A STUDY OF METHODS TO DETECT STORAGE DETERIORATION OF CORN AND WHEAT

by

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TABLE OF CONTENTS

INTRODUCTION

The importance of cereal grains in human nutrition is a well-known fact. To preserve their nutritional value, proper storage of cereal grains is of utmost importance. Dry grains may be easily stored for several years with only little decrease in quality. But modern combine harvesting, as well as unfavorable weather often results in an increased moisture content which, together with high summer temperatures, creates special problems in regard to grain storage. The need of storing surplus grains for extended periods particularly in the United States, has prompted questions on the effect of storage on the quality of cereal grains and their products.

It has been long recognized that the value of stored grain both for feed and industrial processing decreases in proportion to the physical, chemical and biological changes it undergoes during storage (Beleny, 1948). A series of tests have been developed, including the determination of moisture content, number and kinds of molds present, viability, germ damage, fat acidity and baking test, to predict storage behavior and the extent of actual damage to the stored grain. The moisture content is used to indicate present and/or future danger, mold count the presence and degree of fungal invasion taken place, viability developing incipient deterioration, germ damage and fat acidity the extent of actual damage and finally, test baking the possible decrease of baking quality.

All of the above tests have their limitations. In some cases the techniques involved may be inconvenient and time-consuming, yet often the accuracy to predict deterioration remains questionable. Hence, numerous attempts have been made to develop simpler and more reliable tests for this purpose. Recently, (Linko, 1960b; Linko and Sogn, 1960), glutamic acid decarboxylase

activity has been shown to be a good index of storage deterioration of wheat, and a simple and rapid method for its determination developed (Linko, 1961a). This method has now been extended to detect the degree of deterioration of artificially dried and stored corn.

Damage to wheat proteins indicated by a decrease in the activity of certain enzymes, in baking quality and in sedimentation value, among others, can not be detected by Kjeldahl nitrogen determination. Thus it was of interest to investigate if the determination of protein content by means of dye absorption techniques would depend on the degree of deterioration. For this purpose, several modifications of the techniques were developed and investigated in detail.

Finally the present study involves comparison of the various methods to detect degree of deterioration of wheat and corn during storage at various conditions, with a special emphasis on the determination of glutamic acid decarboxylase activity.

REVIEW OF LITERATURE

Factors That Influence the Chemical, Physical, Biological and Nutritive Changes During Storage of Cereal Grain

Moisture and Temperature. Of the various factors influencing the rate of deterioration of cereal grains in storage, moisture is by far the most important. Extensive basic work has been conducted (Bailey, 1917; Coleman and Fellows, 1925; Fenton and Swanson, 1930; Smith and Bartz, 1932; Swanson, 1934; Kuhl, 1940; Thomas, 1941; Kretovich, 1945; Semeniuk and Gilman, 1944) to establish a definite moisture limit below which various grains or grain products may be stored safely.

However, moisture content and temperature are closely interrelated with each other in their effects on grain deterioration. Moisture content which, in turn, is related to interstitial relative humidity of the grain, controls the metabolic processes, while temperature largely determines the rate of the reactions involved. Thus, grains at moisture levels unsafe for storage at summer temperatures may often be stored with little deterioration under artificial refrigeration or at similar temperatures prevailing in winter (Papavizas et al., 1957; Glass et al., 1959; Trisvyatskie, 1960).

Effect on Respiration and Heating. The respiratory rate of sound grain, at moisture levels in equilibrium with 75 percent relative humidity of less, is low and relatively constant, and is due mainly to biological processes of the seed itself (Milner and Geddes, 1946a). An increase in moisture content is followed by a gradual increase in respiratory rate, until a certain "critical" moisture level is reached (Bailey and Gurjar, 1918; Bailey, 1940; Kretovich and Ushakova, 1940; Bottomley and Christensen, 1952). At this level the respiratory rate increases rapidly, eventually resulting in heating of the grain. The "critical" moisture level generally assigned for wheat is 14.6 percent and for corn 13.7 percent (Snow, 1944; Tuite and Christensen, 1955; Papavizas and Christensen, 1957).

Respiration, regardless of its origin, depends upon chemical reactions, and is therefore accelerated by an increase in temperature until it may be retarded by factors such as thermal inactivation of the enzymes involved, exhaustion of the substrate or substrates, accumulation of inhibitory concentrations of carbon dioxide, etc. (Bailey and Gurjar, 1918; Milner and Geddes, 1945b, 1946a; Milner, Christensen and Geddes, 1947a, b). Generally, however, the respiratory rate of stored seeds increases with increasing moisture

content and temperature (Bailey and Gurjar, 1918; Milner, et al., 1947a, b; McDonald and Milner, 1954).

Effect on Carbohydrates. Earlier studies (Leavitt et al., 1909; Geddes, 1935; Bottomley et al., 1950) have shown that during storage of grain alpha- and beta-amylases attack starch granules converting starch to dextrins and maltose. The extent of such changes depends on the moisture content temperature. Montgomery and Smith (1956) postulated that the quantity of soluble carbohydrates in wheat is likely to depend on the highest moisture level to which the grain has been exposed. This theory has been supported by the studies with rice of Houston et al. (1957, 1959), and Fukui and Nikuni (1959). Similarly, Watanabe (1954) observed that the change in maltose value of stored wheat depends on temperature. Recently, Linko, Cheng and Milner (1960) showed with wheat embryos that non-reducing sugars decrease and reducing sugars increase before the moisture level necessary for germination is reached.

Täufel (1958) studied extensively the behavior of sound and sprouted wheat, rye, barley and oats during storage. He observed that sucrose decreased during germination but maltose increased both during germination and storage. On legume seeds, Täufel et al. (1960a) noticed that during one month of normal storage, there is practically no change in small molecular carbohydrates. But with high temperature and humidity the maltose of germinated soybeans completely disappears during storage. In their study with corn, Täufel et al. (1960b) found that the lower saccharides decreased in concentration during storage, the decrease becoming more pronounced as storage conditions became increasingly unfavorable.

Dubois, Geddes and Smith (1960) observed noticeable changes in the composition of sugars on exposing wheat embryos to water vapors for

approximately one day, during which time the moisture content increased from 9.2 to about 13.0 percent. Glass and Geddes (1960) found a marked increase in reducing sugars after storage of Marquis wheat for 24 weeks at 30°C at 18.0 percent moisture, whereas Linko, Cheng and Milner (1960) found little change in glucose and fructose of wheat germ stored eight days at 35°C below 18.0 percent moisture levels. It has been suggested (Linko, 1960d) that if water content does not reach the level necessary for the advanced state of germination, free amino acids, formed largely by proteolysis, would react with reducing carbohydrates to form brown pigments characteristic of damaged wheat embryos, thus retarding the accumulation of reducing sugars.

Effect on Fats. During storage fats may undergo either hydrolytic or oxidative changes. The latter are more pronounced in grains rich in oils and in milled products. Zeleny (1954) observed that hydrolysis of fats seemed to be more pronounced in storated grains than protein or carbohydrate hydrolysis. This was supported by the study of Kelly et al. (1942) on the behavior of wheat stored in experimental bins. He found that fat acidity increased and viability decreased long before the appearance of external physical evidence of deterioration. For this reason, the free fatty acid content of grain was judged to be a sensitive index of incipient deterioration. Baker et al. (1957, 1959) and Linko and Sogn (1960) obtained a high positive correlation between fat acidity and germ damage.

Extensive studies have demonstrated that fat acidity increases during storage of cereal grains at high moisture levels and temperatures, conditions favorable to general deterioration (Anon, 1929; Fenton and Swanson, 1930; Zeleny, 1940; Carter and Young, 1945; Milner, Christensen and Geddes, 1947a; Milner et al. 1947b; Christensen et al., 1949; Hunter et al., 1951; Vakar, 1948;

Auerman, 1959). The presence of microorganisms generally accelerates these hydrolytic changes because of their high lipolytic activity (Geddes, 1935; Kirsh, 1935; Goodman and Christensen, 1952; Loeb and Mayne, 1952; Dirks et al., 1955; Janicki, 1960).

Effect on Proteins and Enzymes. Although the protein content of grain as calculated from its nitrogen content is generally assumed to remain unchanged during storage, Shutt (1909, 1911) demonstrated a progressive though small increase in the protein content of wheat during extended storage. This increase in protein on a percentage basis is doubtless the result of a loss in carbohydrates by respiration. Under normal storage of grain, proteolysis proceeds at a slow rate which, however, rapidly increases as grain deteriorates (Zeleny and Coleman, 1938). Takahashi and Shirahama (1928) observed a marked increase in prolamine and a corresponding decrease in water-soluble proteins in barley during the early period of storage, suggesting that the water-soluble proteins would be involved in the formation of prolamine.

Jones et al., (1943) observed that after two years of storage, digestibility, palatability, and nutritive value of corn, wheat and soybean proteins decreased. Milled products suffered greater changes than whole grain with increasing storage time, temperature and moisture content of grain. It was also noted that the gluten of wheat suffers some changes during storage. Barton-Wright (1938) observed that deterioration of gluten quality during storage of flour, especially the low-grade flours, was found to depend on the unsaturated fatty acids resulting from the enzymatic hydrolysis of the flour fat. He found that during storage at room temperature of a 16.0 percent moisture flour, gluten quality decreased as the number of fungi increased. This is in agreement with the observation of Kozmin (1935) who found that

as the flour-aging process progresses, the washed gluten prepared from the flour becomes less extensible and more springy or elastic and, finally, granular and very easily torn. He further observed that removal of the fat from aged flour returned the gluten to its original condition.

Enzymes of grains are either activated or inactivated during storage depending upon the storage conditions. Being biological catalysts, the activation of the various enzyme systems in cereal grains depends on moisture, temperature and other storage conditions (Acker, 1959; Acker and Luck, 1959). Swanson (1935) was first to investigate the relationship between moisture content and enzyme activity in wheat. He observed an increase in amylase activity only after the grain reached 27.0 percent moisture content. He concluded that 27 to 30 percent moisture content is enough to initiate activation and/or secretion of amylase, but that 40 percent moisture level is necessary to carry on the subsequent process. He failed, however, to explain the deterioration of grain at moisture levels as low as 14.0 percent to 15.0 percent. Linko and Milner (1959b, c) showed with wheat embryos that glutamic acid decarboxylase and glutamic acid-alanine transaminase are activated at very early stages of water imbition. Moisture levels as low as or lower than 18.0 percent activate both enzyme systems, the activity increasing rapidly with increasing moisture content up to the level required for germination. Later, Linko (1960d) concluded that certain enzymes become activated at much lower moisture levels, causing an accumulation of vital metabolic intermediates; the "critical" moisture content for the activation of glutamic acid decarboxylase, transaminases, and proteases being between 13 percent and 16 percent. Others, such as the dehydrogenase system and amylases are activated only when the proteins become saturated with water (about 25.0 percent moisture, or

more). On the other hand, Cheng (1959) showed that glutamic acid decarboxylase activity decreased in damp wheat stored in a moisture range of 11.0 percent to 36.0 percent. The decrease in enzyme activity was very pronounced at moisture levels above 18.9 percent.

Inactivation of the enzymes is also temperature dependent (Fleischmann, 1959). Linko (1960d) suggested that approximately 38°C would be the critical temperature below 15.0 percent moisture level. Cheng (1959) showed that samples of air dry Seneca, Ponca and Langdon wheats stored for three months at 4°, 27° and 39°C decreased in glutamic acid decarboxylase activity with increasing temperature.

Prolonged storage will eventually lead to the decrease in enzyme activity in grain as evidenced by the failure of seeds stored at unfavorable conditions to germinate. Sogn (1960) and Linko (1960c, 1961a) observed a rapid decrease in glutamic acid decarboxylase activity under such conditions.

Effect on Vitamins. Although the vitamin content of cereal grains has been extensively studied, little is known about changes in vitamin composition during storage. Bayfield and O'Donnel (1945) observed that at 12.0 percent moisture level, thiamin of wheat changed little during five months of storage. When moisture content was increased to 17.0 percent, about one-third of the thiamin was lost. It is generally believed (Anderson and Alcock, 1954) that all of the B-vitamins, with the possible exception of pantothenic acid, are relatively stable under normal storage of grain. Pomeranz (1957) observed that pantothenic acid decreased concurrently with molding after 2 weeks of storage of damp wheat.

Pelshenke (1960) found no loss in thiamine or tocopherol in wheat stored below 20°C for three to four months, but noticed a 10 percent loss of thiamine

after 19 months of storage. Rye stored for two to four months lost 30 percent of its tocopherol under similar storage conditions.

Rothe et al. (1958) claimed that losses of tocopherols in wheat were accentuated when the moisture content of the sample exceeded 12.0 percent. Similarly, the pro-vitamin A in corn has been shown to decrease considerably during cold storage (Frapps and Kimmerer, 1937).

Effect on Minerals. Minerals of cereal grains and grain products are the most stable constituents during normal storage conditions. However, Moxon and Rhian (1938) showed that grain grown on seleniferous soil may lose as much as 73 percent of its original selenium content during storage because of the high volatility of the compounds involved.

Greaves and Hirst (1925) showed that during storage of cereal grain and, especially, flour, inorganic phosphorus increased owing to the action of phytase on phytin. Recently, Glass and Geddes (1959) observed a decrease in phytic acid accompanied by an increase in inorganic phosphorus in wheat stored at 18.0 percent moisture at 20°C for 24 weeks. They suggested that the ratio of the released to the total phosphorus may prove useful as an index of storage damage to grains.

Effect on the Baking Quality. The bread baking quality of flour normally tends to improve during aging within a certain period of time, depending on the nature of the flour and storage conditions. Similarly, the potential bread baking quality of freshly harvested wheat appears to improve somewhat during storage, although at a much slower rate than in the case of flour (Saunders, 1909; Fitz, 1910; Saunders et al., 1921). On the other hand Shellenberger (1939) showed that, although the bread baking quality of wheat improved during storage after harvest, the extent of the improvement was

generally quite small. In extended storage, however, baking quality eventually begins a gradual decline, the rate of which again depends on storage conditions (Kozmin, 1935; Sullivan et al., 1936, Halton and Fisher, 1937; Swanson, 1941; Semeniuk et al., 1947; and Auerman, 1960).

Several workers have shown the importance of temperature and moisture in the aging process of wheat and flour. Swanson (1935) has observed a marked decrease in loaf volume and loss of fine silky texture of bread baked from wheat stored at 20.4 percent moisture for 13 weeks at room temperature. Pomeranz et al., (1956) found a marked drop in water absorption, loaf volume and loss of fineness of the crumb with flour from wheat stored at 23.5 percent moisture at 20° to 21°C for four weeks. The deterioration in baking quality became apparent at the stage when the mold population became very high. Furthermore, Kozmin (1935) observed that gluten quality of flour stored at 30° and 45°C, as measured by baking test, drastically decreased within three months of storage while those stored at 15°C remained almost unchanged. These findings were supported by Fifield and Robertson (1945) who obtained very satisfactory bread made from wheat stored for 9 to 22 years under nearly ideal storage conditions. According to Swanson (1941) wheat may be stored at 41°F at relatively high moisture levels for several months without suffering any damage to its milling and baking qualities. However, in practice, commercial millers prefer to mill their wheat before it is more than a year old to avoid possible deterioration.

Mold Growth

Although deterioration of stored grain by fungi was described more than 40 years ago (Duvel, 1909; Shanahan, 1910) only recently the problem has

become recognized as one of some importance.

Fungi in seeds are commonly classified as field fungi or storage fungi. The major field fungi, Alternaria sp., Helminthosporium sp., and Fusarium sp., seldom cause storage deterioration with the exception of the last one, whereas the storage molds Aspergillus sp. and Penicillium sp. are principally associated with decrease in quality during storage. The important role played by these saprophytic fungi in respiration, heating, chemical deterioration, and loss of viability of stored grains is well recognized (Ramstad and Geddes, 1942; Carter and Young, 1950; Hyde, 1950; Hyde, et al., 1951; Milner et al., 1954; and Christensen, 1957).

Effect on Respiration and Heating. The number of molds on cereal grains increases with increasing moisture content, thereby establishing a relationship of fungi with the respiratory activity of grain (Christensen and Gordon, 1948; Gilman and Semeniuk, 1948; Del Prado and Christensen, 1952; Tuite and Christensen, 1955; and Christensen, 1957).

Moisture contents in wheat below about 14.5 percent (corresponding to a relative humidity of 74-75 percent) yield low and constant respiratory rates over extended time invervals, indicative of purely seed respiration (Milner et al., 1947a). However, as early as 1912 (Darsie, 1914), there was fairly conclusive evidence that moldy seeds produce more heat than non-moldy seeds when moistened sufficiently to germinate. It was Gilman and Barron (1930) who first showed the importance of molds in the relationship between moisture content and respiratory activity of cereal grains. They observed that the "critical" moisture level for a sharp increase in respiratory rate coincides with the moisture requirements of certain saprophytic fungi. This is well substantiated by later work of Christensen and Gordon (1948) who found

that at moisture contents favorable to their growth, molds caused the temperature of stored wheat and corn to rise to within a few degrees of the maximum temperature the molds could endure.

Recently, Hummel et al. (1954) have shown that the respiratory rates of mold and insect-free wheat at 35°C and moisture levels ranging from 15 to 31 percent were low and constant, while the respiration of moldy wheat markedly increased after a few days.

Reduction in Germination of Grain. Indirect and direct evidence has indicated that large decrease in germination and development of germ-damage in wheat, barley and corn could be associated with the invasion of the germ by various storage fungi (Christensen and Drescher, 1954; Hummel et al., 1954; Semeniuk et al., 1954; Christensen, 1955; Tuite and Christensen, 1955; and Sorger-Domenigg et al., 1955a). Recent work of Papavizas and Christensen (1957) emphasized the importance of molds in rapid death and discoloration of the germ at moisture contents of 14.7 to approximately 20.0 percent at 25°C.

