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QUALITY OF STORED WHEAT

Simple and Rapid Manometric Method for Determining Glutamic Acid Decarboxylase Activity as Quality Index of Wheat

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Recent studies have indicated that glutamic acid decarboxylase activity gives a good estimate of the storage condition of wheat. Hence a simple and rapid manometric method was developed for its determination. Ground wheat was mixed with glutamic acid in phosphate buffer at pH 5.8, and the carbon dioxide evolution due to the decarboxylation of glutamic acid was measured with Sandstedt and Blish pressuremeters using ethyl lactate as manometer liquid. The correlations between germination percentage and the observed pressure increase ($r = +0.841$***) or $\log_{10}$ pressure increase ($r = +0.928$***), respectively, were highly significant. Estimate of the storage condition of wheat was equal to or better than that by fat acidity determination.

THE IMPORTANCE of quick and reliable tests to estimate the quality of stored wheat is generally recognized. Numerous attempts have been made to determine the storage condition of cereal grains (8). Fat acidity has been shown to be a good index of the extent of deterioration (3, 19) and is widely used by the cereal industry to evaluate wheat. Although relatively simple, the standard method involved is somewhat time-consuming. A rapid method for determining fat acidity has been developed (2), but the technique requires prior drying of grain below the 10% moisture level. It was shown recently that characteristic changes in the composition of free amino acids of wheat take place shortly after the beginning of water imbibition, because of the activation of certain enzymes, such as glutamic acid decarboxylase, transaminases, and proteases, at low moisture levels (8, 12, 13). These changes continue during storage, particularly at elevated moisture levels and temperatures. Subsequently, tech-niques based on the study of paper chromatogram (10) and, particularly, electrophoresis (9) patterns of free amino acids of wheat seemed promising as a convenient means of estimating the degree of deterioration.

On the other hand, enzymes themselves begin to lose activity during storage of grain under unfavorable conditions, and glutamic acid decarboxylase activity decreases during storage of wheat, especially at moisture levels above 15% (4, 8, 15). Glutamic acid decarboxylase activity, as determined by Warburg manometric (14), colorimetric (11), or electrophoretic (11) techniques, appears to be a good index of the storage condition of wheat. All these methods, however, are either relatively time-consuming or require elaborate and expensive equipment. The present paper describes a method suitable for rapid and accurate routine determination of the glutamic acid decarboxylase activity as an index of the quality and storage condition of wheat.

Experimental

Materials. The 60 wheat samples investigated included several varieties of relatively high viability from 1956 to 1959 crops, and a number of commercial wheats at various stages of deterioration. Their moisture content varied from 9.9 to 12.9%, but it seemed likely that the moisture content at one time of storage had been much higher in some of the samples. The viability range covered was from 0 to 99% germination, and that of fat acidity from 10.2 to 79.8. Moisture, germination, fat acidity, and Warburg manometric analyses were performed as described earlier (14).

Apparatus. Sandstedt and Blish (16) pressuremeters were employed to determine the glutamic acid decarboxylase activity. However, instead of being filled with mercury, the manometers were filled to the 300-mm. level with ethyl lactate colored with crystal violet (18). This provided the necessary sensitivity for short-term operations.
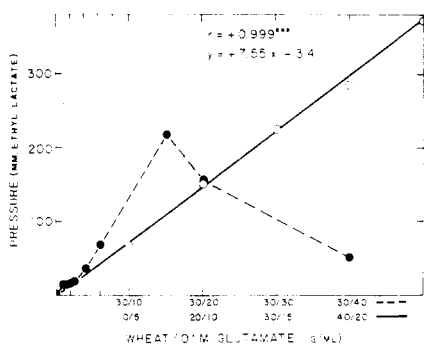
Figure 1. Effects of quantities of wheat and substrate solution on glutamic acid decarboxylase activity of Seneca wheat

30 min., 30° C.

