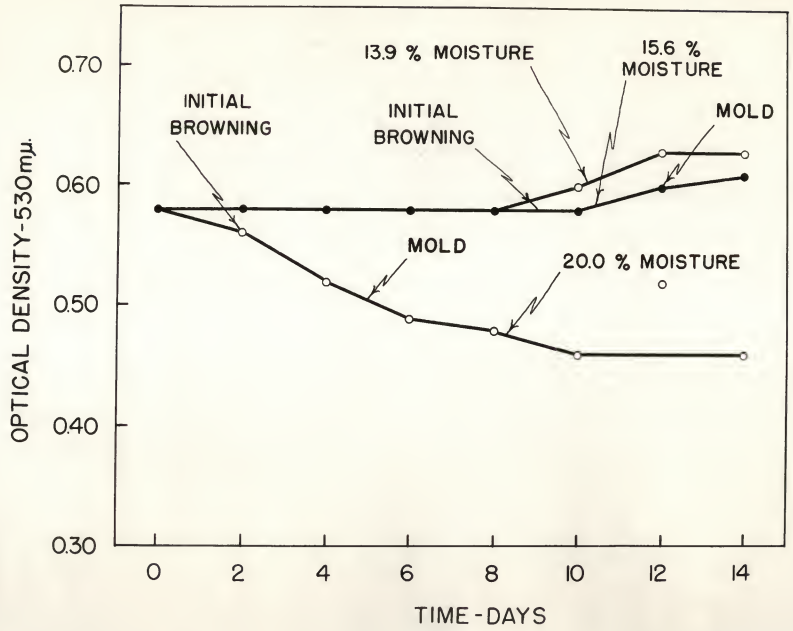
PLATE IX


20.0 % MOISTURE
15.6 % MOISTURE
13.9 % MOISTURE
INITIAL BROWNING (20.0 % MOISTURE)
MOLD
INITIAL BROWNING (15.6 % MOISTURE)
MOLD
OPTICAL DENSITY-245 mμ
1.00
0.90
0.80
0.70
0.60
0
2
4
6
8
10
12
14
TIME-DAYS

EXPLANATION OF PLATE X

Influence of storage time on optical density of peptized protein suspensions of granular wheat germ at various moisture contents at 30 C.

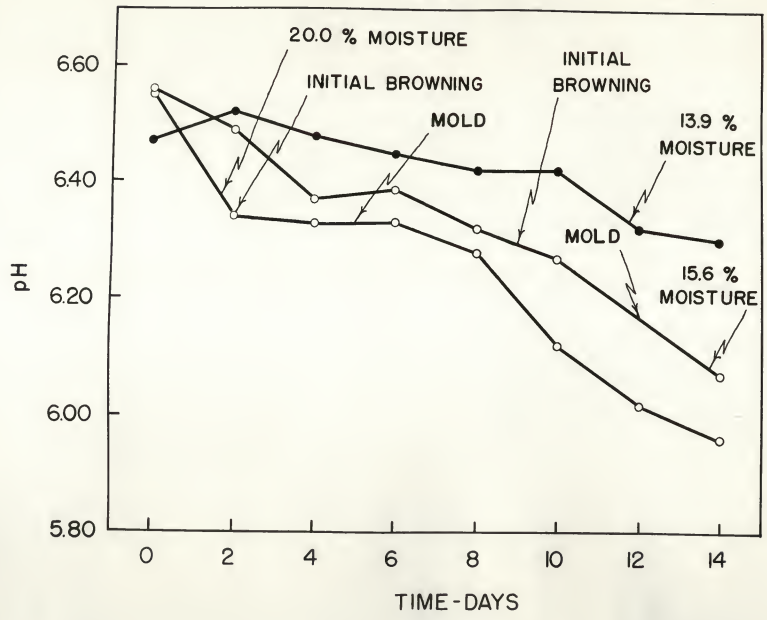
PLATE X


0.70
0.60
0.50
0.40
0.30
OPTICAL DENSITY-530mμ.
INITIAL BROWNING
INITIAL BROWNING
13.9 % MOISTURE
15.6 % MOISTURE
MOLD
MOLD
20.0 % MOISTURE
0
2
4
6
8
10
12
14
TIME-DAYS

EXPLANATION OF PLATE XI

Change in pH of granular wheat germ at various moisture contents at 30°C.

PLATE XI


20.0 % MOISTURE
INITIAL BROWNING
MOLD
INITIAL BROWNING
13.9 % MOISTURE
MOLD
15.6 % MOISTURE
pH
6.60
6.40
6.20
6.00
5.80
0
2
4
6
8
10
12
14
TIME - DAYS

storage experiment at 30 C., aliquots were removed from three samples at various time intervals while gas exchange data were determined on the remaining three samples.

The initial rate of carbon dioxide evolution of the sample of 20.4 percent moisture as shown in Fig. 1, was very high on the first day, and dropped rapidly during the next three days. The rate of oxygen absorption by this sample was also high on the first day and dropped rapidly during the next two days before leveling off. The R. Q. values of this sample as shown in Fig. 2, rose from 1.8 on the first day to 2.9 on the third day, and then dropped during the next three days before leveling off at an abnormally low value of about 0.5. The initial phenomenon of decline in rate of carbon dioxide evolution was also noticed, but to a much lesser extent, in the other two samples of 16.4 percent and 13.9 percent moistures. The rate of oxygen absorption of the sample of 16.4 percent moisture rose on the second day, and then declined during the next three days before leveling off. The rate of oxygen absorption by the lowest moisture sample remained relatively constant during the experiment. The R. Q. of the 16.4 percent sample (Fig. 2) was 1.0 on the first day, and leveled off on the second day at about 0.5 while the R. Q. values of the 13.9 percent moisture samples was 4.5 on the first day, then declined for six days before leveling off at about 0.4

The temperature of the experiment, 40°C., appeared to be unfavorable for the growth of molds. However, molds finally did begin to grow on the sample of 16.4 percent moisture on the

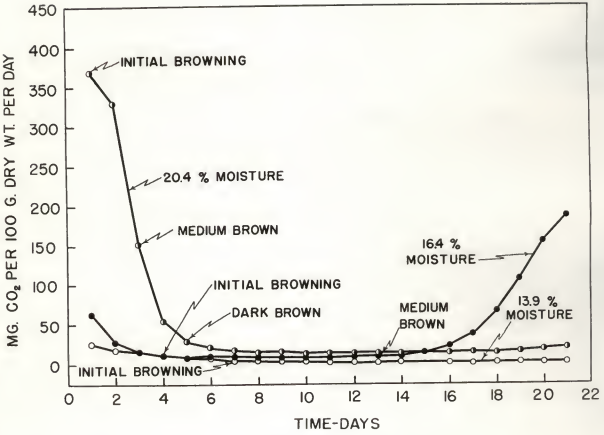


Fig. 1. Carbon dioxide production from granular wheat germ at various moisture contents at 40 C.