Discoloration of the Germ. Thomas (1937) believed that toxic compounds secreted by fungi would be the primary cause of germ damage. In general, invasion of the germs of stored wheat by fungi is known to be commonly associated with germ-damaged or "sick" wheat (Milner et al., 1947; Christensen, 1955; Sorger-Domenigg, 1955; Papavizas and Christensen, 1957). The extent of discoloration has been found to depend on the pathogenecity of the fungi, as well as the variety of wheat, and storage conditions (Papavizas and Christensen, 1960). However, germ damage can also develop in the absence of fungi (Matz and Milner, 1951; Swanson, 1934), particularly during conditions inhibitory to fungal growth. However, Christensen (1955) stated that in

laboratory experiments, storing wheat under carbon dioxide and nitrogen at temperatures of 35°C or higher and by exposure to fumigants, could cause what appears to be typical sick wheat but which is not likely to have the biochemical properties of sick seed encountered in commercial storage. He believed that sick wheat generally would be a result of the invasion of the germ by species of Aspergillus, and if other factors would be involved, they presumably were of minor significance in commercial wheat storage.

Biochemical Changes. As indicated before, biochemical changes in grain during storage are accelerated by the presence of fungi (Milner and Geddes, 1946a; Milner et al., 1947b; Bottomley et al., 1952; Loeb and Mayne, 1952). The most pronounced of these changes are an increase in fatty acids, a decrease in non-reducing sugars, followed by an increase in reducing sugars, and an increased respiration (Barton-Wright, 1940; Bottomley et al., 1950; Fenton and Swanson, 1930). Golubchuk et al. (1956) have demonstrated the importance of molds in the deterioration of grain stored at moisture contents as low as 12.0 percent.

Swanson (1934) observed that the increase in mold population in wheat stored at high moisture levels resulted in a decrease in test weight, thiamine, and nicotinic acid, and in an increase in fat acidity and riboflavin. Pomeranz et al. (1956) showed that the addition to flour of cultures of Aspergillus flavus and A. ochraceus caused a marked deterioration as indicated by undesirable changes in water absorption, dough properties, and bread quality.

Storage Time

Papavizas and Christensen (1957) have indicated that the moisture content which permits damaging invasion of seed by storage fungi is a function of both

time and temperature with decreasing temperature, moisture content may be increased without danger of deterioration. Thus, wheat at a moisture level of 14.5 to 15.0 percent may be stored safely at 20 to 25°C for a few months, but generally not for a year whereas at the same moisture level it presumably could be stored for a year without serious damage from molds at temperatures of 10 to 15°C (Christensen, 1957).

Carter and Young (1945) found that germ damage increases with increasing moisture content, temperature, and time of storage. Recently, Kozlova, and Nekrasov (1956) confirmed that during prolonged storage of wheat the original quality gradually decreases. With samples stored in wooden and brick warehouses at 1 to 20°C and 42 to 80 percent relative humidity, the seeds lost their original viability during 15 years of storage. The same was true with rice (Kondo, 1933). Similarly, Shvetsova (1958) observed that germination dropped to zero during prolonged hermatic storage at 20.0 percent moisture at 18 to 20°C.

The interrelationships of storage time and storage conditions on seed viability were studied very recently by Roberts (1960, 1961) who presented evidence showing that the life duration of cereal seeds appears to bear a simple relationship to temperature and moisture content during storage. His data included a range in moisture content from 11 to 25 percent in temperature 15° to 25° and storage time from a few days up to 123 years.

METHODS TO DETERMINE STORAGE DETERIORATION AND QUALITY OF CEREAL GRAINS

Physical Methods

Severe deterioration in stored grain is noticeable by dull appearance,

increased temperature due to spontaneous heating, and a musty or sour odor. Sprouted or externally damaged kernels can be easily noticed. Extent of insect infestation may be determined by counting insect fragments (Harris et al., 1952; Shellenberger et al., 1953) by x-ray techniques (Milner et al., 1951), by observing stained insect egg-plugs (Frakenfeld, 1948; Milner et al., 1950; Goossens, 1949) or by visual inspection of grains after rendering them transparent by soaking in a mixture of phenol, lactic acid and glycerine (Koura, 1958).

Grain stored at elevated moisture levels usually develops extensive fungal growth (Christensen, 1957) resulting in a marked increase in temperature of the bulk of grain (Smith and Bartz, 1932). A detectable increase in temperature is often considered by the elevator operator as the first evidence of deterioration, and it is generally assumed that if a given bulk of grain is not heating, it is not deteriorating. However, some damage may also take place without detectable heating (Carter and Young, 1950).

Christensen and Drescher (1954) and Milner et al. (1957) showed both with small and large lots of grain that the moisture content is likely to increase with increasing fungal growth. Zeleny (1954) suggested that electrical conductivity of grain may be is a better index of storage behavior than its moisture content. As grain deteriorates, the ratio of free to bound water increases causing an increase in the electrical conductivity.

Protein Content

Determination of nitrogen content of grain does not directly give an estimate of deterioration, but it nevertheless gives a very valuable index of wheat quality. However, although actual protein content may decrease

during extended storage, relative Kjeldahl method is accepted as the standard procedure for quantitative protein determination, and was first used by a flour mill in 1900, as reported by Whitcomb and Bell (1926).

Pinkney (1949) published a colorimetric method based on the Biuret reaction for determination of wheat protein. The method has been modified by Williams (1961), Jennings (1961), and Pinkney (1961). Zeleny (1941) developed a simple photometric method involving the peptization of gluten proteins by very dilue alkali. Feinstein (1959) extended the sulfosalicyclic acid method widely used in clinical chemistry to determine the protein content of wheat.

Fraenkel-Conrat and Cooper (1944) developed techniques employing the ability of certain dyes to form an insoluble complex with proteins, for measuring their ocidic and basic groups. The dyes used were safranin O, a quarternary ammonium salt, and orange G, a disulfonic acid dye. Udy (1954) applied the method to fractions of wheat flour proteins and later extended it to quantitative estimation of the protein content of wheat and wheat flour (1956). However, orange G, the dye adopted by Udy for quantitative protein analysis, shows great differences in absorption by different proteins, necessitating the use of separate standard curves with wheat flour. Plum et al. (1955) studied a number of dyes for quantitative determination of serum proteins finding amido black 10 B the most suitable. Other workers have shown the superiority of this dye over orange G in determining the protein content of milk (Schoeber and Hetzel, 1956; Dolby, 1961). Gorringe (1957) reported recently that the wool dye lissamin green SF 150 is absorbed identically by various serum proteins. This dye, however, does not have seemed to be used for quantitative protein analysis.

Mold Count

Fungal growth is an important factor in the deterioration of bulk grain in storage, and the extent of mold invasion is now frequently employed as one of the criteria for detecting and evaluating the degree of spoilage. Thus several methods for the quantitative determination of total mold spores and/or mycelium have been developed.

Christensen and co-workers (Christensen, 1951; Bottomley et al., 1952; Christensen et al., 1954) developed microbiological techniques for measuring the external and internal microflora of cereal grains, as well as the number of seeds deeply invaded by molds. Recently, Golubchuk et al. (1960) proposed a colorimetric method involving determination of the chitin content of wheat. They found that the chitin content shows a good correlation with mold count, viability, and fat acidity. Pomeranz et al. (1956) observed the increase in mold growth was accompanied by a decrease in fat content and an increase in fat acidity. However, Golubchuk et al.(1956) concluded that mold count alone cannot be a reliable index of present or future deterioration, unless wheat is sampled before the majority of fungal spores have died.

Germ Damage

Germ damage "sick wheat" is generally detected by tan, brown or black discoloration of the embryo after removal of the pericarp. Christensen and Qasem (1959) made the pericarp transparent by bleaching kernels in boiling 2 percent sodium hypochlorite before visual inspection.

Swanson (1934) and Matz and Milner (1951) have shown that germ damage can develop in prolonged storage under conditions when fungal growth is inhibited. Some evidence has associated "sick" wheat development with anaerobic

conditions (Carter and Young, 1945; Oxley and Hyde, 1955). Thomas (1937) on the other hand, believed that toxic compounds secreted by storage fungi may be responsible for germ damage. The Maillard-type nonenzymatic browning reaction, involving condensation of reducing sugars with amino acids and proteins, which is a well known cause of discoloration of many other food products of relatively low moisture content (Ramsey et al., 1933; Olcott and Dutton, 1945; Stadtman, 1948; Tarassuk, 1950) has been also suggested as the course of germ damage in wheat. An increase in fluorescence, characteristic to certain intermediates of maillard type browning, has been reported in germ-damaged wheat (Pearce, 1943; Cole and Milner, 1953; McDonald and Milner, 1954). Further evidence in support of this theory has been recently obtained (Zeleny and Coleman, 1939; Ramstad and Geddes, 1942; Bottomley et al., 1952; Houston et al., 1957; Rohrlich, 1957; Glass et al., 1959; Linko and Milner, 1959; Linko, 1960; Linko et al., 1960). Linko and Sogn (1960) reported a high positive correlation between percent germ damage and fluorescence ($r = +0.804^{***}$).

Germination Test

In seed testing, germination is defined as the emergence and development from the seed embryo of those essential structures which, for the seed in question, are indicative of the embryo's ability to produce a normal plant under favorable conditions (Justice, 1948). Germination test, performed by wet filter paper, by sterilized, wet sand, or by "rag doll" techniques, is still recognized as the best and most objective method for determination of viability and vigor of seeds. Several factors, such as proper moisture content, temperature within a suitable range, oxygen for respiration, light, and dormancy factor, have to be considered while evaluating results from a

germination test (Crocker and Barton, 1958). Linko and Sogn (1960) found with commercial wheats at various levels of deterioration, a high correlation between germination percentage and glutamic acid decarboxylase activity ($r = +0.921^{***}$), with fluorescence ($r = -0.758^{***}$), with TTC ($r = +0.990^{***}$) and with fat acidity ($r = -0.906^{***}$). Similarly, Houston et al., (1957) reported a high correlation between germination percentage and non-reducing sugars, and of free acidity, respectively, with rice stored at high moisture and temperature.

Fat Acidity

As a result of a detailed investigation, Zeleny (1940) concluded that at any given moisture level, the rate of heating of corn tends to increase with increasing fat acidity values. Bottomley et al. (1950), however, were unable to accurately estimate deterioration by measuring any one of the biochemical changes they investigated including fat acidity. In non-aerated samples, they observed little change with fat acidity or non-reducing sugars with an increase in moisture content and storage time, despite decreasing viability. On the other hand, Baker, Neustadt and Zeleny (1937, 1950), Linko and Sogn (1960) and Sorger-Domenigg et al., (1955) obtained a high positive correlation between fat acidity and percentage germ-damage. Wertzel (1958) and Olaru (1961) found fat acidity as a good index of bread baking quality of flour.

The pioneering attempts of Besley and Boston (1914) to standardize the determination of acidity as a measure of corn soundness were followed by several improvements (see Zeleny, 1954), resulting in the present fat acidity test (Am. Assoc. Cereal Chem., 1957). However, aside from the official method,

a rapid and simple method was developed by Baker et al., (1961) utilizing a grinder-extractor to shorten the extraction time. A slight modification of Baker's method was introduced by Bolling (1961) wherein the titration of the fat extract in alcoholic phenolphthalein was done potentiometrically. Baker (1961) developed a colorimetric method, involving reaction of fatty acids with copper acetate, to determine fat acidity in grain.

Enzyme Activity

The activity of several enzymes, such as amylases, catalase, dehydrogenases, peroxidase and glutamic acid decarboxylase, has been associated with the viability and metabolic rate of various seeds (see Linko, 1960a).

As early as 1922, Turreson, indicated that certain dyes may be enzymotically reduced to colored compounds by viable embryos thus indicating the presence of an active dehydrogenase system. He first employed methylene blue as an indicator of viability. Indigo carmine was used by Russian workers (see Linko, 1961) while others (Eidman, 1937; Gadd and Kjaer, 1940) used selenium salts for this purpose. Lakon extended the use of selenium salts (1940a, 1940b) to distinguish the living portions of the embryo from the non-living. Later, he studied the enzymatic reduction of several tetrazolium salts, for this purpose finding 2, 3, 5-triphenyl tetrazolium chloride into insoluble red formazan. Thus, only viable seeds with an active respiratory system would give a positive reaction.

Baird et al., (1950) suggested Lakon's test as a means of detecting the percentage of dead kernels in corn. However, MacMasters et al. (1954) later reported no correlation between the results from laboratory starch processing and viability as measured by TTC test. This was found to be caused by some obviously dead but heavily moldy kernels giving a positive TTC test (Schenk,

1957), thus indicating viability of fungi rather than viability of corn.

Despite the good correlation between TTC-test with germination percentage with several seeds, Cottrell (1957) still preferred germination test for the determination of seed viability. Similarly, Weith (1959) found the method insufficient in testing viability of heat-damaged grain.

Mar (1944) showed that the amylase activity of soaked oat seeds is correlated with germinability, and French (1959) reported recently a positive correlation between viability and the ability of soaked barley embryos to form starch or amylase. On the contrary to these observations Kretovich (1945) found no relation between amylase activity and viability. Evidently this difference in results is due to different experimental conditions. Soaking of seeds prior to analysis would reveal the ability to activate amylase, though the original activity in grain may remain relatively constant. Kersting (1961) found that diastatic activity in grain sorghum was highly correlated with percent germination.

Crocker and Harrington (1918), Davis (1926) and Leggatt (1933) published evidence indicating that catalase activity could be used as a measure of viability. Opalatka (1956) concluded that the determination of the catalase activity is a better method to determine the mustiness of wheat than respiration measurement. Brücker (1948) reported that peroxidase content decreases rapidly in stored seeds exposed to elevated moisture levels and temperatures.

Recently, Cheng (1959) observed that glutamic acid decarboxylase decreased during storage of wheat especially at moisture levels above 15.0 percent. This was confirmed by Linko and Sogn (1960) who showed a high correlation between viability ($r = +0.921^{***}$) and percentage of germ damage ($r = -0.878^{***}$) respectively, with glutamic acid decarboxylase activity of

wheats containing acid decarboxylase activity of wheats containing various amounts of damage. They concluded that glutamic acid decarboxylase activity may provide a good estimate of the storage background of wheat. Hence, Linko (1961) developed a simple and rapid manometric method for its determination. Aside from providing a simple way to estimate quality of stored wheat, glutamic acid decarboxylase activity of wheat may be used to estimate probable storage time under any given average temperature and moisture levels (Linko, 1961).

Janicki et al. (1958) used vital staining with neutral red to evaluate heat damage on the aleuone cells of grain.

Gustafson (1951) had developed a method for determining viability of barley seeds by treating grain with solutions of phosphoric acid, benzidine and hydrogen peroxide containing sodium chloride and finally skellysolve B. Viable grain remained uncolored, and non-viable appeared blue. Zone (1960) used alcoholic benzidine solution or quaicol to determine germinative power of cotton seeds.

Imbibitional Properties of Wheat and Flour Proteins

Gortner and Doherty (1918) performed one of the earliest extensive investigations on the imbibitional properties of flour proteins. They concluded that moist glutens from flours of varying quality were hydrated to the same extent, but that gluten from weak flour had a much slower rate of hydration. Dilute solutions of lactic, acetic, and phosphoric acids caused gluten to imbibe water strongly and solutions of stronger acids diminished imbitition only slightly.

Although Luers and Ostwald (1919) were the first to determine the viscosity of flour suspensions, Sharp and Gartner (1923) were the first to employ

the viscosity of acidified flour-in-water suspensions as an easy, rapid, and accurate method to investigate the comparative imbibitional properties of flour proteins.

Viscosity values have been useful in evaluating soft wheat flour (Bayfield, 1934); however, their utility in predicting hard wheat flour quality has been questionable (Blish and Sandstedt, 1925; Gortner, 1924, Bayfield, 1932; Rich, 1932). The uncertainty regarding the test is due mainly to various factors which affect it.

Finney and Yamasaki (1946) reported that viscosity was a linear function of protein quantity, but that the regression of viscosity on protein content was different for each variety. Furthermore, viscosity did not evaluate properly the loaf volume potential of varieties.

Berliner and Koopman (1929) devised an interesting method based on swelling capacity to determine gluten quality. The test was performed in a volumetric flask with a graduated neck. They placed a known quantity of moist, especially prepared gluten in the flask with 100 ml N/10 lactic acid. The flask was stoppered, inverted periodically, and the volume of the gluten noted. The difference in volume at 0 and 2 1/2 hour intervals exhibited by one gram gluten was called the "specific swelling factor". The authors considered the method a test of gluten strength, not a comprehensive test of flour quality. Fisher and Halton (1933) concluded that it was not suitable to evaluate flour quality.

Recently, Finney and Yamasaki (1953) developed an alkaline viscosity test using sodium bicarbonate instead of lactic acid for evaluating soft wheat flours. Zeleny (1947) has described a simple sedimentation test similar in principle to the test described by Finney and Yamazaki (1945) and used to

determine protein content for wheat inspection. The method consists essentially of mixing 4 grams of flour with 50 ml. of water in a 100 ml. glass stoppered, graduated cylinder. After the mixture has stood 5 minutes, 25 ml. of dilute lactic acid is added. The cylinder is inverted several times and after 5 minutes, the position of the top limit of the suspended phase is observed. This volume in milliliters is the "sedimentation value" of the flour. It is related to the quantity of flour protein, and is a measure of the extent of hydration of the gluten. Specific sedimentation, or the sedimentation value divided by protein percentage, has been considered a useful measure of gluten quality (Zeleny, 1949).

MATERIALS AND METHODS

Materials

The 62 corn samples consisted of 58 yellow and 4 white dent corn at various stages of deterioration. Their moisture content ranged from 8.5 to 22.5 percent.

The wheats included several commercial wheats from 1956 to 1959 crops. Their viability, ranged from 0 to 100 percent, moisture content from 9.1 to 12.7 percent, protein from 10.4 to 13.0 percent. In addition, a large number of pure varieties were included in a study of protein determination by dye absorption techniques. The Kjeldahl protein in these samples ranged from 10.5 to 18.8 in wheat and from 9.5 to 17.1 in the corresponding flours.

Also, samples of Pawnee (12.5 percent moisture, 90 percent germination) and Bison (12.2 percent moisture, 94 percent germination) were employed in short storage studies at various conditions. These samples were divided into 2,500 gram portions and conditioned to approximately 14 (A), 16 (B), 18 (C),

20 (D) and 25 (E) percent moisture. The actual moisture contents determined 12 hours after conditioning are given in Tables 3 and 4. The samples were stored in air-tight jars at +4° (I), +24° (II), and +38°C (III), respectively. About 500 g aliquots were sampled after 2 (1), 4 (2), 8 (3), 16 (4) and 32 (5) days storage, respectively. The aliquots were immediately dried at +37°C for 24 hours in a forced air oven to safe moisture levels, the final moisture content varying from 9.3 to 13.2 percent for Pawnee and from 9.7 to 13.7 percent for Bison.