Figure 2. Effect of substrate concentration on glutamic acid decarboxylase activity of Seneca wheat

30 min., 30° C.

Figure 3. Relation between reaction time and decarboxylation of glutamate by Seneca wheat

0.1M glutamic acid, 30° C.

Glutamic Acid Reagent. The substrate solution generally used was $0.1M$ glutamic acid in $0.067M$ phosphate buffer of pH 5.8, containing 0.1% of 2(3)-benzoxazolone as a preservative. If several analyses were performed daily, it was convenient to use a preservative to prevent microbial growth, rather than to keep the substrate solution in a deep-freeze. Without a preservative, fungal growth was visible in buffered glutamic acid solution after a couple of days even at $+4°$ C.

The presence of 0.1% 2(3)-benzoxazolone, 0.1% potassium sorbate, or toluene (saturated) failed to affect the rate of decarboxylation. However, potassium sorbate appeared to be relatively ineffective as a preservative under the experimental conditions. Although some species of fungi were controlled, mold growth was apparent even at $+4°$ C. as quickly as it was in the control, and bacterial count was, at times, even increased. On the other hand, 2(3)-benzoxazolone reduced microbial growth rate so that no growth was visible after 2 weeks at $+27°$ C., and the growth rate remained slow on subsequent malt-salt-agar (7) cultures. When the solution was stored at $+4°$ C., microbial count remained zero for 4 weeks. When the substrate solution was saturated with toluene, little, if any, microbial growth was evident during 2 weeks at $+27°$ C. However, using toluene was somewhat inconvenient in automatic pipets.

Analytical Procedure. Wheat was ground for 2 minutes with a Waring Blendor shortly before the experiment. After thorough mixing, 30 grams (wet weight basis) of the ground material were weighed in one side of the aluminum cup of the pressuremeter. It was convenient to prepare 10 samples simultaneously. Fifteen milliliters of glutamic acid solution were measured from an automatic pipet (or buret) in the empty side of the cups at 2.5-minute intervals. The contents were mixed well immediately by means of a heavy
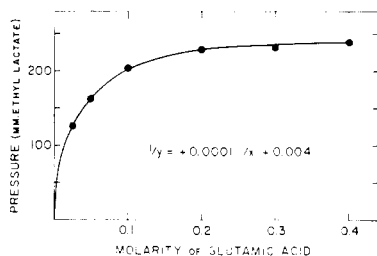
glass rod, the lids were screwed on tightly, and the pressuremeters were placed in a water bath at $30° \pm 0.02°$ C. After 5 minutes' equilibration, the manometers were adjusted to zero. The pressure, in millimeters of ethyl lactate, was recorded after a 30-minute incubation period.

Results and Discussion

Figure 1 shows that increasing the relative quantity of substrate solution (or moisture content) resulted in a steady increase in glutamic acid decarboxylase activity, beginning from approximately 3 to 4 ml. per 30 grams (ca. 20% moisture content) and reaching a maximum at about 15 ml. per 30 grams (ca. 40% moisture content). This tends to confirm the fact that glutamic acid decarboxylase is activated in wheat embryos at approximately 15% moisture level, reaching its maximum activity at 40% moisture (8, 13). The apparent decrease in carbon dioxide evolution following a further increase in substrate solution was probably due to the retention of carbon dioxide because of lack of shaking, since chromatographic studies had shown glutamic acid decarboxylase activity to remain steady above 40% moisture level (8). Figure 1 also shows that at a constant moisture level decarboxylase activity increases linearly with the increase in enzyme (wheat) concentration. Furthermore, as can be seen from Figure 2, the effect of substrate concentration on glutamic acid decarboxylase activity was such as could be expected from the earlier findings (5). The increase in carbon dioxide evolution was very pronounced up to $0.1M$ glutamic acid. A further increase in glutamic acid concentration brought about little increase in decarboxylation. Figure 3 shows the relationship of carbon dioxide evolution with reaction time. Thirty minutes appeared satisfactory so far as gas evolution was concerned, and 30-gram sample size was chosen because of the amount of
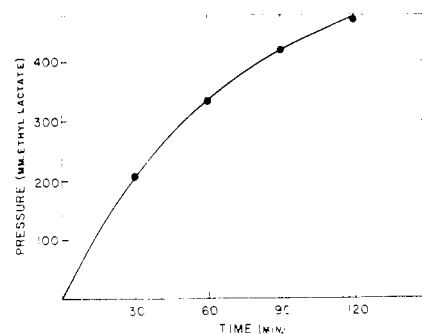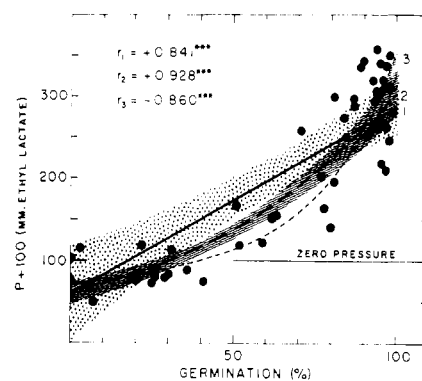