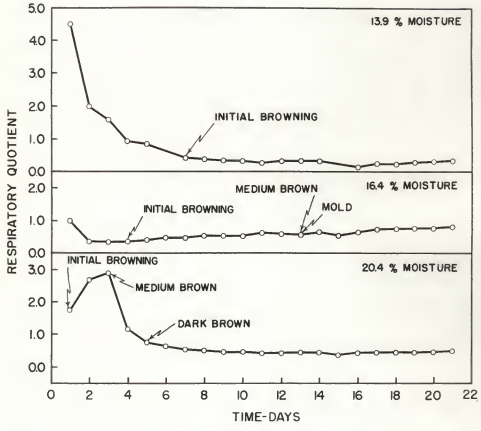


Fig. 2. Respiratory quotient values of granular wheat germ at various moisture contents at 40°C.

thirteenth day, as indicated by the upturn of the respiration curve, and on the 20.4 percent moisture sample on the eighteenth day. On both samples of 16.4 percent moisture, visible mycelia was all concentrated on the side of the flask facing the light as shown in Fig. 3. Grey, white, and green molds were growing on these two samples when the experiment was terminated.

The fluorescence increased very little until after the wheat germ had lost its yellow color and had begun to brown as shown by Fig. 4. As the wheat germ turned to a darker brown the fluorescence increased, and the samples of the higher moisture levels darkened the fastest and gave the highest fluorescence values. Browning and fluorescence increases were well advanced before any mold growth appeared on the samples. The light absorption of the extracts of all three samples as shown in Fig. 5, intermittently increased during the experiment. The irregular course of the optical density increase could not be accounted for as errors in the procedure since the fluorescence of the same extracts was always increasing. At the end of the run fluorescence determinations were made on very moldy and apparently non-moldy portions of wheat germ obtained from the same flasks containing the two samples with 16.4 percent moisture. The moldy samples were from 5 to 10 fluorescence units higher than the corresponding non-moldy germ. This indicated that mold growth may have slightly accelerated the browning of the wheat germ. The peptizable protein as shown in Plate XII of the three samples

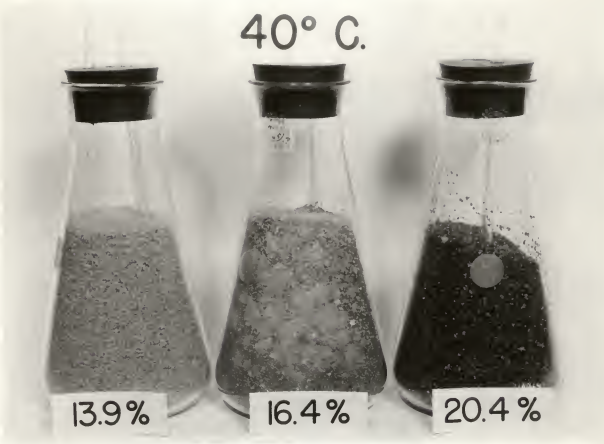


Fig. 3. Wheat germ samples of various moistures content after twenty one days of storage in respirometers at 40°C.

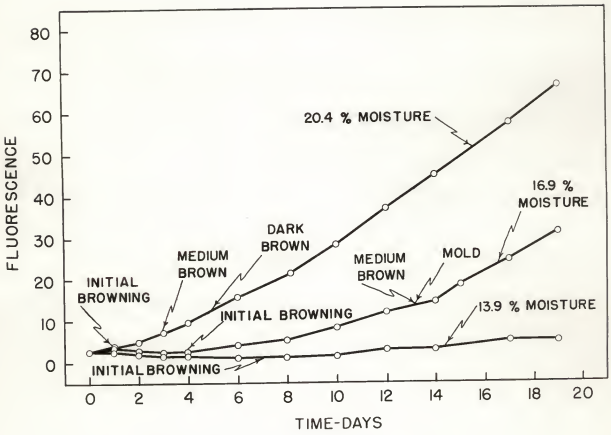


Fig. 4. Change in fluorescence of extracts of germ stored at various moisture contents at 40 C.

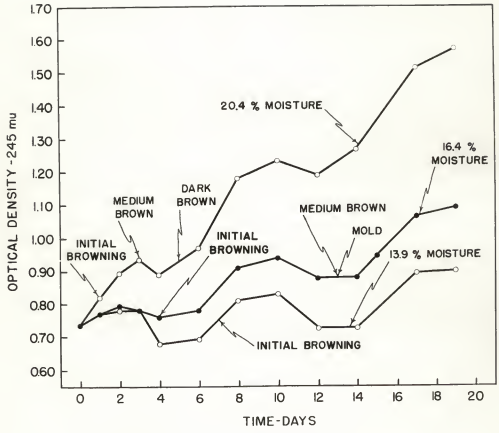
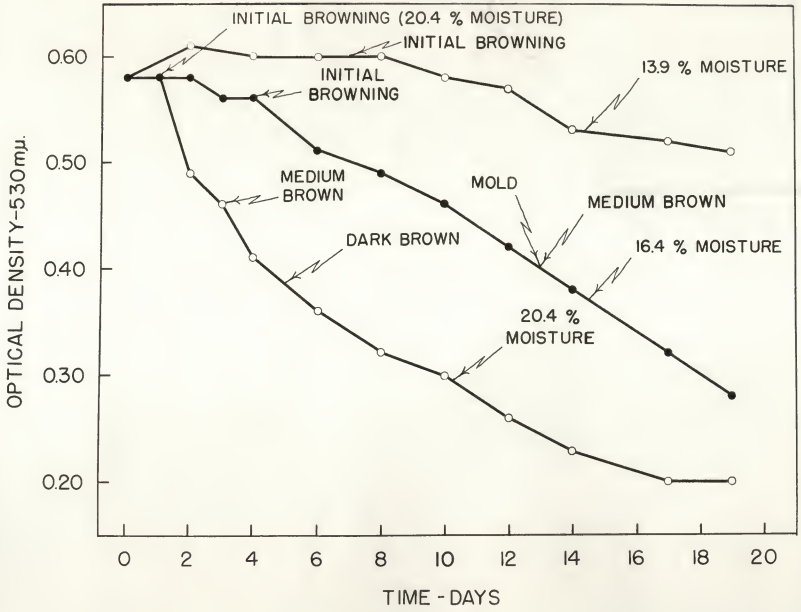


Fig. 5. Change in optical density of germ stored at various moisture contents at 40°C.