Moisture Content

The grains were ground with a Waring Blendor for two minutes. The moisture content was determined by drying samples for one hour at 130°C (Am. Assoc. Cereal Chemists, 1957). All samples were stored in air-tight flasks at room temperature during the time the tests were carried out.

Kjeldahl Protein

Slightly modified AACC (1957) method was used. One gram of material (wet weight basis) ground by a Waring Blendor for two minutes was weighed into a 500 ml. Kjeldahl flask and mixed well with 10 grams of a mixture of potassium and sodium sulfate (60:40), 0.5 g mercuric oxide, and a small amount of pumice. The final mixture was then digested with 20 ml of concentrated sulfuric acid on a 500-watt burner until clear (30 to 45 minutes). The cooled digest was diluted with tap water to 250-300 ml. The solution was then made alkaline by addition of 50 ml of sodium hydroxide solution of 1.47 sp. gr., containing 100 g of sodium thiosulfate per liter, and immediately distilled into 50 ml of 0.1253 N sulfuric acid. When

titrated[1] with 0.1253 N sodium hydroxide, one ml of sodium hydroxide was equivalent to 1 percent protein. Percent protein was obtained by subtracting from 19.0 the number of milliliters of sodium hydroxide used. An automatic burette was set up so that percent protein could be read directly.

Dye Absorption Test for Quantitative Protein Determination

Flour or wheat, ground two minutes in a Waring Blendor was mixed five minutes with 25 ml. of a buffered dye solution[2] in a Waring Blendor equipped with a semimicro monel metal container. The base of the container was surrounded by copper tubing for cooling water. The mixture was centrifuged 15 minutes at 2100 rpm (1400 x g), an aliquot of the supernatant was diluted with distilled water or with a suitable buffer, and the absorbance was measured by a Beckman DU spectrophotometer.

Experimental details for each of the three dyes used are given below.

	Amidoblack 10B	Lissamine green SF 150	Orange G
Wheat	500 mg	500 mg	500 mg
Flour	500 mg	500 mg	600 mg
Concentration of dye solution	0.1 %	0.25 %	0.10 %
Diluent	Water	Phos. buffer (pH 6.0)	Water
Dilution	1:50	1:100	1:50
Wave length	610 mu	635 mu	470 mu

[1] A mixture of sodium alizarin sulfate and methylene blue as an indicator.

[2] McIvaine's citrate buffer of pH 2.2 was used; made of citric acid monohydrate (20.7 g/l) and disodium phosphate (0.57 g/l) in proportion of 49.1.

Except for orange G which is reported as pure dye, the concentrations given refer to dyes used without further purification.

Germination Test

Grains were surface sterilized in 0.1 percent mercuric chloride solution for two minutes, and rinsed five times in tap water before they were laid out for germination. Samples of 100 kernels each were placed crease down on moist sterile quartz-sand (15 ml water to 40 ml sand) in petri dishes, covered with wet filter paper and stand at room temperature (+23°-25°C). The filter paper was kept moist by adding water about every six hours. Germinated grains were counted and removed every second day. All kernels which showed normal sprouts after seven days were considered viable.

Fluorescence

A slightly modified procedure of Cole and Milner (1953) was employed (Linko, 1960). One gram (wet weight basis) of ground kernels (by Waring Blendor for two minutes) was weighed into a 125 ml Erlenmeyer flask containing 25 ml of 0.2 M hydrochloric acid. The mixtures were allowed to stand overnight at about +25°C, shaking them by hand at certain time intervals. After filtering through Whatman No. 5 paper, the clear solutions were diluted, if necessary, and used for fluorescence determinations. Measurements were made with a Coleman Photoelectric Fluorometer with B_1-S and PC-1 filters; the instrument was standardized to read 60 with 0.1 ppm sodium fluorescence solution.

Fat Acidity

Benzene Extraction Method. Generally, fat acidity was determined by the benzene extraction method (Zeleny, 1940). Twenty grams of freshly ground (2 minutes with Waring Blendor) material were weighed into 250 ml glass-stoppered Erlenmeyer flasks and shaken with 250 ml of benzene for 15 minutes by means of a magnetic stirrer. The mixtures were then allowed to stand for five minutes followed by filtering through fluted Whatman No. 5 paper. The funnel was covered with a watchglass to avoid evaporation. A 25 ml aliquot of the filtrate was then mixed with 25 ml of 0.05 percent alcoholic phenolpthalein solution, and the mixture titrated with about 0.01 N alcoholic potassium hydroxide. Fat acidity is reported as mg of KOH required to neutralize free fatty acids in 100 g (dry weight basis) of ground material.

Colorimetric Method. The colorimetric method developed by Baker (1961) was employed. A 40 g sample of grain was ground for one minute in a Waring Blendor, followed by addition of 50 ml of benzene and extraction by Waring Blendor for four minutes. After filtering as in the benzene extraction method, 10 ml of the filtrate were pipetted to a centrifuge tube containing 2 ml of 5 percent (w/v) cupric acetate, the tube shaken rapidly by hand 50 times, and the solution centrifuged for 15 minutes at 2100 rpm (1400 x g). The absorbance of the clear supernatant was read against benzene at 640 mu using a Beckman DU spectrophotometer. Results were correlated with fat acidity values obtained with the benzene extraction method.

Glutamic Acid Decarboxylase Activity

Glutamic acid decarboxylase activity was determined by Sandstedt and Blish (1934) pressuremeters, using the method of Linko (1961). Grain was

ground for two minutes with a Waring Blendor shortly before the experiment, followed by thorough mixing. Thirty grams (wet weight basis) of the ground material were weighed in the aluminum cup of the pressuremeter. Ten to 16 samples were prepared simultaneously. Fifteen milliliters of 0.1 M glutamic acid in 0.067 M phosphate buffer of pH 5.8, containing 0.1 percent of 2(3)-benzoxazolone as a preservative, were then added from a burette in each cup. After mixing well with a heavy glass rod, the lids were screwed on tightly and pressuremeters were placed in a water bath at +30° ± 0.02°C. After five minutes' equilibration, the manometers were adjusted to zero. The pressure, in millimeters of ethyl lactate, was recorded after a 30-minute incubation period.

Pyruvic Acid Decarboxylase Activity

The method for glutamic acid decarboxylase activity was followed except that 0.1 M pyruvic acid in 0.067 M phosphate buffer of pH 5.8, without a preservative, was used as substrate.

Sedimentation Value

Sedimentation value was determined with 3.2 g of experimentally milled (Brabender cane-burr mill) flour, otherwise following the AACC method (1957). The flour, (wet weight basis) was weighed into a 100 ml glass-stoppered graduated cylinder, having a distance between 0 and 100 ml marks of 180 to 185 mm. Fifty milliliters of distilled water, containing 4 mg of bromphenol blue per liter, were added by means of an automatic pipette to the cylinder, and the timing was started simultaneously. The mixture was then shaken thoroughly by hand moving the stoppered cylinder horizontally lengthwise,

alternately right and left, through a space of 7 inches, 12 times in each direction in five seconds. Care was taken that the flour was completely swept into suspension during the mixing. The cylinder was then allowed to shake for five minutes in a special shaker (Fred Stein Laboratories), after which 25 ml of dilute lactic acid[3] were added and the cylinder mixed again in the shaker for exactly five minutes. After standing for exactly five minutes, the volume of the sediment was read, giving the sedimentation value in milliliters.

Acridine Orange Staining Method

One gram of wheat ground for two minutes in a Waring Blendor, was shaken in a 15 ml centrifuge tube with 3 ml of 0.015 percent acridine orange dye in McIlvaine's citric acid buffer of pH 2.0 for 30 minutes, using a wrist-action shaker. Twelve milliliters of distilled water were then added, followed by thorough mixing of the contents. The solution was then allowed to stand for 30 minutes, centrifuged for 15 minutes at 2,000 rpm (1333 x g). The absorbance of the supernatant was measured with Beckman DU spectrophotometer at 497 mu.

Baking Test

The water absorption of flour was determined by the farinograph using a 10-g bowl (Shogren and Shellenberger, 1954). Ten grams of flour (wet

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[3]Prepared by mixing thoroughly 180 ml. of lactic acid stock solution (250 ml 85 percent L.A. U.S.P. grade, diluted to 1 liter and refluxed for six hours), 200 ml of isoprophyl alcohol (99-100 percent N.F.), and distilled water to make 1 liter. The final solution is approximately 0.5 N in respect to lactic acid and contains 20 percent isopropyl alcohol. It is allowed to stand 48 hours before using.

weight basis) were used. Using the values thus obtained, a number of farinographs were run with a 50 g bowl to study curve characteristics (Figs. 4 to 6). The following straight dough formula was used for baking tests:

Flour (wet weight basis)	100 g
Cerelose	6 g
Dry Milk solids	3 g
Salt	2 g
Malted wheat flour	1/4 g
$KBrO_3$ (1.4 mg/ml)	0.5 ml
Yeast (80 g/l)	25 ml
Water	as needed

The dough was mixed to optimum consistency. Total fermentation time was three hours (85°F; 85% R.H.). The total dough was molded, proofed 45 minutes (98°F; 95% R.H.) and baked 25 minutes at 410°F. The loaf volume was measured immediately after baking. The bread was generally scored four hours after baking.

RESULTS

Storage Deterioration of Commercial Wheats

The results obtained with commercial samples are summarized in Tables 1 and 2.

Germination Percent. The germination percentage varied from 0 to 100 percent, with nearly half of the samples in the range from 90 to 100 percent. Table 2 shows some of the correlations between the various measurements.

Table 1. Extent of deterioration of commercial wheats.

Sample No.	Moisture	Germination	Fluorescence	Fat Acidity: Benzene Extraction	Fat Acidity: $Cu(Ac)_2$ Method	GADA[1]	PyrDA[2]	Acridine orange test: Wheat	Acridine orange test: Flour
	%	%	E/g	mgKOH/100 g	E/g	mm Ethyl lactate + 100		E/dil.1:0	E/dil 1:10
1-G	10.0	83	16.5	23.33	0.077	206	207	0.620	0.185
2-G	10.0	93	22.0	16.96	0.037	276	251	0.550	0.155
3-G	9.3	9	47.0	43.50	0.135	77	103		
4-G	9.9	95	17.0	24.11	0.063	235	244	0.595	0.170
5-G	9.5	61	27.0	45.40	0.083	169	205	0.480	0.135
6-G	9.8	93	18.0	20.89	0.049	293	260		
7-G	9.6	95	18.0	21.25	0.045	251	207	0.580	0.159
8-G	9.7	92	21.0	15.50	0.038	292	280	0.450	0.120
9-G	10.1	96	18.5	16.67	0.032	287	283	0.450	0.130
10-G	9.7	78	24.0	18.30	0.039	259	228	0.550	0.150
11-G	10.2	97	21.0	14.99	0.039	302	296		
12-G	9.2	95	24.0	16.01	0.040	386	323	0.450	0.117
13-G	9.2	89	25.0	14.82		308	322	0.400	0.120
14-G	9.9	95	24.0	15.33	0.042	280	281	0.465	0.130
15-G	10.2	9	32.0	45.71	0.166	102	105	0.619	0.150
16-G	10.0	91	18.5	16.46	0.057	323	244	0.580	0.145
17-G	9.2	12	44.0	43.79	0.164	107	122	0.595	0.180
18-G	10.5	96	16.0	22.36	0.055	199	219	0.580	0.162
19-G	9.7	78	16.0	19.39	0.060	210	232		
20-G	9.2	4	35.5	49.70	0.153	82	127	0.590	0.170
21-G	9.5	97	20.5	18.94	0.030	252	292		
22-G	9.7	100	20.5	17.40	0.040	254	291	0.478	0.135
23-G	9.9	95	21.0	21.32	0.042	257	214	0.580	0.162
24-G	9.6	99	20.0	17.99		296	269		
25-G	10.8	9	41.0	42.47	0.162	126	117	0.570	0.156

[1]GADA = glutamic acid decarboxylase activity

[2]PyrDA = pyruvic acid decarboxylase activity

Table 1, (cont.)

Sample No.	Moisture	Germination	Fluorescence	Fat Acidity: Benzene Extraction	Fat Acidity: $Cu(Ac)_2$ Method	GADA[1]	PyrDA[2]	Acridine orange test: Wheat	Acridine orange test: Flour
	%	%	E/g	mgKOH/100g	E/g	mm Ethyl lactate + 100		E/dil.1:0	E/dil.1:10
26-G	10.0	76	19.5	27.72	0.040	253	231	0.540	0.140
27-G								0.500	0.156
28-G	9.5	93	18.0	13.99	0.026	305	273	0.438	0.115
29-G	9.5	95	23.0	14.58	0.032	355	324	0.470	0.120
30-G	9.7	94	25.0	25.56	0.07	314	325		
31-G		2	65.9	79.66	0.069	84	106		
32-G	9.7	97	16.0	25.34		198	258		
33-G	9.6	98	22.0	21.86		362	332	0.435	0.135
34-G	9.1	92	23.5	23.00		365	316	0.457	0.157
35-G	10.1	96	17.5	17.18		192	255	0.510	0.130
36-G	9.6	1	71.0	90.17		73	96	0.535	0.140
37-G	10.0	97	22.0	16.46	0.045			0.482	0.135
38-G	9.5	97	20.0	20.53	0.037	294	249	0.585	0.150
39-G	9.2	94	20.0	18.19	0.043	289	276	0.510	0.150
40-G	10.3	94	19.0	24.12		296	282	0.510	0.140
41-G	9.9	97	22.0	13.50	0.037			0.520	0.137
42-G	9.9	1	63.0	88.50		86	108	0.460	0.135
43-G	9.9	97	18.0	20.82	0.034	331	273	0.530	0.145
44-G	9.7	94	18.0	19.38	0.045	316	253	0.555	0.160
45-G	9.2	92	19.0	14.88	0.030	337	234	0.535	0.134
46-G	9.1	97	20.0	18.57	0.054	326	223		
47-G	9.8	59	26.0	42.00	0.074	190	173		
5-S		93					322		
6-S		89					268		
7-S		92					279		
9-S		95					282		
78-L	8.6	84	21.0	28.10		205	201		
79-L	10.8	83	18.0			171	205		

Table 1, (concl.).

Sample No.	Moisture	Germination	Fluorescence	Fat Acidity: Benzene Extraction	Fat Acidity: $Cu(Ac)_2$ Method	GADA[1]	PyrDA[2]	Acridine orange test: Wheat	Acridine orange test: Flour
	%	%	E/g	mgKOH/100g	E/g	mm Ethyl lactate + 100		E/dil.1:0	E/dil.1:10
80-L	9.9	72	25.0	37.38		171	164	0.570	0.150
81-L	9.7	87	16.5	33.90		216	184		
83-L	12.2	79	27.5			182	177		
84-L	9.6	58	27.0			152	132		
87-L	9.7	72	21.0			168	151		
88-L	11.4	56	26.0			151	172		
89-L	11.7	61	21.5	34.77	0.063	118	174	0.550	0.177
90-L	10.0	31	27.0	53.98		65	107		
92-L	11.0	65	23.0	33.98	0.083	117	170		
93-L	10.6	84	20.0	28.92	0.072	163	219		
94-L	9.8	36	23.0			149	121		
95-L	10.2	46	27.0			153	120		
96-L	10.0	36	28.5	50.87	0.147	117	136	0.568	0.175
97-L	11.1	6	26.0	51.42	0.190	68	90	0.590	0.213
99-L	11.6	17	24.0	56.35	0.163	76	97		
100-L	11.2	0	25.0	85.13	0.350	90	127	0.560	0.175
101-L	9.7	22	27.5	68.93		63	97		
102-L	12.7	65	21.0	44.50	0.127	152	187		
103-L	10.2	32	28.0	50.31	0.157	88	127		
104-L	9.4	35	47.0	42.00		111	181		
105-L	10.2	51		42.80		182	142	0.538	0.163
106-L	9.8	56	26.0	46.20		99	152		
107-L	11.0	25	28.0	62.64	0.207	89	131		
108-L	9.7	13	31.0	53.39		68	86		
109-L	10.4	19	35.0	57.41		79	94	0.555	0.155
110-L	9.3	15	30.0	62.38	0.195	78	148	0.550	0.182

Table 2. Simple correlation coefficients between various viability determinations with commercial wheat samples.

Correlation	Correlation Coefficient	Degrees of Freedom	Regression Equation	y	x
% germination vs. log GADA	r = +0.906***	71			
% germination vs. log GADA	r = +0.920***	250[1]	Log y = 0.00757x + 1.780	GADA (P=180)	% germination
% germination vs. log PyrDA	r = +0.927***	76	Log y = 0.00476x + 1.969	PyrDA (P=100)	% germination
Fat acidity (Std. method) vs. % germination	r = -0.731***	66			
Fat acidity (Std. method) vs. % germination	r = -0.754***	115[1]	y = 394x + 58.2	Fat Acidity	% germination
Fat acidity (Std. method vs. Cu-method)	r = +0.267[ns]				

[1]Pooled with all previous data from this laboratory.

Fluorescence. As shown in Table 1, fluorescence values ranged from 16.0 to 71.0. A great majority of the samples showed little increase in fluorescence, the values falling within a range of 16.0 to 30.0. No statistical calculations were made.

Fat Acidity. Fat acidity is reported as mg of potassium hydroxide required to neutralize free fatty acids from 100 grams of grain (dry weight basis). Fat acidity varied from 13.50 to 90.17. As can be seen from Table 2, fat acidity has a highly significant negative correlation with germination percentage ($r = -0.731^{***}$, 66 d.f.). When the results were pooled with data previously obtained in this laboratory, the resulting correlation (Fig. 1; $r = -0.754^{***}$, 115 d.f.) was not significantly different. The correlation between the benzene extraction method and the colorimetric method to determine fat acidity was low and insignificant ($r = +0.267^{ns}$, 48 d.f.).

Glutamic Acid Decarboxylase Activity. Glutamic decarboxylase activity (GADA) was reported as millimeters of ethyl lactate plus one hundred. As shown in Table 1, the values ranged from 58 to 386. As can be seen from Table 2, log GADA showed a highly significant positive correlation with germination percentage ($r = +0.906^{***}$, 71 d.f.). Again, a numerically higher, but not significantly different correlation coefficient (Fig. 2; $r = +0.920^{***}$, 250 d.f.) was obtained when the results were pooled with previous data from this laboratory.