Figure 4. Relation between glutamic acid decarboxylase activity and germination percentage

Shaded areas indicate 95% confidence belts for sample means
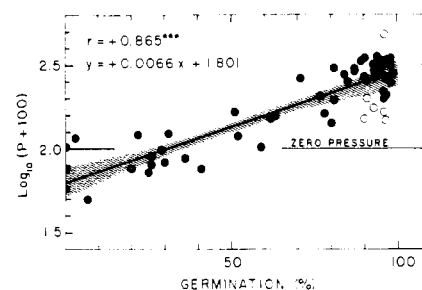

Figure 5. Relation between log glutamic acid decarboxylase activity and germination percentage

White circles represent Langdon (above regression line) and Panca (below regression line) wheats. Shaded area shows 95% confidence belt for sample mean

carbon dioxide evolution, convenience in handling, and good statistical reproduciblity as compared with the 300 to 500 mg. used in Warburg manometric analyses.

According to Figure 4 the pressure increase due to the enzymatic decarboxylation of glutamic acid was highly correlated with germination percentage.

The regression equations and corresponding correlation coefficients for the curves presented in Figure 4 are:

$$y = 2.28x + 60$$
$$(r = +0.841^{***}) \quad (1)$$

$$\log y = 0.0068x + 1.798$$
$$(r = +0.928^{***}) \quad (2)$$

$$1/y = -0.000117x + 0.0145$$
$$(r = -0.860^{***}) \quad (3)$$

Although the linear correlation coefficient was as high as $r = +0.841^{***}$, it seemed that the regression was likely to be curvilinear. Accordingly, the correlation coefficient of the logarithmic regression ($r = +0.928^{***}$) was found to be significantly higher (at 5% level) than that of the linear regression. Furthermore, as shown by Figure 4, the 95% confidence belt for the sample mean was much narrower when logarithmic regression was used.

The present techniques were developed primarily for simple and rapid routine estimation of the quality of stored wheat. Hence, no correction was made for the relatively small (5) oxygen uptake by wheat, for the oxygen uptake due to fungi and other microorganisms present, particularly in wheats at advanced states of deterioration, nor for the retention of carbon dioxide. The latter is small at pH 5.8 (18), but may be affected by the lack of shaking. Furthermore, the decrease in enzyme activity during storage apparently is a result of a number of processes, depending largely on the storage conditions involved. However, if enzyme inactivation is considered as a simple independent process, the logarithmic regression may be easily explained, the slight deviations being largely due to the factors mentioned above, plus wheat's being weighed on a wet weight basis.