EXPLANATION OF PLATE XII

Influence of storage time on optical density of protein suspensions from granular wheat germ samples of various moisture content at 40°C.

PLATE XII


INITIAL BROWNING (20.4 % MOISTURE)
INITIAL BROWNING
13.9 % MOISTURE
INITIAL BROWNING
MEDIUM BROWN
MOLD
MEDIUM BROWN
16.4 % MOISTURE
DARK BROWN
20.4 % MOISTURE
OPTICAL DENSITY-530mμ.
0.60
0.50
0.40
0.30
0.20
0
2
4
6
8
10
12
14
16
18
20
TIME - DAYS

decreased in an analogous manner to their increase in fluorescence and optical density.

The pH of all three samples of 13.9 percent, 16.4 percent, and 20.4 percent moisture content decreased gradually as shown in Plate XIII in a somewhat similar manner during the twenty-one day storage period. Mold growth did not appear to greatly influence the pH trend in the 16.4 percent moisture sample. A total decrease of almost one pH unit occurred during the storage experiment.

Storage of Wheat Germ in Respirometers at 52°C.

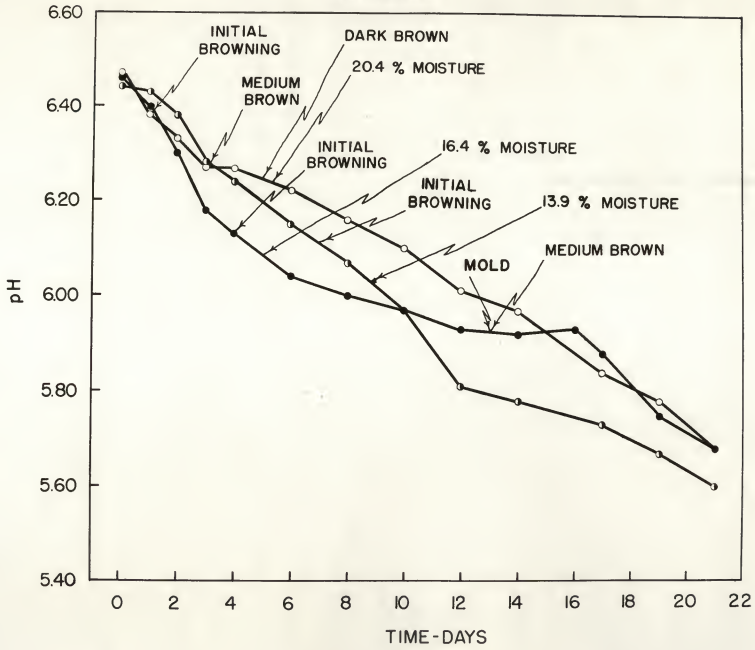
Six granular wheat germ samples of various moistures were stored for eleven days in respirometers at 52°C. The carbon dioxide evolution only was determined during the experiment. The initial rate of carbon dioxide evolution as shown in Plate XIV was extremely high for the sample of 19.6 percent moisture and then dropped rapidly during the next three days. A similar but much smaller drop in rate of carbon dioxide evolution was obtained for all the samples except that of lowest moisture content (10.6 percent) whose respiration rate was low and constant during the entire experiment. As indicated by the respiration curves, molds did not grow on any of the samples during the experiment.

The fluorescence and optical density of extracts of the wheat germ samples after the storage experiment are shown in

EXPLANATION OF PLATE XIII

Change in pH of granular wheat germ at various moisture contents at 40°C.

PLATE XIII


6.60
6.40
6.20
6.00
5.80
5.60
5.40
pH
0
2
4
6
8
10
12
14
16
18
20
22
TIME-DAYS
INITIAL BROWNING
DARK BROWN
20.4 % MOISTURE
MEDIUM BROWN
INITIAL BROWNING
16.4 % MOISTURE
INITIAL BROWNING
13.9 % MOISTURE
MOLD
MEDIUM BROWN

EXPLANATION OF PLATE XIV

Carbon dioxide production from granular wheat germ stored at various moisture contents at 52°C.

PLATE XIV

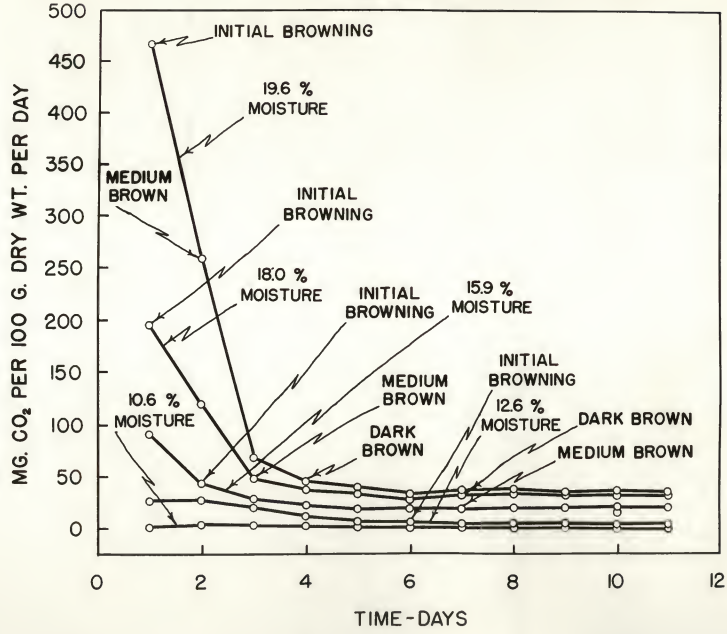

MG. CO_2 PER 100 G. DRY WT. PER DAY
500
450
400
350
300
250
200
150
100
50
0
0
2
4
6
8
10
12
TIME - DAYS
INITIAL BROWNING
19.6 % MOISTURE
MEDIUM BROWN
INITIAL BROWNING
18.0 % MOISTURE
INITIAL BROWNING
15.9 % MOISTURE
INITIAL BROWNING
MEDIUM BROWN
10.6 % MOISTURE
12.6 % MOISTURE
DARK BROWN
DARK BROWN
MEDIUM BROWN

Table 2. As seen from Table 2, the amount of browning that occurred at 52°C. was markedly influenced by the moisture content of the wheat germ sample.