Pyruvic Acid Decarboxylase Activity. Pyruvic acid decarboxylase activity (PyrDA), in mm ethyl lactate, was generally of the same order of magnitude throughout the range than GADA. Table 1 shows that the values ranged from 90 to 332. According to data presented in Table 2 and Fig. 3, log PyrDA correlated highly with germination percentage ($r = +0.927^{***}$, 76 d.f.). This

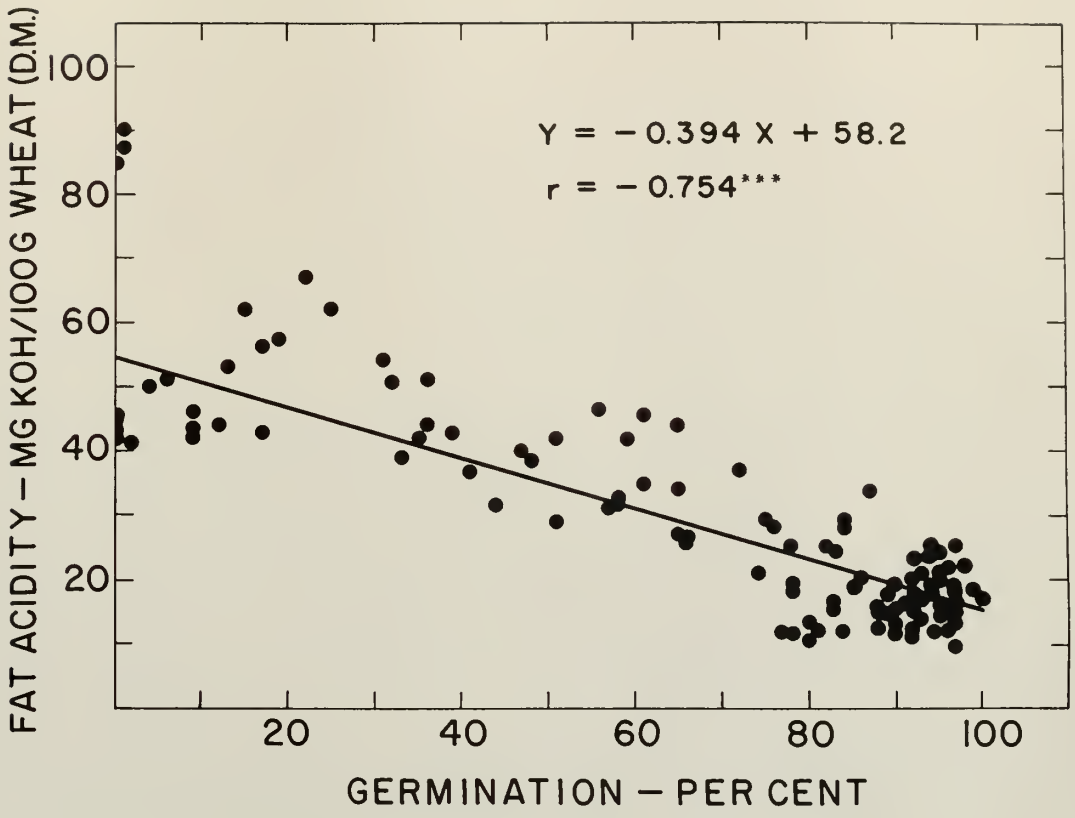

Fig. 1. Relation between fat acidity and germination percentage of commercial wheat.

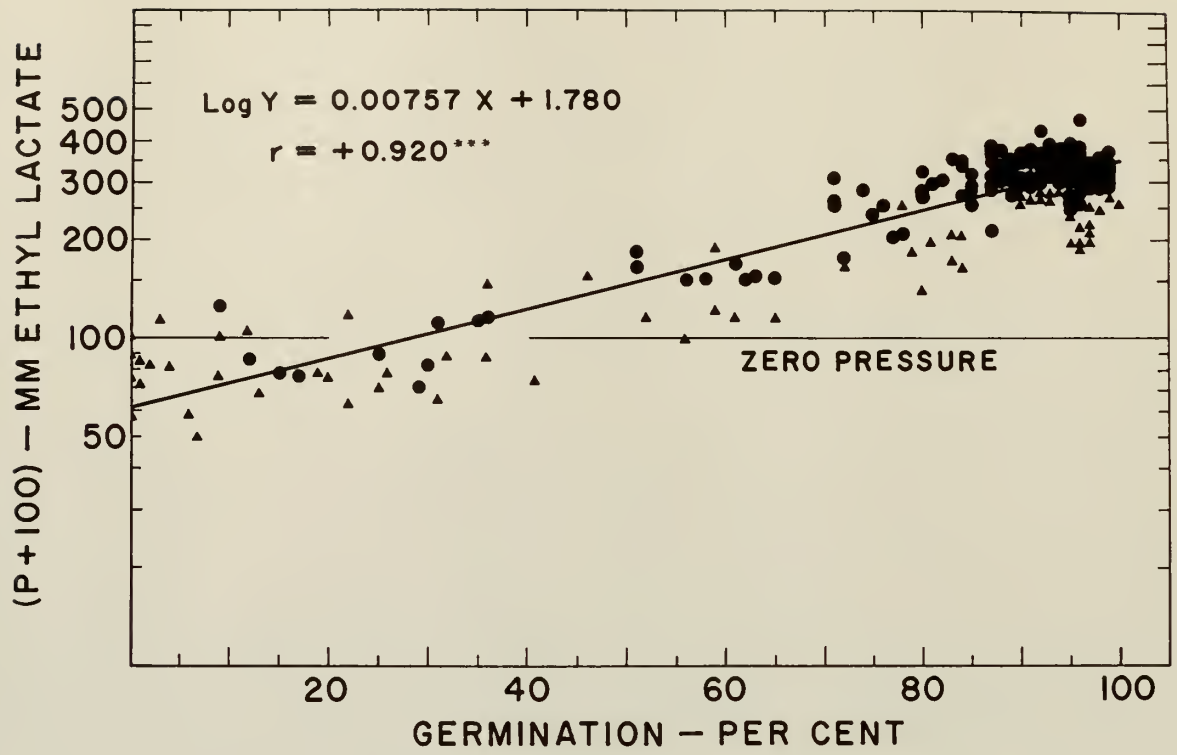

Fig. 2. Relation between log glutamic acid decarboxylase activity and germination percentage of commercial wheat.

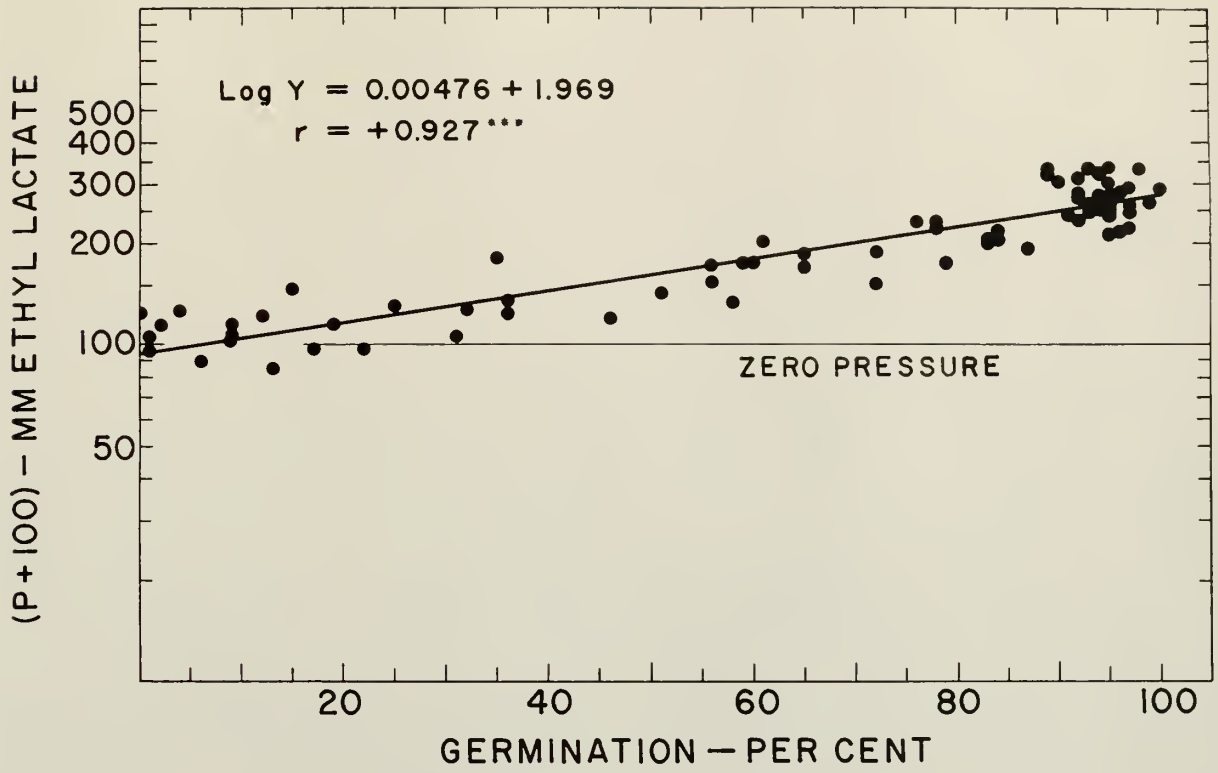

Fig. 3. Relation between log pyruvic acid decarboxylase activity and germination percentage of 78 wheat samples.

correlation was not significantly different from that involving the glutamic acid decarboxylase activity.

Absorption of Acridine Orange by Wheat and Flour. The values were reported as absorbance at 470 mu of the clear supernatant from reaction of acridine orange with one gram of ground wheat or flour, respectively. Thus, the readings are negatively correlated with the actual absorption of acridine orange. The supernatant for wheat was read without dilution, while that of flour required a 1:10 dilution owing to relatively smaller absorption of the dye. The values, as presented in Table 1, ranged from 0.400 to 0.590, and from 0.115 to 0.213, respectively, for ground wheat and flour. The absorbance readings with wheat had a low insignificant correlation with fluorescence ($r = -0.280^{ns}$, 43 d.f.) and a low correlation with fat acidity ($r = +0.339^{*}$, 43 d.f.), significant at 5 percent level. A highly significant negative correlation ($r = -0.604^{***}$, 43 d.f.) was obtained with log GADA, indicating a positive correlation between the absorption of acridine orange and the soundness of wheat.

Effect of Short Storage on Quality of Pawnee and Bison Wheats

The results are summarized in Tables 3 and 4.

Moisture Content. The moisture content of the samples as determined immediately after conditioning, after storage, and after final drying at 37°C are given in Table 3 (for Pawnee) and Table 4 (for Bison).

In the case of Pawnee, the moisture content after conditioning ranged from 13.6 to 25.7 percent for samples subsequently stored at +4°C (I), 13.6 to 26.5 for +24°C (II) and 13.6 to 23.8 percent for the +38°C (III), instead of the intended 14 to 25 percent range. Similarly in case of Bison, moisture

Table 3. Extent of deterioration of Pawnee wheat after short storage.[1] Storage time 2 days.

Sample No.	Moisture: After conditioning	Moisture: After storage	Moisture: After drying	Germination	GADA	Fat Acidity	Sedimentation value	Loaf volume	Water Absorption
	%	%	%	%	mm E.L.+100	mgKOH/100 g	ml	cc	%
P- I-A-1	13.5	12.9	9.3	95	304	10.08	29	780	61.0
B-1	14.5	14.0	9.2	96	304	9.50	28	800	61.5
C-1	16.4	16.2	9.9	94	300	10.43	26	730	62.0
D-1	17.7	17.5	10.0	95	302	9.86	28	765	62.0
E-1	19.5	20.5	10.6	96	308	9.92	30	795	61.0
F-1	25.7	24.4	10.6	94	310	9.93	29	845	61.0
P- II-A-1	13.6	13.4	9.6	99	360	10.37	28	700	62.0
B-1	14.6	14.4	9.4	94	353	11.57	27	815	62.0
C-1	17.1	16.4	10.0	89	288	11.01	29	840	63.0
D-1	17.8	18.7	10.0	96	294	10.46	29	875	61.5
E-1	21.0	20.2	10.5	93	298	10.22	31	825	61.5
F-1	26.4	24.4	11.6	74	270	12.18	29	830	61.0
P-III-A-1	13.6	13.3	9.6	94	308	10.42	28	725	63.0
B-1	14.7	14.0	9.9	97	317	10.37	27	760	63.0
C-1	16.4	15.4	10.0	97	324	10.16	28	745	63.0
D-1	18.2	16.5	10.1	92	324	10.48	27	720	62.0
E-1	19.4	17.1	10.5	90	276	10.82	27	780	58.5
F-1	23.8	24.2	11.7	91	262	11.27	29	805	64.0

[1] I, stored at +4°C; II stored at +24°C; III, stored at +38°C,
GADA, glutamic acid decarboxylase activity; E.L., ethyl lactate.

Table 3, (cont.). Storage time 4 days.

Sample No.	Moisture: After conditioning	Moisture: After storage	Moisture: After drying	Germination	GADA	Fat Acidity	Sedimentation value	Loaf volume	Water Absorption
	%	%	%	%	mm E.L.+100	mgKOH/100 g	ml	cc	%
P- I-A-2	13.5	12.7	10.2	95	320	10.49	30	815	62.0
B-2	14.5	13.6	11.1	94	319	10.89	28	785	62.0
C-2	16.4	16.5	11.10	94	298	10.89	24	755	64.5
D-2	17.7	17.8	11.1	98	290	11.46	28	840	61.5
E-2	19.5	19.3	12.5	96	294	11.69	29	660	61.5
F-2	25.7	24.0	12.3	96	284	11.34	30	850	59.5
P- II-A-2	13.6	12.9	10.2	92	323	10.79	29	820	61.5
B-2	14.6	13.7	10.4	97	353	10.51	27	810	61.0
C-2	17.1	16.1	10.9	94	292	10.57	30	810	61.0
D-2	17.8	16.7	11.0	95	296	10.27	29	850	60.5
E-2	21.0	20.8	12.8	93	288	11.11	30	850	60.5
F-2	26.4	24.9	10.8	77	270	10.55	29	875	58.0
P-III-A-2	13.6	12.5	10.5	96	325	10.52	28	790	60.0
B-2	14.7	13.7	10.3	92	317	11.09	26	805	60.5
C-2	16.4	16.7	10.8	90	324	11.16	27	780	60.0
D-2	18.2	18.7	11.2	79	322		25	835	59.5
E-2	19.4	21.2	10.2	80	280	13.62	27	815	59.0
F-2	23.8	23.7	11.6	82	262	16.74	26	870	58.5

Table 3, (cont.). Storage time 8 days.

Sample No.	Moisture: After conditioning	Moisture: After storage	Moisture: After drying	Germination	GADA	Fat Acidity	Sedimentation value	Loaf volume	Water Absorption
	%	%	%	%	mm E.L.+100	mgKOH/100 g	ml	cc	%
P- I-A-3	13.5	13.8	10.5	97	350	11.12	29	865	62.5
B-3	14.5	14.6	10.9	96	352	11.47	28	860	61.5
C-3	16.4	18.2	12.4	98	345	11.67	25	830	61.0
D-3	17.7	19.1	12.0	94	355	11.30	28	785	62.0
E-3	19.5	19.8	12.6	97	360	11.39	28	795	60.5
F-3	25.7	24.8	13.2	97	349	11.46	29	840	59.5
P- II-A-3	13.6	13.6	11.3	93	390	12.12	27	795	60.5
B-3	14.6	15.0	12.1	94	393	11.97	25	825	59.0
C-3	17.1	16.7	12.1	93	310	12.87	29	820	60.5
D-3	17.8	19.2	11.9	81	383	16.79	27	825	60.5
E-3	21.0	20.2	12.7	73	322	18.18	27	795	59.5
F-3	26.4	25.2	12.6	33	294	20.62	28	870	59.0
P-III-A-3	13.6	13.5	11.1	91	370	12.10	27	770	61.0
B-3	14.7	14.9	11.2	93	335	12.72	32	930	61.5
C-3	16.4	16.8	11.7	86	308	21.01	27	820	60.5
D-3	18.2	18.8	12.0	60	250	37.92	25	850	60.5
E-3	19.4	21.2	12.4	52	238	35.61	24	825	59.0
F-3	23.8	24.4	13.0	31	147	40.80	24	820	59.5

Table 3, (cont.). Storage time 16 days.

Sample No.	Moisture: After conditioning	Moisture: After storage	Moisture: After drying	Germination	GADA	Fat Acidity	Sedimentation value	Loaf volume	Water Absorption
	%	%	%	%	mm E.L.+100	mgKOH/100 g	ml	cc	%
P- I-A-4	13.5	13.6	9.5	95	347	11.30	30	825	61.5
B-4	14.5	15.2	9.8	97	342	11.63	29	850	61.5
C-4	16.4	17.4	10.4	98	330	12.61	28	870	60.5
D-4	17.7	18.6	14.7	95	340	13.24	27	825	64.5
E-4	19.5	20.3	11.1	97	350	12.10	27	790	61.0
F-4	25.7	26.7	9.3	98	340	13.35	27	855	60.5
P- II-A-4	13.6	14.0	10.0	94	320	12.84	27	730	61.0
B-4	14.6	15.0	10.0	93	269	11.96	27	720	59.0
C-4	17.1	18.4	10.6	93	290	16.25	28	715	60.0
D-4	17.8	19.9	10.6	69	232	33.99	26	735	59.0
E-4	21.0	21.1	10.7	51	261	47.58	27	730	59.0
F-4	26.4	26.2	12.5	21	243	47.94	19	745	57.5
P-III-A-4	13.6	13.6	9.4	92	285	14.85	23	770	61.0
B-4	14.7	14.5	9.6	86	174	15.47	26	765	61.0
C-4	16.4	15.8	10.0	39	157	51.41	25	800	60.0
D-4	18.2	17.4	9.8	41	157	57.88	25	765	59.5
E-4	19.4	22.2	11.0	29	187	67.13	20	635	55.0
F-4	23.8	25.9	11.0	0	138	67.10	19	695	56.0

Table 3, (concl.). Storage time 32 days.