Although the exact relationship between the time of storage and viability is not known, it is generally accepted that for a given wheat sample at any given set of conditions a certain correlation exists between the storage time and germination percentage—that is. under a given set of conditions a certain storage time may be considered to represent a certain germination percentage. Hence, if we investigate the enzyme inactivation vs. time, the latter may be readily correlated with the loss of viability. On the other hand, at least certain types of enzyme denaturations, such as heat inactivation, appear to be unimolecular in nature (17), and a purely unimolecular reaction is likely to be of the first order. Hence the enzyme activity may be expected to decrease logarithmically with time and, consequently, with viability.

If the decrease in glutamic acid decarboxylase activity were correlated with viability, so that the enzyme activity dropped to zero at the time, and only at the time, of the death of the embryo (a dead kernel here meaning one which does not germinate), a linear regression would be obtained. However, glutamic acid decarboxylase activity decreases markedly before the death of the embryo. If the denaturation of glutamic acid decarboxylase in wheat vs. time of storage is examined in greater detail beginning from harvest, one may easily visualize that the logarithmic denaturation curve will intercept the y (enzyme activity) axis at the point of initial (maximum) enzyme activity. A logarithmic decrease in the decarboxylase activity follows, until death of the embryo occurs. Generally, however, dead embryos still possess some decarboxylase activity, and the logarithmic denaturation curve continues beyond the death point. In a wheat sample we may have individual kernels at different stages of deterioration, with the individual decarboxylase activities representing various points on the denaturation curve. However. in a sample of high germination percentage the majority of the individual decarboxylase activities would be distributed near the y-axis. Similarly, in wheat of poor viability most of the kernels would possess enzyme activities representing points near or beyond the death point. Because the rate of an enzyme reaction is proportional to the total quantity of enzyme present, a logarithmic denaturation curve is obtained if the sum of the enzyme activities of the individual kernels in a sample of wheat is plotted vs. storage time (or per cent dead kernels).

Some variation in glutamic acid decarboxylase activity may be encountered in different varieties of wheat (6, 14). Minor variations, however, are likely to be due to the beginning deteriorative process. One variety has been found to possess an unusually low (Ponca) and one an unusually high (Langdon) glutamic acid decarboxylase activity, but these differences appear to be significant only in wheats of high viability. If one sample of Langdon wheat (germination 96%; pressure increase 376 mm. of ethyl lactate per 30 minutes), and five samples of Ponca wheat (germination 90 to 96%; pressure increase 49 to 99 mm. of ethyl lactate per 30 minutes) were included in a total population of 60 samples, the linear correlation ($r = +0.755^{***}$) between decarboxylase activity and viability was lower at 7% level than logarithmic correlation ($r = +0.865^{***}$; Figure 5). Even in this case the correlation between the logarithm of the pressure increase and germination percentage was relatively high, further illustrating the usefulness of the logarithmic plot.

It was thought that the varietal differences in glutamic acid decarboxylase activity might have been caused by differences in pyridoxal phosphate in wheat, and that the decrease in pyridoxal phosphate during storage might result in leveling off the varietal differences in enzyme activity. However, when 0.01% of pyridoxal phosphate was added in the substrate solution, the correlation. between the decarboxylase activity and viability ($r = +0.797^{***}$) was not significantly different from that obtained without pyridoxal phosphate ($r = +0.841^{***}$).

Deviations from the logarithmic regression may have been partially due to wheat's being weighed on a wet weight basis. However, when results were corrected for moisture content. the correlation coefficient obtained between the decarboxylase activity and germination percentage ($r = +0.869^{***}$) was not significantly higher than that obtained from results on a wet weight basis. Hence it appeared that when moisture content was within the limits of approximately 9 to 13%, wheat could be weighed on a wet weight basis. Preliminary evidence has indicated that the error contributed by weighing wheats up to 18% moisture level on a wet weight basis is insignificant from any practical point of view and could, if desired, be rapidly corrected by determining the moisture content by an electrical moisture meter. Generally, however, a moisture determination prior to an analysis is unnecessary, another time-saver.