Table 2. Fluorescence and optical densities of wheat germ at various moisture contents stored for 11 days at 52°C.

	Moisture Content of Sample					
	10.6	12.6	14.4	15.9	18.0	19.6
Fluorescence	5.0	10.7	48.1	69.2	116	133
Optical Density (245 mu)	0.76	0.80	1.01	1.21	1.89	2.14

Sodium Bisulfite Inhibition of Browning During Storage of Wheat Germ in Respirometers at 40°C.

According to Hodge and Rist (10), one of the characteristics of non-enzymatic browning is its inhibition by sodium bisulfite. Wheat germ samples were tempered with distilled water and sodium bisulfite solutions in such a manner that the resulting samples contained zero, one, and five percent sodium bisulfite on the basis of the dry weight of germ. An attempt was made to temper all samples to 18.5 percent moisture (on the as is basis after the weight of sodium bisulfite is subtracted). The 5 percent sodium bisulfite sample contained 19.5 percent moisture while the other two samples contained the desired moisture content of 18.5 percent.

Duplicate sample pairs of the three sodium bisulfite levels were placed in the respiration apparatus at 40°C. In the course of the experiment aliquots from one sample of each sodium bisulfite level were removed at various time intervals for fluorescence, optical density, and peptizable protein measurements. Gas exchange data were obtained daily on the remaining three samples.

The browning in these wheat germ samples was completely inhibited by 5 percent sodium bisulfite, as shown in Fig. 6, and delayed for one day by 1 percent bisulfite.

The phenomenon of high initial carbon dioxide evolution on the addition of water to wheat germ, which appears in normal germ, was greatly inhibited by the addition of sodium bisulfite as shown in Plate XV. The evolution of carbon dioxide from the untreated sample started at a high level and dropped for six days before again rising due to the onset of mold growth. The rates of carbon dioxide evolution from the 1 percent sodium bisulfite sample which started at a relatively high value and the 5 percent sodium bisulfite sample which started at a low level dropped for three days before leveling off at low almost identical rates. The initial rate of oxygen absorption was little effected by the presence of sodium bisulfite, as is shown in Plate XVI. The initial rate of oxygen absorption of the 5 percent sodium bisulfite sample of the higher moisture content, 19.5 percent, was higher than the other two samples of 18.5 percent moistures. The rate of oxygen absorption of all three samples dropped for five days before leveling off on the sixth day. After leveling

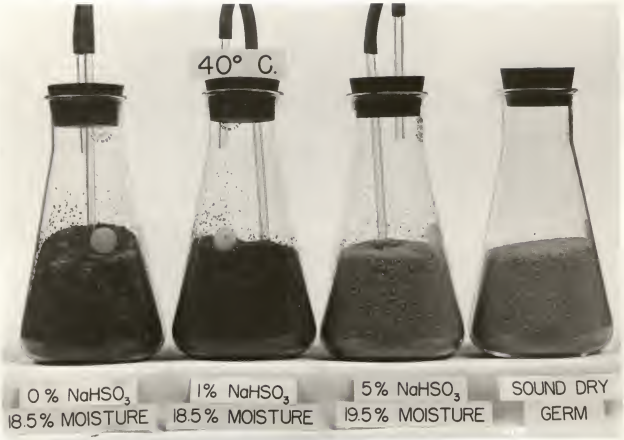
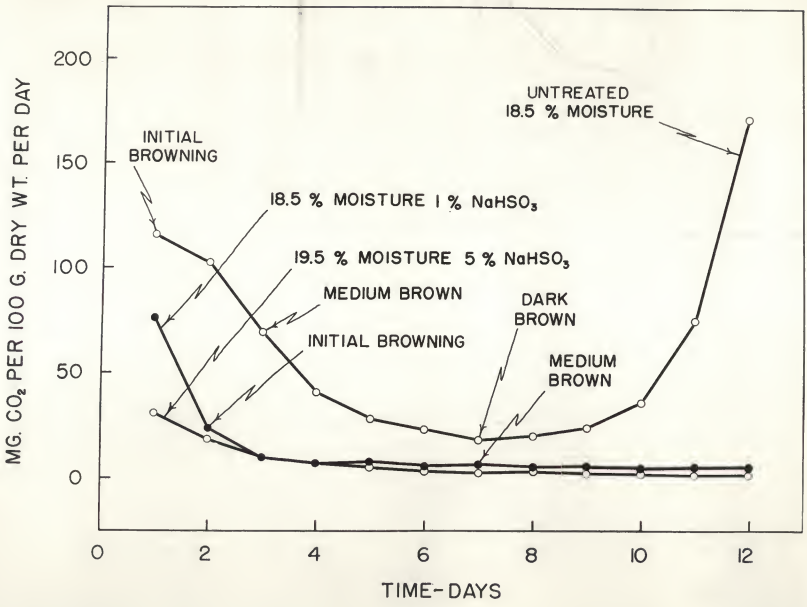


Fig. 6. Wheat germ samples containing various quantities of sodium bisulfite after twelve days of storage at 40°C.

EXPLANATION OF PLATE XV

Carbon dioxide production from granular wheat germ containing various quantities of sodium bisulfite and stored at 40°C.