Sample No.	Moisture: After conditioning	Moisture: After storage	Moisture: After drying	Germination	GADA	Fat Acidity	Sedimentation value	Loaf volume	Water Absorption
	%	%	%	%	mm E.L.+100	mgKOH/100 g	ml	cc	%
P- I-A-5	13.5	12.8	9.9	97	350	10.45	30	810	62.0
B-5	14.5	14.3	10.7	97	350	12.35	27	820	64.0
C-5	16.4	17.6	12.3	90	320	13.19	26	780	61.5
D-5	17.7	19.2	11.1	95	310	11.20	28	775	60.0
E-5	19.5	19.8	10.6	90	350	11.44	26	695	54.5
F-5	25.7	23.5	11.1	87	315	13.01	28	840	57.5
P- II-A-5	13.6	12.9	9.4	98	340	12.47	26	735	61.0
B-5	14.6	14.2	9.3	97	283	18.38	27	750	61.5
C-5	17.1	17.3	11.9	75	285	18.92	30	835	64.0
D-5	17.8	18.4	11.7	33	210	43.88	27	805	61.0
E-5	21.0	19.7	12.6	33	215	56.33	27	800	61.0
F-5	26.4	27.3	14.9	0	192	70.20	10	505	57.5
P-III-A-5	13.6	11.7	10.2	88	230	16.77	25	765	64.0
B-5	14.7	13.6	10.4	48	120	23.12	25	790	61.0
C-5	16.4	15.5	10.0	7	85	52.59	26	810	61.0
D-5	18.2	20.2	10.1	0	100	66.13	16	650	57.0
E-5	19.4	22.1	11.2	0	90	71.77	14	515	58.0
F-5	23.8	27.7	11.9	0	60	99.87	13	360	58.0

Table 4. Extent of deterioration of Bison wheat after short storage.[1] Storage time 2 days.

Sample No.	Moisture: After conditioning	Moisture: After storage	Moisture: After drying	Germination	GADA	Fat Acidity	Sedimentation value	Loaf volume	Water Absorption
	%	%	%	%	mm E.L.+100	mgKOH/100 g	ml	cc	%
B- I-C-1	16.5	15.7	10.6	88	340	9.03	35	785	60.0
D-1	18.0	16.5	9.7	95	331	8.63	35	845	60.5
E-1	19.4	20.6	11.0	91	300	9.07	35	860	64.0
F-1	26.1	26.2	11.5	88	287	9.17	34	960	64.0
B- II-A-1	13.5	13.5	10.0	96	315	8.96	35	835	60.5
B-1	15.2	14.9	10.0	97	320	9.26	35	825	63.0
C-1	17.3	16.5	10.0	89	320	10.07	36	805	63.0
D-1	19.2	19.8	10.2	92	327	10.19	36	820	63.0
E-1	20.7	21.0	11.2	92	335	10.30	35	885	63.0
F-1	24.5	24.9	11.9	75	305	11.59	36	930	62.0
B-III-A-1	13.6	14.6	10.5	96	330	10.22	35	855	63.3
B-1	14.7	15.5	9.5	90	317	9.81	36	850	63.0
C-1	16.8	16.7	9.8	88	314	10.13	35	845	61.5
D-1	19.3	18.8	10.2	95	325	10.19	35	880	61.5
E-1	22.2	21.6	11.1	94	320	10.59	35	845	61.0
F-1	23.8	28.0	12.4	87	262	10.74	31	880	60.5

[1] I, stored at +4°C; II, stored at +24°C; III, stored at 38°C,
GADA, glutamic acid decarboxylase activity; E.L., ethyl lactate

Table 4, (cont.). Storage time 4 days.

Sample No.	Moisture: After conditioning	Moisture: After storage	Moisture: After drying	Germination	GADA	Fat Acidity	Sedimentation value	Loaf volume	Water absorption
	%	%	%	%	mm E.L.+100	mgKOH/100 g	ml	cc	%
B- I-C-2	16.5	16.6	10.4	89	309	10.96	34	835	61.0
D-2	18.0	18.0	10.9	95	314	11.47	35	890	62.0
E-2	19.4	20.5	11.7	95	317	10.97	30	865	61.5
F-2	26.1	24.4	13.7	91	310	11.53	35	880	61.5
B- II-A-2	13.5	12.8	10.4	93	312	11.11	34	940	62.0
B-2	15.2	14.0	11.2	94	313	11.21	35	900	62.0
C-2	17.3	17.5	11.8	91	310	12.19	34	900	61.5
D-2	19.2	18.4	11.2	90	310	12.12	35	905	61.5
E-2	20.7	20.6	11.4	92	311	12.13	35	925	62.0
F-2	24.5	24.1	11.3	82	310	13.04	36	925	63.0
B-III-A-2	13.6	12.8	10.5	92	325	10.52	34	875	61.5
B-2	14.7	13.5	11.0	95	328	11.18	35	855	62.0
C-2	16.8	16.5	10.9	92	323	11.47	33	860	61.5
D-2	19.3	18.9	12.2	90	330	11.94	34	895	62.0
E-2	22.2	20.8	12.6	84	250	13.84	33	905	61.5
F-2	23.8	24.6	13.0	83	210	13.92	31	925	61.5

Table 4, (cont.). Storage time 8 days.

Sample No.	Moisture: After conditioning	Moisture: After storage	Moisture: After drying	Germination	GADA	Fat Acidity	Sedimentation value	Loaf volume	Water absorption
	%	%	%	%	mm E.L.+100	mgKOH/100 g	ml	cc	%
B- I-C-3	16.5	16.1	10.7	89	330	11.15	33	890	61.5
D-3	18.0	17.0	12.0	94	335	11.92	35	855	61.5
E-3	19.4	21.9	11.6	86	320	12.17	35	855	61.5
F-3	26.1	24.2	12.5	85	312	11.98	36	970	61.0
B- II-A-3	13.5	13.8	11.3	93	346	11.82	37	860	63.0
B-3	15.2	15.6	12.4	93	325	12.58	36	815	65.0
C-3	17.3	16.8	10.8	94	324	12.07	31	925	61.5
D-3	19.2	20.7	10.7	89	335	15.05	36	860	61.5
E-3	20.7	20.6	11.4	72	310	17.91	31	875	60.5
F-3	24.5	24.8	12.1	32	287	19.88	36	910	60.5
B-III-A-3	13.6	13.7	10.4	91	351	13.21	35	905	62.0
B-3	14.7	15.3	11.7	94	354	13.70	33	865	62.0
C-3	16.8	18.0	11.0	77	322	19.94	27	810	61.5
D-3	19.3	20.1	12.3	63	258	20.24	33	835	61.5
E-3	22.2	20.8	12.7	56	224	24.33	34	790	61.5
F-3	23.8	24.3	13.3	44	138	30.40	29	800	60.0

Table 4, (cont.). Storage time 16 days.

Sample No.	Moisture: After conditioning	Moisture: After storage	Moisture: After drying	Germination	GADA	Fat Acidity	Sedimentation value	Loaf volume	Water absorption
	%	%	%	%	mm E.L.+100	mgKOH/100	ml	cc	%
B- I-C-4	16.5	17.5	10.4	90	331	13.51	30	870	57.5
D-4	18.0	18.5	11.9	93	309	13.73	34	875	60.0
E-4	19.4	22.3	11.1	95	306	12.10	36	900	60.0
F-4	26.1	26.8	10.5	91	262	13.22	35	895	60.5
B- II-A-4	13.5	13.8	10.2	92	354	12.58	34	860	60.5
B-4	15.2	14.1	9.7	90	335	13.09	34	845	60.0
C-4	17.3	17.3	11.5	88	325	17.01	34	885	60.0
D-4	19.2	17.9	11.9	57	303	17.70	33	870	60.0
E-4	20.7	19.5	12.2	48	300	29.42	32	840	60.0
F-4	24.5	26.5	13.6	31	240	38.00	27	775	57.5
B-III-A-4	13.6	15.8	10.1	93	261	13.46	31	970	62.0
B-4	14.7	16.5	9.8	79	206	15.81	34	885	61.5
C-4	16.8	17.7	11.0	38	150	47.14	30	820	60.5
D-4	19.3	18.3	10.9	28	150	51.91	26	760	59.0
E-4	22.1	21.9	9.9	33	182	50.78	24	615	57.0
F-4	23.8	26.7	11.8	0	132	54.27	24	485	57.5

Table 4, (concl.). Storage time 32 days.

Sample No.	Moisture: After conditioning	Moisture: After storage	Moisture: After drying	Germination	GADA	Fat Acidity	Sedimentation value	Loaf volume	Water absorption
	%	%	%	%	mm E.L.+100	mgKOH/100	ml	cc	%
B- I-C-5	16.5	17.3	11.1	83	320	12.41	34	870	62.0
D-5	18.1	18.9	11.9	90	350	12.21	36	930	63.0
E-5	19.4	21.3	11.6	90	340	11.26	34	990	59.5
F-5	26.1	24.1	13.2	80	310	13.94	36	990	60.5
B- II-A-5	13.5	13.0	10.8	89	351	12.66	35	840	62.5
B-5	15.2	14.6	10.5	87	290	13.53	35	885	64.0
C-5	17.3	17.1	14.4	60	282	28.27	31	865	68.5
D-5	19.2	19.8	12.6	36	235	40.95	32	795	63.0
E-5	20.7	21.0	12.2	26	230	41.99	23	630	60.5
F-5	24.5	28.0	13.5	0	184	49.76	12	510	57.5
B-III-A-5	13.6	11.8	9.9	96	240	18.70	33	920	63.0
B-5	14.7	14.1	12.6	4	138	28.31	31	825	65.0
C-5	16.8	16.5	11.2	18	122	43.29	28	865	62.0
D-5	19.3	20.2	11.1	0	115	48.42	23	675	58.0
E-5	22.2	21.3	11.5	0	121	48.60	18	440	58.0
F-5	23.8	24.9	11.2	0	110	51.52	17	355	58.5

content ranged from 16.5 to 26.1 for +4°C (I), 13.5 to 24.5 percent for +24°C (II), and 13.6 to 23.8 percent for +38°C (III), instead of the intended range from 16 to 25 percent in case of +4°C storage and 14 to 25 percent for higher temperatures. In spite of airtight storage, the moisture content changed somewhat with time.

After 32 days of storage, the moisture content for Pawnee wheat ranged from 12.8 to 23.5 at +4°C, from 12.9 to 27.3 at +24°C and from 11.8 to 27.7 percent at +38°C. Similarly with Bison, the moisture content after 32 days of storage varied from 17.3 to 24.1 at +4°C, from 13.1 to 28.0 at +24°C, and from 11.8 to 24.9 percent at +38°C. Generally, as can be seen from Table 3 for Pawnee and Table 4 for Bison, the high moisture samples stored at higher temperatures showed a consistent increase in moisture content, whereas low-moisture wheats exhibited a decrease.

Moisture content after final drying at +37°C ranged from 9.2 to 13.0 percent for Pawnee and from 9.6 to 13.7 percent for Bison wheats.

Germination Percent. After 32 days of storage at +4°C, germination percent for both Pawnee and Bison wheats remained virtually unchanged (Tables 3 and 4). At +24° and +38°C, germination percent decreased progressively, with increasing moisture content, temperature, and storage time. After 32 days of storage at +24°C, the germination percentage ranged from 0 to 98 percent for Pawnee and from 0 to 89 percent for Bison. At +38°C, germination percentage was still lower, 0 to 7 percent for Pawnee and 0 to 18 percent for Bison.

Fat Acidity. Wheats stored at +4°C showed little increase in fat acidity up to 32 days of storage, regardless of moisture content (a range of 10.08 to 13.01 for Pawnee and from 9.03 to 13.94 for Bison). An increase in storage temperature brought up drastic increases in fat acidity particularly at higher

moisture levels, up to 70.20 at +24°C and 99.87 at +38°C with Pawnee and up to 49.76 at +24°C and 51.52 at +38°C with Bison after 32 days of storage.

Glutamic Acid Decarboxylase Activity. Generally, little change in GADA was observed for the refrigerated samples: from 304 to 350 for Pawnee and from 340 to 320 for Bison at the lowest moisture content, and from 310 to 315 with Pawnee and from 287 to 310 with Bison at the highest moisture level. Even at +24°C, little change was observed at low moisture levels (from 360 to 340 with Pawnee and from 315 to 351 with Bison), whereas an increase in moisture content brought about a progressive decrease in GADA with increasing storage time (ranging after 32 days from 270 to 192 with Pawnee and from 305 to 184 with Bison). At +38°C, even at the lowest moisture level, there was a noticeable decrease in GADA, the magnitude of decrease increasing drastically with an increase in moisture content. Under these conditions, at the highest moisture levels, GADA dropped to zero.

Sedimentation Value. The results of the sedimentation test were reported as milliliters of sediment. As shown in Table 3 (for Pawnee) and 4 (for Bison) no significant changes took place, regardless of moisture content, when wheat was stored at +4°C. Even at elevated temperatures, little decrease was observed at the lowest moisture level, whereas an increase in moisture content brought about marked changes in the sedimentation value. At the highest moisture level, at +24°C, the sedimentation value dropped from 29 to 10 with Pawnee and from 36 to 12 with Bison.

Baking Characteristics. Figures 4 to 6 show the changes of the farinograms exhibited by the flours from Pawnee and Bison wheats indicating the extent of storage damage. For both wheats, water absorption changed little during storage at +4°C and +24°C, whereas at +38°C, there was a slight decline,

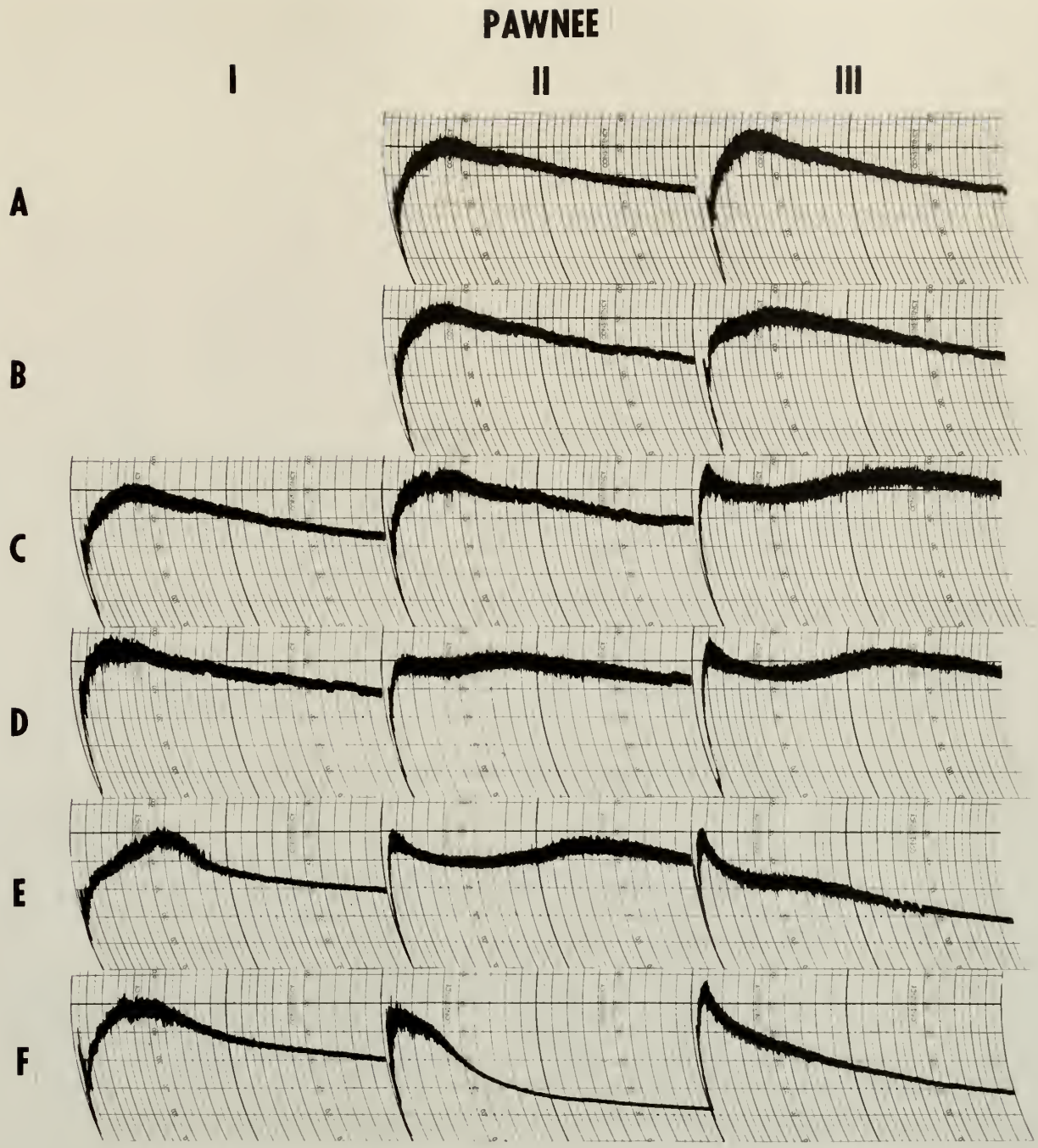

Fig. 4. Farinograms for Pawnee wheat stored at various temperature and moisture levels for 32 days. For details see Table 3.