The results were correlated with Warburg manometric determinations. as well as with fat acidity analyses. Correlation between the present method and Warburg techniques was high ($r = +0.904^{***}$). The correlation coefficient between fat acidity and germination percentage ($r = -0.860^{***}$) was of the same order of magnitude as the linear correlation between glutamic acid decarboxylase activity and viability. Similar observations were previously made regarding Warburg manometric techniques (14). However, the correlation between germination percentage and the logarithm of decarboxylase activity was higher at the 12% level than that involving fat acidity. This indicated that the present method gives as good as or a better estimate of the degree of deterioration than fat acidity does. Furthermore, less than 1.5 hours is necessary for a complete analysis of 10 samples, including grinding and weighing.

Figure 4 reveals that with few exceptions only samples with germination above approximately 80% gave a pressure increase of more than 100 mm. of ethyl lactate per 30 minutes at 30° C. Because germination above 75% may be considered normal for wheat used for milling purposes (7), the test appears to provide a simple way to estimate the quality of wheat. At present, there is no definite explanation for the few samples of germination percentage near or above 80 possessing unexpectedly low enzyme activity. The characteristically low

pressure readings of Ponca wheat of high viability have been mentioned. Many of the commercial wheat samples used in this study were either of unknown variety or mixtures of several varieties. A number of other possibilities, such as the effect of protein content on enzyme activity, are being studied. It appears also that if the nonviable kernels of a wheat sample were badly damaged, the pressure increase produced could be unusually low, particularly with commercial mixtures of wheat.

Germination percentage has been used in this study as a primary standard for the deterioration of wheat in storage. It is recognized, however, that germination percentage is apparently not a sufficient index as far as the suitability of wheat for bread making is concerned. Work is in progress to prepare standards for different varieties of wheat, and to correlate the glutamic acid decarboxylase activity of a given wheat sample with its actual baking quality.

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FISH COMPOSITION

Proximate Composition of Nine Species of Sole and Flounder

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In order that adequate data could be provided for the commercially important and palatably desirable group of West Coast marine fishes known as sole and flounder, the composition of nine of the more common species was determined. This investigation included the analysis of more than 200 specimens of varying size collected over a period of 3 years in different seasons and preserved in different ways. Large variations were found among the nine species and among the fish within a given species. The group as a whole was low in oil and ash, about average in protein, and high in sodium. Variations for all specimens grouped according to size of fish, season of capture, and manner of preservation were not large. Both the nonedible parts and the whole fish for the various species were sufficiently high in oil, protein, and minerals to be commercially important as an animal food, or as a supplement in poultry feed when converted to meal.

More than a dozen species of sole and flounder inhabit the waters along the Pacific Coast of the United States (1, 4). Mature specimens of these fishes range in length from 25 to 70 cm. but the average is about 40 cm. They are taken by commercial fishermen at all seasons of the year. In 1957, the combined catch from California. Oregon, and Washington amounted to 48 million pounds (3). The catches of other important species taken during the same year, also in millions of pounds, were Pacific salmon 61, rockfish 28, halibut 22, and Pacific cod 12.

Sole and flounder usually are marketed as fresh or as frozen fillets, and their processing is an important part of the West Coast fishing industry. The edible portion of sole and flounder represents approximately 28% of the total weight of the fish, and the remainder is frozen and sold primarily for use as feed for fur-bearing animals.

Because sole and flounder are used as food for humans and as sources of feed for animals, it is important to know the nutritive value of the whole fish, of the edible flesh, and of the nonedible parts of the fish. Very few data are available in the literature regarding the proximate composition of these fishes. The reports that have been published deal with abnormal conditions (2, 6) or compare the composition of the right and left sides of the fish (5). The object of the present investigation therefore was to establish the proximate composition of several species of sole and flounder from the west coast of the United States.

Experimental

In order that data could be provided for this purpose, chemists from the Seattle Technological Laboratory of the Bureau of Commercial Fisheries analyzed more