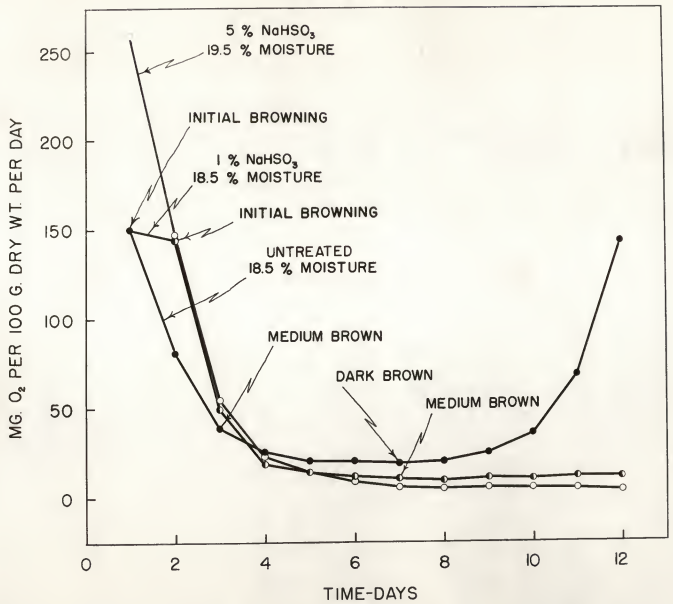
PLATE XV


MG. CO_2 PER 100 G. DRY WT. PER DAY
200
150
100
50
0
0
2
4
6
8
10
12
TIME-DAYS
UNTREATED
18.5 % MOISTURE
INITIAL
BROWNING
18.5 % MOISTURE 1 % $NaHSO_3$
19.5 % MOISTURE 5 % $NaHSO_3$
MEDIUM BROWN
DARK
BROWN
INITIAL BROWNING
MEDIUM
BROWN

EXPLANATIONS OF PLATE XVI

Oxygen absorption at 40°C. by granular wheat germ containing various quantities of sodium bisulfite.

PLATE XVI


5 % NaHSO3
19.5 % MOISTURE
INITIAL BROWNING
1 % NaHSO3
18.5 % MOISTURE
INITIAL BROWNING
UNTREATED
18.5 % MOISTURE
MEDIUM BROWN
DARK BROWN
MEDIUM BROWN
MG. O2 PER 100 G. DRY WT. PER DAY
250
200
150
100
50
0
0
2
4
6
8
10
12
TIME-DAYS

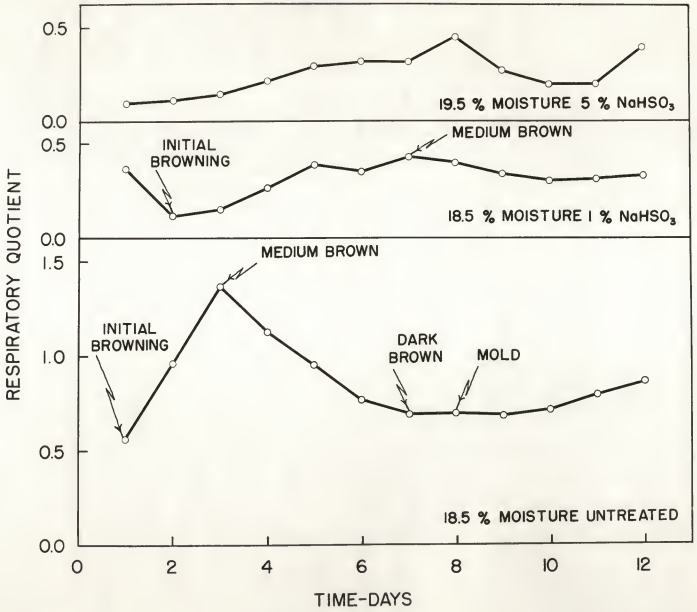
off the rate of oxygen absorption was slightly inhibited by the bisulfite. The rate of oxygen absorption of the untreated sample began increasing on the seventh day because of mold growth. The R. Q. values of the samples containing sodium bisulfite as shown in Plate XVII, started at abnormally low values of 0.4 for the 1 percent sodium bisulfite sample and .09 for the 5 percent sodium bisulfite sample. The values rose a little during the experiment but were abnormally low during the entire twelve days of the storage experiment. The control, which contained no sodium bisulfite, showed low initial R. Q. value of 0.6 which rose for two days to an abnormally high value of 1.3, and then dropped for three days before leveling off at about 0.7. The R. Q. of this sample began to rise on the ninth day because of mold growth.

The fluorescence of extracts of the untreated and 1 percent sodium bisulfite-treated samples increased gradually as shown in Plate XVIII during the experiment at an almost identical rate, whereas the fluorescence of extracts of the 5 percent sodium bisulfite treated germ did not increase. The optical density of acid extracts of the untreated and 1 percent sodium bisulfite samples as shown in Plate XIX, increased gradually in a somewhat intermittent pattern during the storage period, while the optical density of the extracts of the 5 percent sodium bisulfite increased very little. The increase in optical density of the untreated sample extracts was about twice the increase obtained

EXPLANATION OF PLATE XVII

Respiratory quotient values at 40°C. of granular wheat germ containing various quantities of sodium bisulfite.

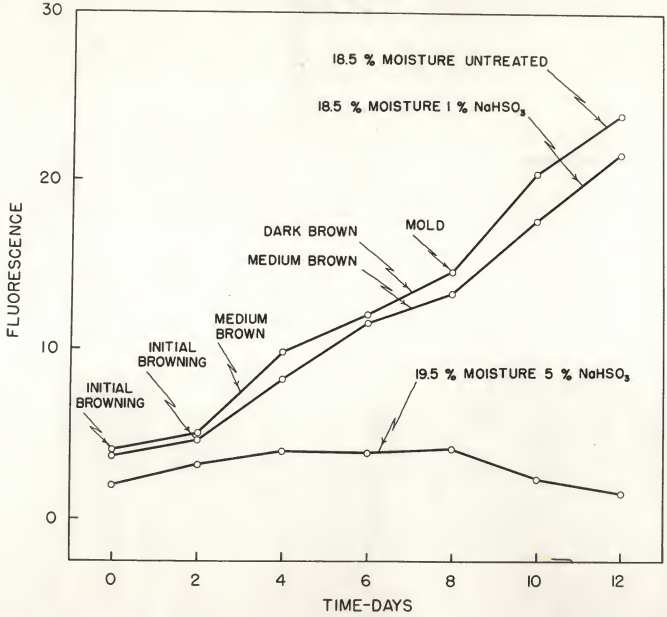
PLATE XVII


RESPIRATORY QUOTIENT
TIME-DAYS
19.5 % MOISTURE 5 % NaHSO3
18.5 % MOISTURE 1 % NaHSO3
18.5 % MOISTURE UNTREATED
INITIAL BROWNING
MEDIUM BROWN
INITIAL BROWNING
MEDIUM BROWN
DARK BROWN
MOLD
0.0
0.5
1.0
1.5
0
2
4
6
8
10
12

EXPLANATION OF PLATE XVIII

Change in fluorescence at 40°C. of extracts of wheat germ containing various quantities of sodium bisulfite.