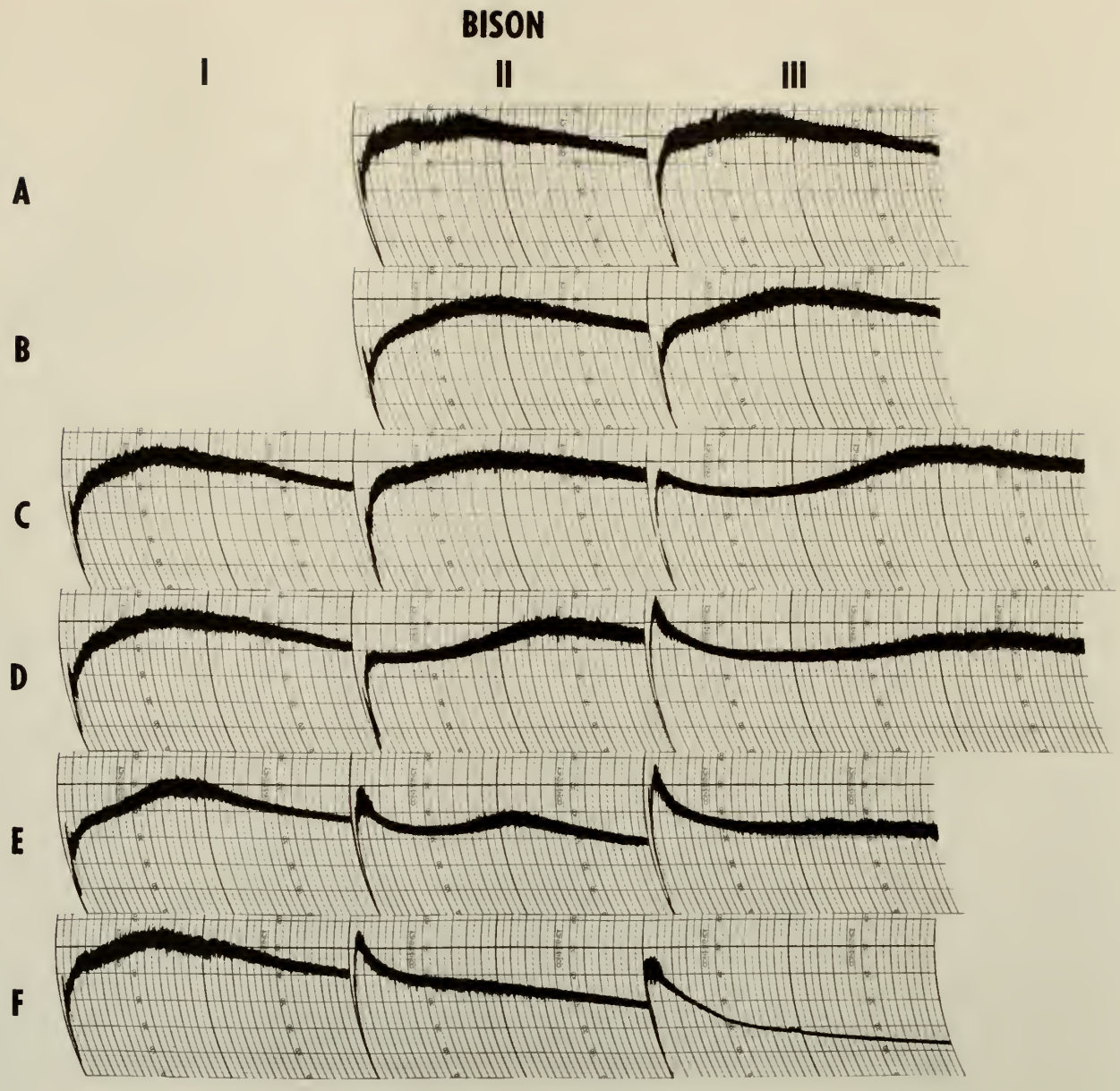

Fig. 5. Farinograms for Bison wheat stored at various temperature and moisture levels for 32 days. For details see Table 4.

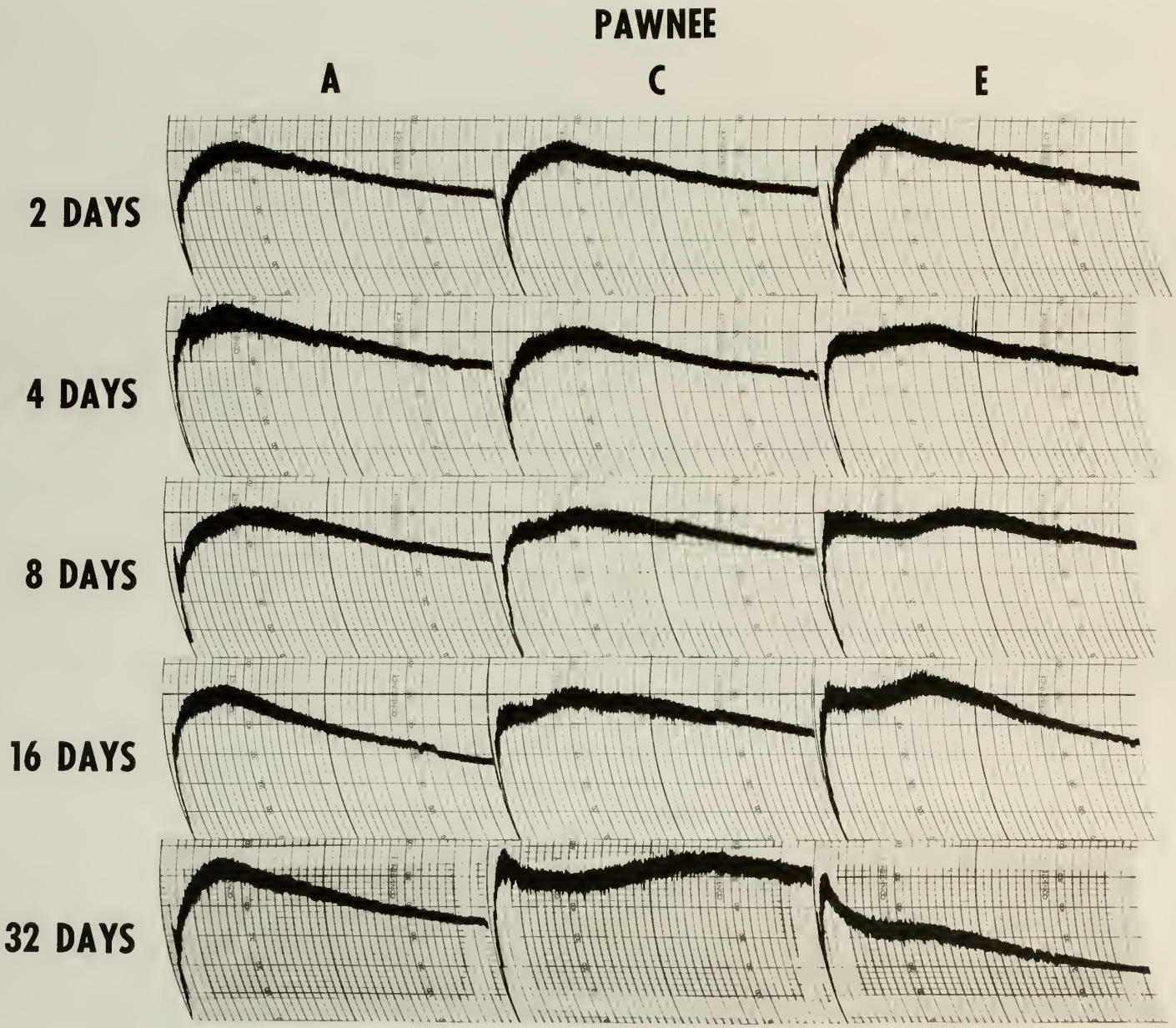

Fig. 6. Farinograms for Pawnee wheat stored at various moisture levels for various periods of time at 38°C. For details see Table 3.

the magnitude depending on moisture content. At the highest moisture level, the absorption dropped from an original of 64.0 percent to 58.0 percent with Pawnee and from 60.5 percent to 58.5 percent with Bison.

Changes in loaf volume followed the same general pattern as observed with various indexes of deterioration. No significant changes were observed at +4°C at any moisture level up to 32 days storage. However, an increase in temperature to +24°C and, particularly, to +38°C caused marked decreases in loaf volume after about two weeks of storage at moisture levels above approximately 18 percent moisture content. At +24°C at the highest moisture level loaf volume dropped from the original of 830 to 745 cc with Pawnee and from 930 to 775 with Bison after 16 days of storage, and from 745 to 505 cc with Pawnee and 775 to 510 with Bison after 32 days storage. Similarly, at +38°C, the loaf volume decreased from 805 to 695 cc with Pawnee and from 880 to 485 with Bison after 16 days and from 695 to 360 with Pawnee and 485 to 355 with Bison after 32 days storage.

Figures 7 to 9 illustrate some examples on the effect of moisture, temperature, and storage time on loaf volume of Bison wheat. There was a marked darkening of crumb color at +24°C at moisture levels above approximately 16.0 percent, but only after wheat was stored for about one month. At +38°C, the same effect could be observed already after two week's storage. Generally, the degree of darkening increased with an increase in the moisture content of stored wheat.

Quantitative Determination of Wheat Protein

The protein content of the wheats studied ranged from 10.4 to 18.8 percent and that of flour from 9.4 to 17.1 percent, Table 5. Figures 10 to 12

Fig. 7. Loaves baked from Bison wheat stored at 38°C. for 8 days at various moisture levels. Final moisture of wheat after storage: (4) = 13.7% (5) = 15.3% (6) = 18.0% (7) = 20.1% (8) = 20.8% (9) = 24.3%.

Fig. 8. Loaves baked from Bison wheat stored at 38°C for 16 days at various moisture levels. Final moisture after storage: (10) = 15.8% (11) = 16.5% (12) = 17.7% (13) = 18.3% (14) = 21.9% (15) = 26.7%.

Fig. 9. Loaves baked from Bison wheat stored at 24°C for 32 days at various levels. Final moisture after storage: (12) = 13.1% (13) = 14.6% (14) = 17.1% (15) = 19.8% (16) = 21.0% (17) = 28.0%.

Table 5. Simple correlation coefficients between dye absorbance and Kjeldahl protein with wheat and flour.

Dye	Material	Correlation Coefficient	Degrees of Freedom	Regression Equation: Y = Kjeldahl Protein X = Dye Absorption
Amido black 10B	Flour	r = 0.914***	105	y = 17.15x + 19.69
	Wheat	r = 0.921***	190	y = 19.89x + 20.28
	Flour + wheat	r = 0.924***	297	y = 17.67x + 19.64
Lissamine green SF 150 (with water)	Flour	r = 0.951***	32	y = 42.54x + 30.48
	Wheat	r = 0.949***	68	y = 39.10x + 29.02
	Flour + wheat	r = 0.932***	102	y = 39.07x + 29.03
Lissamine green SF 150 (with buffer pH 6.0)	Flour	r = 0.993***	37	y = 23.57x + 30.06
	Wheat	r = 0.992***	68	y = 23.20x + 29.71
	Flour + wheat	r = 0.995***	107	y = 23.51x + 29.97
Orange G	Flour	r = -0.929***	182	y = 46.45x + 37.64
	Wheat	r = -0.890***	74	y = 47.71x + 40.66

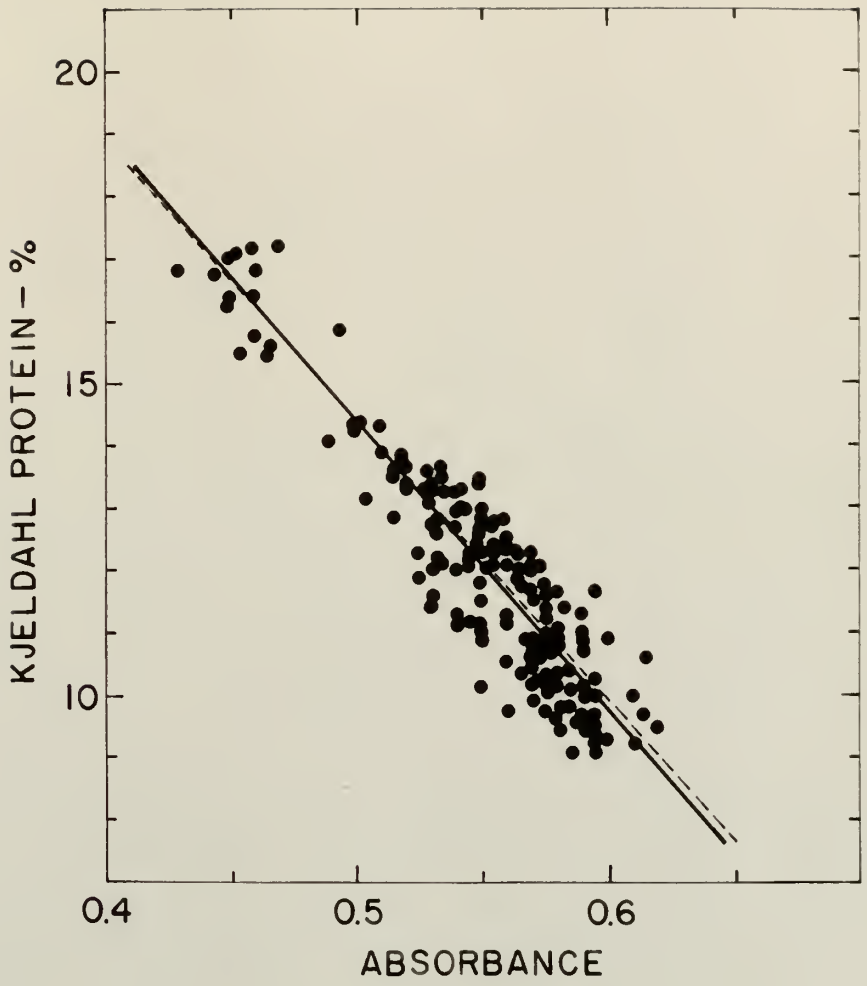

Fig. 10. Relation between the absorbance of excess orange G and Kjeldahl protein with wheat flour. Dotted line represents that obtained by Udy (1956).

$y = -46.45x + 37.64$ ($r = -0.929^{***}$).

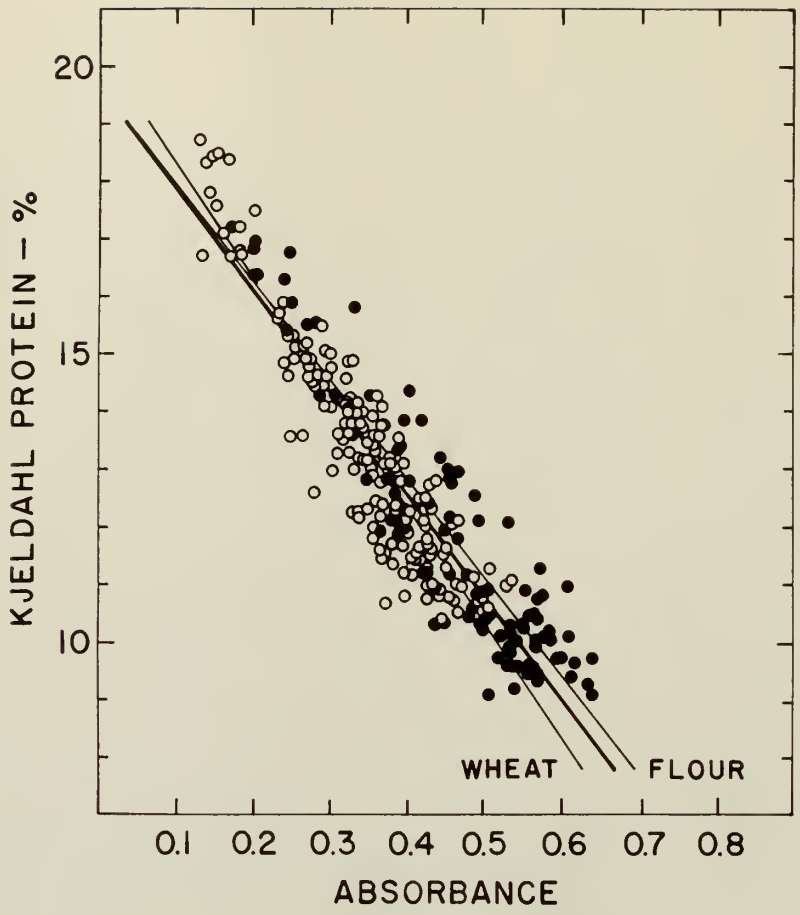

Fig. 11. Relation between the absorbance of excess amido black 10 B and Kjeldahl protein with wheat and wheat flour.

• Flour o Wheat Heavy line represents total regression.

Flour: $y = -17.15x + 19.69$ ($r = -0.914^{***}$).

Wheat: $y = -19.89x + 20.28$ ($r = -0.921^{***}$).

Total: $y = -17.67x + 19.64$ ($r = -0.924^{***}$).

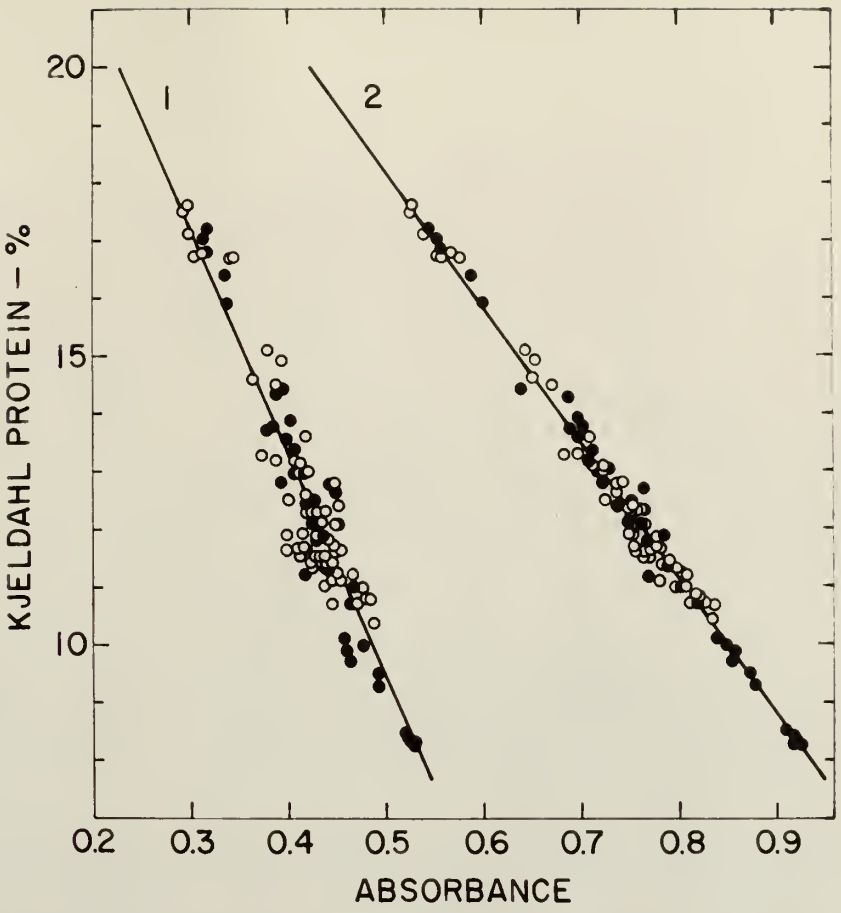

Fig. 12. Relation between the absorbance of excess lissamine green SF 150 and Kjeldahl protein with wheat and wheat flour. • Flour o Wheat Lines represent total regression. (1) Diluted with water (2) Diluted with phosphate buffer pH 6.0.

(1) $y = -39.07x + 29.03$ ($r = -0.932^{***}$).

(2) $y = -23.51x + 29.97$ ($r = -0.995^{***}$0.

summarize the results from dye absorption protein determination. Table 5 shows some correlations obtained between Kjeldahl protein and the dye absorption method with different dyes.

Figure 10 shows results obtained using Orange G with wheat flour, the correlation between the absorbance of excess dye and Kjeldahl protein being -0.929^{***}. The correlation obtained with wheat was even lower ($r = -0.890^{***}$). As illustrated by Fig. 11, similar results were obtained with amido black 10 B ($r = -0.914^{***}$ with flour; $r = -0.921^{***}$ with wheat), except that the regression coefficients representing wheat and wheat flour differed from each other significantly less. Significantly high correlation was obtained between Kjeldahl protein of wheat and flour samples together and the observance of excess amido black 10 B ($r = -0.924^{***}$, 297 d.f.).

As shown in Fig. 12 (1), $r = -0.951^{***}$ with flour; $r = -0.949^{***}$ with wheat was observed between Kjeldahl protein and absorbance of excess lissamine green SF 150.

The effects of time and pH on the absorbance of lissamine green SF 150, are shown in Table 6.

Table 6. The effect of time on absorbance at 635 mu of lissamine green SF 150 solutions after dilution by various media. Final dye concentration 0.0025%.

Time, minutes	Diluent		
	Citrate buffer (pH 2.2)	Water (pH 3.3)	Phosphate buffer (pH 6.0)
2	0.505	0.560	1.10
5	0.402	0.670	1.34
10	0.302	0.750	1.35
15	0.262	0.795	1.35
20	0.240	0.840	1.35
25	0.235	0.865	1.35
30	0.234	0.885	1.35
45	0.232	0.930	1.35
60	0.238	0.940	1.35
120	0.240	0.855	1.35

Figure 12 (2) illustrates results obtained with phosphate buffer of pH 6.0 as diluent. Table 5 shows the regression equations, and correlation coefficients with Kjeldahl protein ($r = -0.993^{***}$ with flour; $r = -0.992^{***}$ with wheat; $r = -0.995^{***}$ with flour plus wheat) which are significantly higher (at 0.1 percent level) than those involving the other two dyes. Table 5 also shows that the regression coefficients in case of flour and wheat are practically the same.

Storage Deterioration of Corn

Germination Percentage. The germination percentage varied from 0 to 93 percent (Table 7), with only few samples possessing a germination percent above 90. Table 8 and Figs. 13 and 14 illustrate correlations between the different measurements.

Fluorescence. As shown in Table 7, fluorescence values ranged from 19 to "infinity", with majority of the samples lying in the range of 20 to 30.

Fat Acidity. As shown in Table 7, fat acidity varied from 15.80 to 143.5. Figure 13 shows the relationship obtained between fat acidity and germination percentage of corn. A low correlation coefficient ($r = -0.433^{***}$, 60 d.f.) was obtained. The correlation between the benzene extraction and the colorimetric methods to determine fat acidity was relatively low ($r = +0.443$, 32 d.f.).

Glutamic Acid Decarboxylase Activity. GADA of corn, in mm. ethyl lactate, was generally somewhat higher throughout the germination range than GADA of wheat of same germination, as shown in Fig. 14. Table 7 shows that the values of GADA ranged from 67 to 443, while Fig. 14 shows the relationship between

Table 7. Extent of deterioration of corn samples.

Sample No.	Moisture	Germination	Fluorescence	GADA[1]	Fat Acidity: Benzene Extraction	Fat Acidity: $Cu(AC)_2$ method
	%	%	E/g	mm E.L.[2]+100	mgKOH/100 g	E/g
C- 1	15.1	30	27.0	136	76.67	
C- 2	14.8	93	77.0	394	15.80	0.340
C- 3	15.2	77	35.0	387	25.31	0.454
C- 4	13.3	68	20.0	307	23.29	0.072
C- 5	12.6	40	19.0	261	16.85	0.042
C- 6	16.1	71	86.0	255	59.69	0.695
C- 7	11.2	18	22.0	155	47.71	
C- 8	13.9	34	25.0	187	43.46	0.147
C- 9	12.8	12	23.0	127	38.90	0.255
C-10	14.9	10	45.0	84	109.64	1.240
C-11	16.4	62		266	81.03	0.980
C-12	18.40	11		126	21.31	
C-13	15.6	65	39.0	281	90.18	0.960
C-14	13.9	81	22.0	347	40.79	
C-15	14.7	93	23.0	443	27.48	
C-16	11.8	26	39.0	162	49.87	0.345
C-17	16.0	52	41.0	238	130.34	0.960
C-18	12.7	53	25.0	256	37.23	
C-19	12.3	57	25.0	265	42.90	
C-20	11.9	79	26.0	378	28.52	0.107
C-21	13.7	19	29.0	139	55.36	
C-22	14.7		37.0	79	85.00	0.790
C-23	15.6		42.5	147	42.21	1.350
C-24	16.1		24.0	115	98.97	1.350
C-25	12.5		23.0	152	31.32	0.235

[1]GADA = glutamic acid decarboxylase activity.

[2]E. L.= ethyl lactate.

Table 7, (cont.).

Sample No.	Moisture	Germination	Fluorescence	GADA[1]	Fat Acidity: Benzene Extraction	Fat Acidity: $Cu(AC)_2$ method
	%	%	E/g	mm E.L.[2]+100	mgKOH/100 g	E/g
C-26	12.1		25.0	121	39.93	
C-27	12.2		23.0	121	35.13	
C-28	22.1		49.0	67	143.49	
C-29	12.4		44.0	123	70.12	
C-30	14.2		26.0	417	23.47	0.138
C-31	15.2		23.5	422	16.41	0.128
C-32	12.6		31.0	114	58.41	
C-33	12.4		32.5	136	37.40	0.445
C-34	12.3	31	40.0	192	34.87	
C-35	12.3	84	21.0	357	17.55	
C-36	16.4	98	43.0	460	18.29	
C-37	11.5	21	28.0	153	73.31	
C-38	10.2	0	26.0	80	80.41	
C-39	11.5	68	26.0	228	28.60	
C-40	17.5	55		328	109.81	0.430
C-41	14.8	12	42.0	147	32.24	
C-42	17.6	83	79.0	308	52.45	
C-43	13.4	66	24.0	284	21.24	
C-44	14.2	60	30.0	251	20.74	0.730
C-45	11.6	71	22.0	257	37.04	
C-46	11.4	35	23.0	181	19.12	
C-47	11.0	0	46.0	115	88.71	
C-48	12.4	36	23.0	168	18.83	
C-49	8.6	74	24.0	242	30.62	
C-50	8.5	38	24.0	157	45.98	

Table 7, (concl.)

Sample No.	Moisture	Germination	Fluorescence	GADA[1]	Fat Acidity: Benzene Extraction	Fat Acidity: $Cu(AC)_2$ method
	%	%	E/g	mm E.L.[2]+100	mgKOH/100 g	E/g
C-51	11.7	39	23.0	173	32.30	0.305
C-52	11.7	36	23.0	188	47.56	0.180
C-53	11.7	33	22.5	206	50.20	0.225
C-54	12.0	38	23.5	170	44.06	0.195
C-55	11.6	30	27.0	174	35.48	0.175
C-56	11.6	32	25.0	184	39.80	0.150
C-57	11.3	10	26.0	123	38.96	0.300
C-58	11.0	30	24.0	168	43.79	0.480
C-59	22.5	43		160	42.48	0.640
C-60	16.7	27	39.0	152	62.88	1.300
C-61	11.4	19	28.0	123	73.89	0.590
C-62	11.8	15	33.0	118	76.17	0.570

Table 8. Simple correlation coefficients between various viability determinations with corn samples.

Correlation	Correlation Coefficient	Degrees of Freedom	Regression Equation	y	x
Log GADA vs. % germination	r = +0.949***	60	Log y = 0.00675x + 1.991	GADA	% germination
Fat acidity (Std. meth.) vs. % germination	r = -0.433***	60	Log y = -0.453x + 68.6	F.A.	% germination
Fat acidity (Std. meth. vs. Cu- meth.)	r = +0.443**	32			

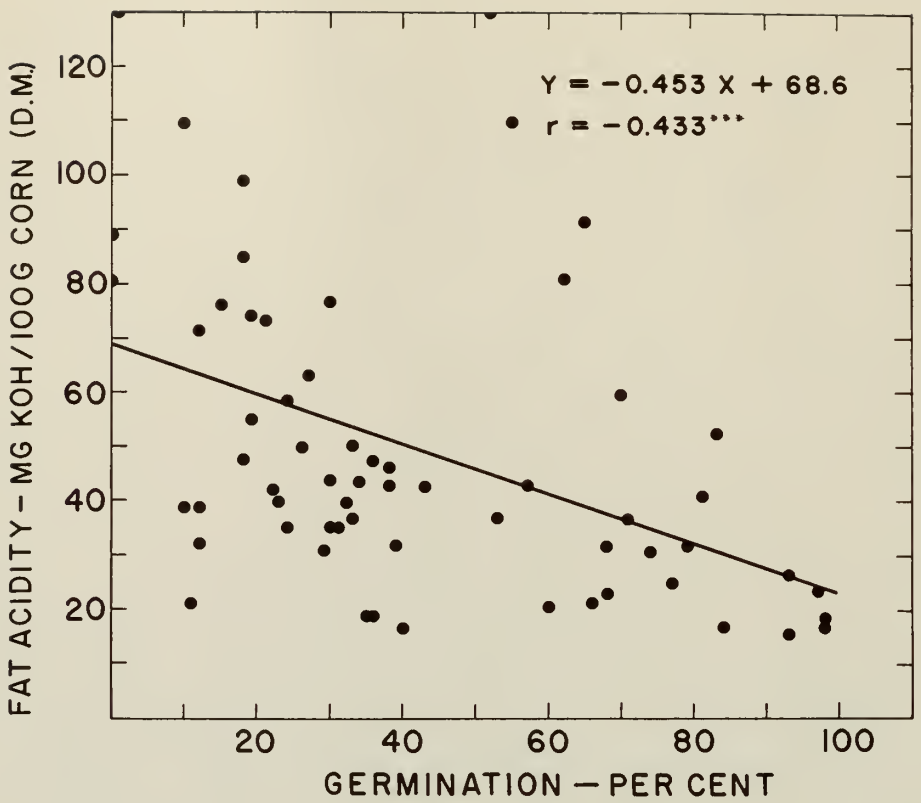

Fig. 13. Relation between fat acidity and germination percentage of 62 corn samples.

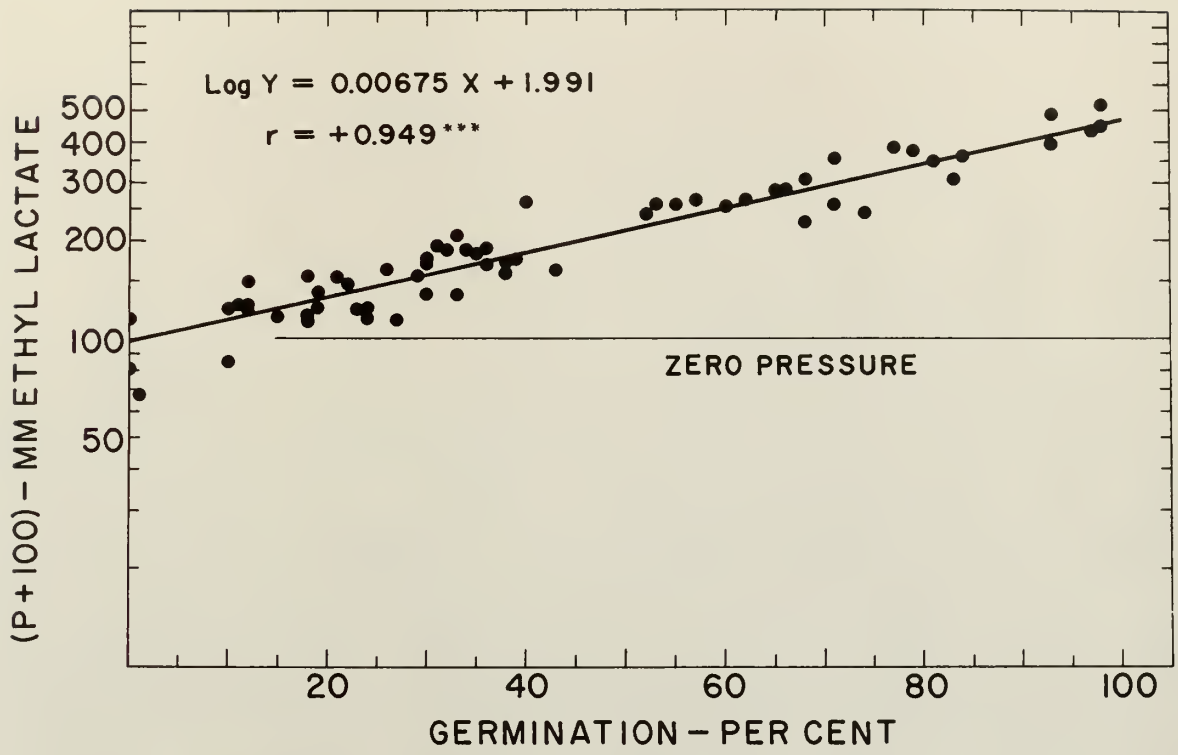

Fig. 14. Relation between log glutamic acid decarboxylase activity and germination percentage of 62 corn samples.

log GADA and germination percentage of 62 corn samples. The correlation coefficient ($r = +0.949^{***}$, 60 d.f.) was of the same order of magnitude as with wheat ($r = +0.920^{***}$, 250 d.f.) and significantly higher at 0.1 percent level than the correlation involving fat acidity.

DISCUSSION

In the present study, the germination percentage was used as a primary standard in determining the viability of the wheat and corn samples (Sogn, 1960). Thus germination percent was, in turn, correlated with the various tests to determine viability and storage deterioration.

Pomeranz and Shellenberger (1961) had shown a definite decrease in the acridine orange absorption by wheat germs stored at elevated moistures and temperatures. In the present study, a low correlation was obtained between the absorbance of excess acridine orange and fluorescence ($r = -0.280^{ns}$), and fat acidity ($r = 0.339^{*}$), respectively.

However, a moderately high significant correlation was obtained between the absorption of acridine orange with log GADA ($r = -0.604^{***}$) of wheat samples of varying degree of deterioration. This suggested that the absorption of acridine orange by wheat may be related to the protein deterioration during storage of commercial wheats. Apparently, however, other factors are also involved, resulting in a relatively low correlation coefficient from a point of view of practical application. It seems that germ proteins are mainly involved (Pomeranz and Shellenberger, 1961), but some variation in dye absorption by flour, too, decreases the usefulness of the test for evaluating wheats.

These results with acridine orange gave reason to believe that dyes

commonly used for quantitative protein determination might be absorbed differently, depending on the degree of deterioration of wheat. However, orange G, amidoblack 10 B, and lissamine green SF 150 did not show any significant evidence of being affected by the storage deterioration of wheat proteins, as evidenced by the relatively high correlations obtained with Kjeldahl protein determinations with wheats widely varying in quality and degree of deterioration.

However, the high correlations ($r = +0.997$ with flour; $r = +0.992$ with wheat) reported by Udy (1956) could not be reproduced with orange G in the present work. Figure 10 shows results obtained with wheat flour, the correlation between the absorbance of excess dye and Kjeldahl protein being -0.929^{***}. The correlation obtained with wheat was even lower ($r = -0.890^{***}$). As illustrated by Fig. 11, similar results were obtained with amidoblack 10 B, except that the regression coefficients representing wheat and wheat flour differed from each other significantly less.

The first experiments performed with lissamine green SF 150 were encouraging (Fig. 12 (1); $r = -0.951^{***}$ with flour; $r = -0.949^{***}$ with wheat). Unfortunately, replicate determinations performed during different days occasionally showed some unexpected variations. Since it was reported previously (Gorringe, 1957) that lissamine green SF 150 does not fade at pH 6.0 or less, the final supernatant was diluted with distilled water as in case of the other two dyes. The pH increased during dilution about to 3.3. It was then observed that the absorbance increased under these conditions very rapidly during the first 30 minutes after dilution. Table 6 shows the effect of time and pH on the absorbance of diluted lissamine green SF 150 solutions. Best results were obtained using 0.067 M phosphate buffer of pH 6.0 as diluent. After reaching a maximum within 10 minutes, the color remained stable for at least

two hours.

Figure 12 (2) illustrates results obtained with phosphate buffer of pH 6.0 as diluent. The regression equations, and correlation coefficients with Kjeldahl protein were as follows:

Flour: $y = -23.57\ x + 30.06$; $r = -0.993^{***}$

Wheat: $y = -23.20\ x + 29.71$; $r = -0.992^{***}$

Total: $y = -23.51\ x + 29.97$; $r = -0.995^{***}$

where y = Kjeldahl protein and x = absorbance of the excess dye in dilution 1:100. The durations between different standard curves are well within the experimental error.

Although dye absorption techniques hardly can compete with Kjeldahl protein determination in large scale routine testing, they may be of great value where expensive equipment is not available. Thus, e.g., at the grain elevator a rapid and accurate estimate of the protein content of wheat can be obtained within minutes.

In commercial wheats, the values of fluorescence varied little with the exception of extensively deteriorated samples, the majority of the samples falling within the range of 16 to 30. This is in good accordance with the results from the earlier work of Linko and Sogn (1960) who observed little increase in fluorescence down to about 20.0 percent germination, whereafter fluorescence occasionally showed a drastic increase. Thus, Sogn (1960) obtained a relatively low correlation ($r = -0.637^{***}$) between fluorescence and GADA as determined by Warburg manometric techniques. A somewhat higher correlation ($r = -0.758^{***}$) was found between fluorescence and germination percentage which agreed well with the results obtained by Cole and Milner (1953; $r = -0.775^{***}$) and Sorger-Domenigg et al. (1955b; $r = -0.663^{***}$).