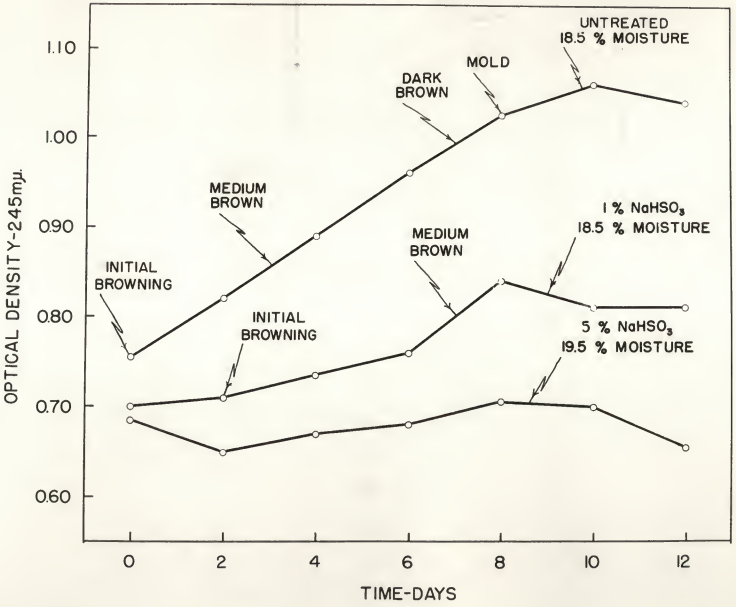
PLATE XVIII


30
20
10
0
FLUORESCENCE
18.5 % MOISTURE UNTREATED
18.5 % MOISTURE 1 % NaHSO3
DARK BROWN
MOLD
MEDIUM BROWN
MEDIUM BROWN
INITIAL BROWNING
INITIAL BROWNING
19.5 % MOISTURE 5 % NaHSO3
0
2
4
6
8
10
12
TIME-DAYS

EXPLANATION OF PLATE XIX

Change in optical density at 40°C. of extracts of wheat germ containing various quantities of sodium bisulfite.

PLATE XIX

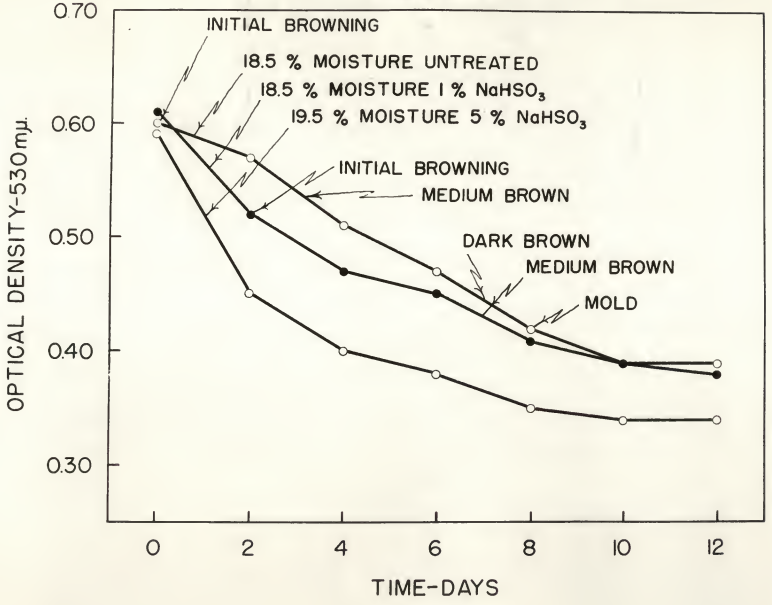

UNTREATED
18.5 % MOISTURE
MOLD
DARK
BROWN
MEDIUM
BROWN
INITIAL
BROWNING
1 % NaHSO3
18.5 % MOISTURE
MEDIUM
BROWN
INITIAL
BROWNING
5 % NaHSO3
19.5 % MOISTURE
OPTICAL DENSITY-245mμ.
1.10
1.00
0.90
0.80
0.70
0.60
0
2
4
6
8
10
12
TIME-DAYS

with the 1 percent sodium bisulfite sample. The decrease in peptizable protein as shown in Plate XX, followed an unexpected pattern as the decrease appeared to be independent of the presence of sodium bisulfite and dependent upon the amount of moisture present. The rate of decrease of the peptizable protein of the untreated and 1 percent sodium bisulfite samples was almost identical, while the rate of decrease of the 5 percent sodium bisulfite sample which is higher in moisture content, was slightly greater. Determination of nitrogen soluble in 1 N. trichloroacetic acid in these germ samples by the Kjeldahl method showed that only an insignificant change in soluble nitrogen had occurred even though peptizable nitrogen had decreased in the 5 percent sodium bisulfite sample without producing a brown color in the germ. Thus amino acids and short chain peptides which may have been produced by hydrolysis of the protein, had not accumulated in this sample during storage.

EXPLANATION OF PLATE XX

Influence of storage time on optical density of peptized protein suspensions at 40°C. of granular wheat germ containing various quantities of sodium bisulfite.

PLATE XX


0.70
0.60
0.50
0.40
0.30
OPTICAL DENSITY-530mμ.
0
2
4
6
8
10
12
TIME-DAYS
INITIAL BROWNING
18.5 % MOISTURE UNTREATED
18.5 % MOISTURE 1 % NaHSO3
19.5 % MOISTURE 5 % NaHSO3
INITIAL BROWNING
MEDIUM BROWN
DARK BROWN
MEDIUM BROWN
MOLD

DISCUSSION AND CONCLUSIONS

Some evidence obtained in the storage experiment with autoclaved germ suggested that mold growth was related to browning. These results were not entirely conclusive since browning associated with mold growth in germ which has undergone drastic physical and chemical changes during autoclaving, may not be the same as that occurring on untreated granular wheat germ. Subsequent evidence indicated that browning of natural germ is not related to mold growth.

Negative results obtained for phenol oxidase activity suggest that the brown pigment was probably not due to enzymatic causes. Additional evidence that the normal brown pigment of deteriorated wheat germ was not produced by oxidizing enzymes is the fact that it was shown to be largely water soluble. This property is not a characteristic of the melanin pigments produced by some plant oxidases.

Good evidence has been presented indicating that the brown pigment formed in wheat germ is the result of a non-enzymatic browning reaction between reducing sugars and nitrogenous compounds. Since Cole and Milner (4) have shown that browning in commercial germ as well as in germs of intact wheat kernels are apparently identical, it is suggested that the deterioration in stored wheat known as "sick" wheat is as far as the color of the germ is concerned, a non-enzymatic browning reaction of the Maillard type.

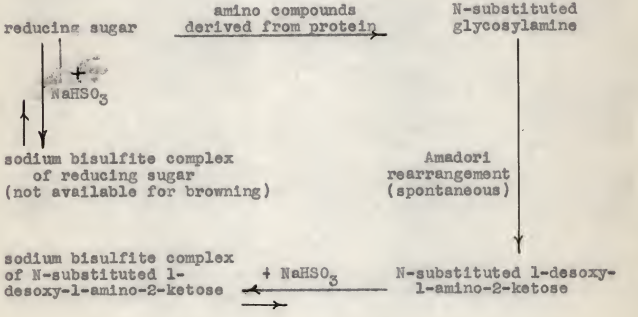
Storage studies have shown that the normal browning occurring in wheat germ is greatly retarded when the water soluble fraction of the germ is removed. Reducing sugars which are water soluble are involved in the non-enzymatic formation of brown colors in many food products. Wheat germ contains high percentages of the free sugars sucrose and raffinose which are water soluble and may be hydrolyzed by mold or wheat germ enzymes to form reducing sugars. Some reducing sugars are already available, as DuBois (6) has recently shown that sound wheat germ contains small amounts of glucose and fructose.

The present work indicates that the rate of increase in fluorescence and optical density of extracts of wheat germ samples coincident with browning during storage increases with corresponding increases in temperature and moisture. This is to be expected since at higher moisture values the solubility and mobility of the reactants become greater and at higher temperatures the rates of chemical reactions increase.

A decrease in peptizable protein has been shown to take place during the normal browning of wheat germ. This decrease may mean one or more of three things are occurring. In the course of the browning reaction the protein may have been denatured, hydrolyzed or combined with other compounds to form non-peptizable nitrogen containing compounds. Since the decreases in peptizable protein followed a pattern analogous to the increases of fluorescence and light absorption, which are characteristic properties of non-enzymatic browning, the protein was probably used

intact, or indirectly as amino acids or peptides, in the formation of the brown pigment.

The development of the brown substance in wheat germ during storage under unfavorable conditions was completely inhibited by the addition of 5 percent sodium bisulfite. During storage the fluorescence and optical density of extracts of a sample so treated did not increase as is normal for untreated germ, but the peptizable protein of the germ nevertheless did decrease. The fact that brown colors did not appear and yet peptizable protein did decrease might be explained by resorting to the mechanism of non-enzymatic browning proposed by Hodge and Rist (10) as follows:



Most of the aldehyde or ketone reducing groups of the sugars or their derivatives are inactivated by the formation of their sodium bisulfite complexes. However, small amounts of reducing

sugars are free in the equilibrium reaction with sodium bisulfite, and these sugars can react with proteins or related amino compounds. The N-substituted glycosylamine produced would spontaneously rearrange to nitrogen-containing amino ketoses, and these compounds would then accumulate as the sodium bisulfite complex. By such a mechanism non-peptizable protein would decrease, but dark brown compounds whose extracts exhibit fluorescence and light absorption properties would not be produced because of the bisulfite complexing of intermediate compounds required to form the brown fluorescent materials.

As demonstrated in Plate VI and Fig. 1, mold growth, as indicated by respiratory acceleration, always appeared after browning even at temperatures, moistures, and storage periods favorable to mold growth. Also, browning occurred before mold growth on three other wheat germ samples during a storage experiment (which is not presented in this dissertation) at 31°C. in respirometers. This may indicate that some mold inhibitory material in the germ or its biological source is destroyed during the initial stages of browning. Thus mold growth in the germs of intact wheat kernels during commercial storage may occur very soon after the initial onset of the "sick" wheat condition. It is also conceivable that the browning reaction is associated with the loss of viability, and consequent chemical dearrangement of the embryo cells which would favor the germination and growth of the ever-present saprophytic fungal spores.

The onset of mold growth during browning of wheat germ did

not appear to greatly increase the rate of browning. This is shown by the fluorescence curve of the sample containing 16.4 percent moisture stored at 40°C. (Fig. 4). However mold growth appeared to have accelerated slightly the browning in this sample as indicated by the fact that very moldy wheat germ taken from the same sample flask as slightly moldy germ gave a somewhat higher fluorescence value. On the other hand any increase in browning may have been due to an increase in moisture content caused by the respiration of the molds.

During the storage experiments with freshly wetted germ an unusual phenomenon appeared in which the carbon dioxide evolution started at an unusually high rate and then declined for three to five days before leveling off. This initial carbon dioxide evolution, which has been reported in literature for other seeds (7) (16) (23), was greatly inhibited by sodium bisulfite. A similar high initial rate of oxygen uptake was also noticed, but this oxygen absorption was not inhibited by sodium bisulfite. This may indicate that the high oxygen uptake was independent of the simultaneous high carbon dioxide evolution. It may be conceived that the source of the initial elevated carbon dioxide production was a carboxylic acid normally produced in the germ cells from aldehyde or ketone intermediates or precursors. Complexing of these intermediates by the bisulfite would therefore inhibit the production of the carboxylic acid which is normally decarboxylated to yield the carbon dioxide.

The results obtained in the hygroscopicity study showed that

the rate of absorption or evaporation of water by granular wheat germ was greater than that for intact wheat seeds. Since usually the bran coat of wheat is thin or even broken at the germ end of the kernel, under practical storage conditions when humidities are high, moisture from the surrounding air could be absorbed via the germ into the wheat kernel. Wheat kernels with broken bran coats over their germ ends would probably absorb moisture much faster than those with intact bran coats. This might account for the fact that in many "sick" wheat samples only part of the wheat kernels, which may have been of higher moisture contents due to broken bran coats, are germ damaged.