Neglecting highly deteriorated samples of 0 percent germination, the correlation decreased significantly. The increase in fluorescence only in extensively deteriorated samples is consistent with the observation by Linko et al. (1960) that primary browning products in "sick" wheat development are not fluorescent; viability may be lost before any browning of germ can be noticed, and visually detectable browning precedes any marked increase in fluorescence. Similar results were obtained with corn (Table 7).

As shown in Tables 1, 3 and 4 for wheat and Table 7 for corn, fat acidity values showed a relatively wide range. A high significant negative correlation was obtained between fat acidity and percent germination, with commercial wheats ($r = -0.731^{***}$, 66 d.f.). This is significantly lower than the correlations obtained by Sogn (1960) who found a very high correlation between fat acidity and germination percentage ($r = -0.906^{***}$, 23 d.f.) with a similar series of wheats, except of smaller population. His values agree well with that of Sorger-Domerigg et al. (1955b); ($r = -0.915^{***}$) with commercial wheats. In both cases, however, there was a relatively greater number of badly damaged wheats. The effect of such wheats in correlations was well illustrated by Linko and Sogn (1960) who showed that the omission of the only badly deteriorated sample from a series of 20 wheats dropped the correlation between fat acidity and germination percentage from $r = -0.920^{***}$ to $r = +0.042^{ns}$.

An attempt to correlate fat acidity, as obtained by the benzene extraction method, with the recently published (Baker, 1961) colorimetric copper acetate method was not successful. Although Baker reported correlations as high as $r = -0.988$ for wheat, and $r = -0.983$ for corn, almost no correlation was found in the present work ($r = +0.267^{ns}$, 48 d.f. with wheat; $+0.433^{**}$, 32 d.f., with corn). However, it was felt that the data obtained were not sufficient as

conclusive evidence.

Storing Pawnee and Bison wheats for about a month at +4°C failed, even at moisture levels up to 26 percent, to result in an increase in fat acidity. But with increasing storage temperature to +24° and +38°C, fat acidity increased rapidly in high moisture samples. The increase was related to temperature, moisture content, time of storage and, generally (by visual inspection), to mold growth. At +24°C and 26.5 percent moisture fat acidity reached a high value of 70.2 in 32 days with Pawnee wheat. At +38°C, a similar increase in fat acidity was reached much sooner again seemingly parallel with mold growth. At 23.8 percent moisture level fat acidity reached values as high as 40.8 in 8 days. After about one month, at +38°C values as high as 99.87 were obtained.

As can be seen from Tables 3 and 4, samples of Pawnee and Bison wheats may be safely stored at all temperature levels used in this study up to one month without a significant fat hydrolysis as long as the moisture content is kept below 15.0 percent.

Figure 13 shows the relationship obtained between fat acidity and germination percentage of corn. The correlation coefficient ($r = -0.433^{***}$, 60 d.f.) was highly significant but low. Although the five samples above 90 percent germination possessed low fat acidity values, also several samples of very low viability showed little if any increase in fat acidity. Furthermore, some samples having a germination percentage as high as 70 to 80, showed fat acidity values from 30 to 60. Although Zeleny and Coleman (1938, 1939) had shown fat acidity to be more reliable as a measure of the degree of soundness than any other test then available, Bottomley *et al.* (1950) found a low correlation ($r = +0.20$) between mold count and fat acidity. Under anaerobic

conditions fat acidity did not exceed value 40, and it was later shown that in nonaerated corn samples fat acidity may remain relatively constant despite a decrease in viability (Bottomley et al., 1952).

It has been shown that glutamic acid decarboxylase activity decreases markedly during storage of moist wheat (Rohrlich, 1957; Cheng, 1959; Linko and Sogn, 1960; Linko, 1960c, 1961a). Due to the activation of glutamic acid decarboxylase by an increase in moisture content (Linko and Milner, 1959c; Linko, 1960d), this phenomenon is preceded by a dissipation of free glutamic acid followed by an increase in free gamma-amino butyric acid (Linko and Milner, 1959a; Linko, 1960a, b, 1961b).

As shown in Table 2 and Fig. 2., the log GADA, in mm ethyl lactate plus 100, of commercial wheats is highly correlated with germination percentage ($r = *0.906^{***}$, 71 d.f.), thus supporting earlier observations. Even a slightly higher, although not significantly different, correlation was obtained when the results were pooled with previous data from this laboratory ($r = +0.920^{***}$, 115 d.f.). Linko and Sogn (1960) obtained a correlation $r = +0.921^{***}$ and Linko (1961) $r = +0.928^{***}$. The work of Linko and Sogn (1960) had suggested that pyruvic acid decarboxylase (carboxylase) activity would be less dependent on varietal differences and thus be an even better indication of the extent of deterioration. The evidence was, however, inconclusive, owing to the small number of samples investigated. In the present work, a high correlation was obtained between log pyruvic acid decarboxylase activity and percent germination ($r = +0.927^{***}$, 76 d.f.). The correlation coefficient was not, however, significantly different from that involving log GADA. Therefore, any further work with pyruvic acid decarboxylase was discontinued.

Table 3 (for Pawnee) and Table 4 (for Bison) show a general decrease in the glutamic acid decarboxylase activity as the germination decreases and as fat acidity increases with an increase in temperature and moisture content. The refrigerated samples showed little change in GADA with an increase in storage time regardless of moisture content. Even samples stored at +24°C, showed little decrease in GADA up to 32 days' storage, except when moisture content exceeded 20 percent. But at +38°C, decrease in GADA became very pronounced at elevated moisture levels. A marked decrease took place at moisture levels above 16 percent in two weeks both with Pawnee and Bison wheats. Glutamic acid decarboxylase activities finally dropped to zero or near nil after one month's storage at +38°C above approximately 18 percent moisture levels. The slight variation in the results between Pawnee and Bison could be explained by varietal differences (Linko and Sogn, 1960).

Again, as could be concluded from fat acidity determinations, samples stored at or below 15.0 percent moisture level could be stored safely up to about one month at all temperature levels studied, without suffering much, if any, deterioration as measured by GADA. At +4°C, wheats up to 25 percent moisture could be stored safely at least up to one month. It should be noticed, however, that increasing the number of moisture levels and extending the storage time might change the safe-storage limits now established.

Figure 14 illustrates the relationship between log GADA and germination percentage of 62 corn samples. The correlation coefficient ($r = +0.949^{***}$, 60 d.f.) was of the same order of magnitude as obtained with wheat ($r = +0.920^{***}$, 250 d.f.). The GADA of corn were generally somewhat higher than those of wheat within the same viability range, but the slopes of the regression lines obtained from populations with similar viability distribution were surprisingly

identical. As shown by Tables 2 and 8 the regression coefficient for wheat was +0.0068 (Linko, 1961b), and for corn +0.00675. The slope tends to increase somewhat with an increase in the number of high viability, high protein wheats in the population (Linko, in press). The higher rate of decarboxylation of glutamic acid with corn may partially explain the early observations by Bailey (1921) and Olafson et al. (1954), that the "respiratory rate" of corn, as indicated by carbon dioxide evaluation, exceeds that of wheat at the same moisture level. Although in the present study, the number of high viability corn samples was limited, Fig. 14 shows that all corn samples possessing a germination percentage above 75 developed a pressure increase above 200 mm ethyl lactate (300 in figure). Thus the techniques for measuring GADA provide not only a quick and reliable way to estimate storage deterioration of corn but will also detect damage caused to proteins by operations such as drying at excessively high temperatures. In addition to protein denaturation, drying at high temperatures also seems to decrease the amount of pyridoxal phosphate, the coenzyme of glutamic acid decarboxylase (MacMasters, et al., 1954). One sample may be analyzed in approximately 45 minutes, or a series of 16 in about two hours, in contrast to the TTC-test which takes a minimum of four hours (Baird et al., 1950). In addition, the method is free of such human errors as may be encountered in the visual inspection required by the TTC test.

Both with wheat and corn, the correlation coefficient between germination percentage and log GADA was significantly higher than that involving fat acidity.

An attempt was also made to correlate sedimentation values with the actual baking quality of wheats stored at various conditions. As shown in Table 3 (for Pawnee) and 4 (for Bison) sedimentation values changed little except for

highly deteriorated samples. For both Pawnee and Bison, no change in the values was observed with samples stored at +4°C regardless of moisture content. The same was true with the samples stored at +24°C up to two weeks' storage. At this temperature, sedimentation value of Pawnee wheat dropped in 16 days from 28 to 19 and that of Bison from 36 to 27. After one month of storage, the sedimentation values had decreased to 10 and 12, respectively. These findings indicate that short storage at room temperature does not alter the sedimentation value of a particular sample if the moisture does not exceed 20 percent. On the other hand, samples stored at +38°C, exhibited marked changes in sedimentation values at moisture levels above approximately 15 percent.

Generally, as can be seen from Tables 3 and 4, a decrease in sedimentation value was followed by a decrease in water absorption and loaf volume. The farinograph and bake mixing times both generally increased with increasing deterioration. When deterioration became very advanced, the farinograph peak representing true mixing time gradually vanished (Figs. 4, 5 and 6). Simultaneously another peak of very short mixing time developed, remaining as the only peak in flours of very advanced deterioration. It is likely that the formation of this secondary peak is, at least partly, associated with water absorption by starch (Pence et al., 1959). Generally, the findings are in good agreement with the observation of several other workers. Zeleny (1947, 1949), in introducing the sedimentation test, found a high correlation between loaf volume and sedimentation value ($r = +0.863$). The correlation coefficient was significantly higher than the correlation between loaf volume and protein content ($r = +0.79$). He assumed that the greater the percentage (up to 50 percent) of wheats with inferior gluten quality in a series, the lower the

correlation between protein content and loaf volume for the entire series, resulting therefore in progressively greater advantage of sedimentation value over protein content as a measure of baking quality. Similarly, other workers observed significant correlations (Capp, 1956, $r = 0.88^{***}$; Harris and Sibbit, 1956, $r = 0.78^{***}$; Shellenberger, 1958, $r = 0.726^{***}$) between loaf volume and sedimentation value, respectively ($r = 0.85^{***}$; $r = 0.54^{***}$; $r = 0.648^{***}$), which are slightly higher than those obtained between loaf volume and protein. Karacsanji (1956) established a correlation of $r = +0.71$ between sedimentation value and dry gluten content.

On the other hand, Miller et al. (1956) obtained a somewhat lower correlation between sedimentation value and loaf volume ($r = +0.62$) but a significantly high correlation between sedimentation value and valorimeter value ($r = +0.84$). They also showed that sedimentation value was highly correlated with protein content ($r = +0.75$), corresponding well to the findings of Zeleny ($r = +0.79$). They concluded that sedimentation test measures part of the protein quality differences among different flours. It may be well worth noticing, however, that wheats included in the study by Miller et al. (1956) all were from the same years' crop, and thus little if any post-harvest deterioration had taken place. With these samples the correlation between protein content and loaf volume was of the same order of magnitude ($r = +0.62$) than that involving sedimentation value.

However, despite some limitations, Zeleny's sedimentation test was found by Fajersson (1961) to be very promising for evaluating Swedish wheat. Furthermore, Schaefer (1957) believes that, if followed carefully, the test can be used to determine commercial value of wheat with sufficient precisions. On the other hand, Haertlein (1956) claims that the sedimentation test as a single

flour-grade score is not sufficiently discriminating and must be supplemented by some of the gluten quality tests.

As shown in Table 3 (for Pawnee) and 4 (for Bison) both loaf volume and sedimentation value of the samples stored at +4°C remained virtually unchanged up to 32 days, except the one stored above 25 percent which exhibited a very slight increase with Bison wheat.

SUMMARY

In the present study, different methods of detecting the degree of storage deterioration have been applied on wheat and corn. The changes occurring in cereal grains during storage have been discussed.

Changes in germination percent, fluorescence, fat acidity, glutamic and pyruvic acid decarboxylase activities, absorption of acridine-orange by wheat and flour and sedimentation value were investigated in relation to storage deterioration and baking quality.

With 73 commercial wheats at various stages of deterioration, it was found that log glutamic acid decarboxylase activity (GADA) correlates significantly higher with percent germination ($r = +0.920^{***}$) than fat acidity with percent germination ($r = -0.731^{***}$). The correlation involving log pyruvic acid decarboxylase activity ($r = +0.927^{***}$) was of same order of magnitude than that between log GADA and viability, and not significantly different from it. It was concluded that log GADA is a better index of storage quality of wheat than fat acidity or fluorescence.

Samples of Pawnee and Bison wheats were stored at different temperature and moisture levels for various periods of time. Here, too, glutamic acid decarboxylase activity decreased with percent germination, as temperature,

moisture level and storage time increased. Sedimentation values decreased in samples stored above 20 percent moisture content at +38°C for 16 to 32 days. Similar behavior was exhibited by loaf volume.

Also with corn, log GADA correlated highly with percent germination ($r = +0.949^{***}$). This correlation was again significantly higher (at 0.1 percent level) than that involving fat acidity ($r = -0.433^{**}$).

The recently published copper acetate method for determination of fat acidity was found to correlate poorly to fat acidity determination by standard techniques, both for wheat and corn.

Dye-binding techniques for quantitative protein determination using amidoblack 10 B, orange G and lissamine green SF 150, were conducted on a large number of wheats to establish the relationship between dye absorption and protein deterioration. No significant effect on the dye absorption by the degree of deterioration could be observed. Lissamine green SF 150 was introduced for quantitative protein determination by dye absorption techniques, using phosphate buffer of pH 6.0 as final diluent. The correlations thus obtained with Kjeldahl protein and lissamine green SF 150 were significantly higher than when using other dyes ($r = -0.992^{***}$ with wheat and $r = -0.993^{***}$ with flour). Furthermore, the same standard curve could be used both for wheat and flour ($r = -0.995^{***}$ for wheat and flour).

The absorbance of excess acridine orange correlated significantly ($r = -0.604^{***}$) with log GADA, suggesting that proteins lose their binding ability with advancing deterioration.

SUGGESTIONS FOR FUTURE RESEARCH

The glutamic acid decarboxylase activity showed promise in evaluating

storage deterioration of wheat and corn. Further work, however, is necessary to establish the behavior of individual varieties of wheat in regard to the test, as well as to establish the relationships of glutamic acid decarboxylase activity to the sedimentation value of stored grain.

Results from preliminary experiments suggested a poor correlation between the absorption of acridine-orange and degree of storage deterioration of wheat. However, storage studies that would establish the exact nature of the relationship between the absorption of basic dyes and protein deterioration could provide valuable information regarding the mechanism of deterioration.

A definite decrease in the sedimentation value during storage of wheat at elevated moisture levels and temperatures was clearly established in the present work. Long term storage studies are, however, necessary to find out the behavior of wheat at relatively low moisture levels. That grain proteins undergo certain changes during storage is well established, although the exact nature of such changes is largely unknown. Electrophoretic studies could bring about valuable information in this regard.

To ascertain the value of the sedimentation test in evaluating storage deterioration and quality, long term storage studies are necessary. The effect on sedimentation value of the moisture content of wheat at the time of testing, of sprout damaged and malted wheat, of drying the wheat at various temperatures of fumigants, etc., needs to be established.

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A STUDY OF METHODS TO DETECT STORAGE DETERIORATION OF CORN AND WHEAT

by

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It has long been recognized that the value of stored grain both for feed and industrial processing decreases in proportion to the physical, chemical, and biological changes it undergoes during storage.

In the present study, different methods of detecting the degree of storage deterioration have been applied on wheat and corn. The changes occurring in cereal grains during storage have been discussed. Changes in germination percent, fluorescence, fat acidity, glutamic and pyruvic acid decarboxylase activities, absorption of acridine-orange by wheat and flour, and sedimentation value were investigated in relation to storage deterioration and baking quality.

With 73 commercial wheats at various stages of deterioration, it was found that percent germination correlates significantly higher (at 0.1 percent level) with log glutamic acid decarboxylase activity (GADA) ($r = +0.920^{***}$) than with fat acidity ($r = -0.731^{***}$). The correlation involving log pyruvic acid decarboxylase activity ($r = +0.927^{***}$) was of same order of magnitude than that between log GADA and viability, and not significantly different from it. It was concluded that log GADA is a better index of storage quality of wheat than fat acidity or fluorescence.

Samples of Pawnee and Bison wheats were stored at different temperature and moisture levels for various periods of time. Here, too, glutamic acid decarboxylase activity decreased with percent germination, as temperature, moisture level and storage time increased. Also with corn, log GADA correlated highly with percent germination ($r = +0.949^{***}$). This correlation was again significantly higher (at 0.1 percent level) than that involving fat acidity ($r = -0.433^{***}$). The recently published copper acetate method for determination of fat acidity was found to correlate poorly with fat acidity

determination by standard techniques, both with wheat and corn. It could be concluded that the techniques for measuring GADA provide, in addition of being quick and reliable in estimating storage deterioration, means to detect damage caused by operations such as drying high moisture grain at excessive temperatures.

An attempt was also made to correlate sedimentation values with actual baking quality of wheats stored at various conditions. Sedimentation value decreased markedly in samples of Pawnee and Bison wheat stored above 20 percent moisture level at 38°C for more than two weeks, the decrease being accompanied by a decrease in water absorption and loaf volume. The farinograph and bake mixing times both generally increased with increasing deterioration. When deterioration became pronounced the farinograph peak representing true mixing time gradually vanished. Simultaneously another peak of very short mixing time developed, remaining as the only peak in flours of very advanced deterioration. It is likely that the formation of this secondary peak is, at least partly, associated with water absorption by starch.

The absorbance of excess acridine-orange correlated significantly ($r = -0.604^{***}$) with log GADA, suggesting that proteins lose their binding ability towards basic dyes with advancing deterioration. This prompted a study of dye binding techniques for quantitative protein determination, using amidoblack 10 B, orange G, and Lissamine green SF 150, on a large number of wheats to establish the relationship between dye absorption and protein deterioration. No significant effect on the dye absorption by the degree of deterioration could be observed. Lissamine green SF 150 was introduced for quantitative protein determination by dye absorption techniques, using phosphate buffer of pH 6.0 as final diluent. The correlations thus obtained

with Kjeldahl protein and lissamine green SF 150 were significantly higher than those involving other dyes ($r = -0.992^{***}$ with wheat and $r = -0.993^{***}$ with flour). Furthermore, the same standard curve could be used both for wheat and flour ($r = -0.995^{***}$ for wheat plus flour). The dye absorption techniques for quantitative proteins analysis may be of great value when expensive and elaborate equipment is not available, and when rapidity is an important factor.