SUMMARY

Experiments with wheat germ were carried out to study the fundamental chemical and physical changes related to the germ deterioration of wheat kernels in storage known in the grain trade as "sick" wheat. The effects of moisture content, temperature, and mold growth during storage on the rate of browning of wheat germ was also investigated. The following observations were made:

1. Browning in damp wheat germ was accompanied by increases in fluorescence and optical density of acid extracts of the germ and by decreases in peptizable protein. The changes in these properties were used to follow the rate of browning in wheat germ.

2. During storage the rate of browning of the germ was

increased by increases in moisture and temperature.

3. Mold growth accelerated only slightly the browning rate in wheat germ. This was shown by the difference in fluorescence of extracts of very moldy and slightly moldy wheat germ from the same sample flask.

4. The browning of wheat germ was inhibited by extraction of the sugars or addition of sodium bisulfite. The bisulfite also inhibited the increase in fluorescence and optical density of extracts of the germ but did not inhibit the characteristic decrease in peptizable protein.

5. An unusual phenomenon was observed in that the initial evolution of carbon dioxide and absorption of oxygen were abnormally high after wetting of the germ. These initial high rates were increased by increases in moisture and temperature. The addition of sodium bisulfite to the germ sample inhibited the high initial carbon dioxide evolution but did not effect the initial high rate of oxygen absorption.

6. The critical moisture content, that is the moisture at equilibrium with 75 percent humidity, was found to be 13.2 percent for whole granular wheat germ and 13.5 percent for Kiowa wheat seeds. The rate of loss or gain of water was found to be greater with granular wheat germ than with intact Kiowa wheat seeds.

The results strongly indicate that a non-enzymatic browning reaction of the Maillard type occurs in wheat germ, and that the darkening associated with deterioration of the germs in commercial

grain referred to as "sick" wheat is also due to the same reaction.

ACKNOWLEDGMENTS

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APPENDIX

SUGGESTIONS FOR FUTURE RESEARCH

The effects of "sick" wheat on the baking qualities of the flour should be determined. The fundamental effects could first be studied by the addition to the baking formula of wheat germ that has been browned in respirometers with and without mold growth. If any effect is noticed then the influence of the constituents of the germ, such as browning products, oxidized fats, glutathione, etc., should be studied. The browning products could conceivably be isolated or obtained synthetically by allowing sugars and proteins isolated from wheat germ to react together.

After the constituents causing the bad effect are known, samples of wheat whose moisture and mold history are known could be allowed to proceed to various degrees of "sickness" in respirometers. By variations in the storage temperatures germ damaged wheat samples should be produced in the presence and absence of mold growth. Germs isolated from these "sick" wheat samples would be added to the baking formula. Also baking tests should be run on flour obtained by experimental milling of these samples. The validity of these results on wheat under normal commercial storage conditions could be checked by determining the baking qualities of numerous commercial "sick" wheat samples.

A study should be conducted to determine how deteriorated germs of "sick" wheat get into the flour during milling. The brittleness and friability of granular wheat germ samples of various degrees of browning should be determined by standardized shaking procedures. The amount of germ contaminating flour samples should be determined on the flours previously obtained

for baking tests by experimental milling of "sick" wheat samples of various degrees of deterioration.

The determination of the causes of the high initial carbon dioxide evolution and oxygen absorption observed on wetting of wheat germ represents a very interesting research problem. The source and mechanism of these gas exchange properties might be determined by chromatography and other qualitative organic techniques.

BROWNING OF WHEAT GERM IN RELATION TO SICK WHEAT

by

CLARENCE E. MCDONALD

B. A., McPherson College, 1950

AN ABSTRACT OF A THESIS

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Large financial losses are suffered each year because of a storage deterioration known in the grain trade as "sick" wheat. "Sick" or germ damaged wheat is that type of deteriorated grain in which the seed loses its viability and the germ darkens in color and becomes rancid. The present study was carried out to determine the fundamental causes and characteristics of this type of germ deterioration.

Since Cole has shown that the browning in commercial granular wheat germ and in germs of intact wheat kernels is apparently identical, these studies were conducted with commercial granular wheat germ.

Preliminary storage studies showed that autoclaving of wheat germ and storing under sterile conditions greatly inhibited browning, but did not completely stop it. This suggested that the discoloration may be due to enzymatic causes. Determination of catechol oxidase activity, which could be responsible for enzymatic browning, showed however that little if any of this enzyme is present in wheat germ.

The removal of water soluble substances, including sugars, from wheat germ greatly retarded the rate of browning during storage while the removal of the ether soluble substances did not retard browning. This indicated the browning reaction may be a non-enzymatic one between sugars and amino compounds.

Hygroscopic equilibria studies were carried out on wheat germ , since this information is not in the literature, in order to carry out controlled storage experiments in which the humidities

around the samples are in equilibrium with the moistures of the samples. Also the hygroscopic characteristics of wheat seeds were determined. The critical moisture content, that is the moisture at equilibrium with 75 percent humidity, above which molds can grow, was found to be 13.2 percent for whole granular wheat germ and 13.5 percent for whole Kiowa wheat seeds. These critical moistures are about 1 percent lower then those obtained for wheat by other writers.

Storage experiments in respirometers, in which the temperature aeration, and humidity were controlled, were carried out with granular wheat germ samples. The rate of browning in the germ was followed by increases in fluorescence and optical density of extracts of the germ, by darkening of the color of the germ, and by decreases in the peptizable protein of the germ samples. Also the rate of carbon dioxide evolution and oxygen absorption, and changes in the pH of the wheat germ samples were determined during the storage experiments.

The rate of browning of wheat germ was shown to increase with corresponding increases in temperature and moisture. Browning of germ in the absence of mold growth was characterized by low and constant carbon dioxide output and with respiratory quotient values below unity. This phase followed a short preliminary period of elevated carbon dioxide production, oxygen absorption, and respiratory quotients, also not associated with fungal activity.

Sodium bisulfite inhibited browning in wheat germ. Fluores-

cence and optical density of extracts of germ, and carbon dioxide production were also inhibited while the oxygen absorption was not effected by the bisulfite.

The changes in pH of the germ samples during storage appeared to be independent of the rate of browning of the wheat germ samples.

Appearance of fungal growth in wheat germ is indicated by an upturn in carbon dioxide production and did not precede the initial formation of the brown color. Fungal growth on wheat germ appeared to only slightly increase the rate of browning.