THE KINETICS AND OCCURRENCE OF WHEAT GLUTAMIC ACID DECARBOXYLASE

by

YU YEN CHENG

B.S., National Taiwan University, Taipei, Taiwan, 1956

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Flour and Feed Milling Industries

KANSAS STATE UNIVERSITY
OF AGRICULTURE AND APPLIED SCIENCE

1959

TABLE CONTENTS

INTRODUCTION 1

REVIEW OF THE LITERATURE 2

The Relationships Among Moisture Content, Mold Growth, Respiration, and Viability with Special Reference to Grain Stored at Elevated Moisture Levels 2

Enzymatic Decarboxylation of Glutamic Acid with Special Reference to Higher Plants 4

MATERIALS AND METHODS 8

Materials 8

Moisture Content 9

Manometric Techniques 9

Respiratory Measurements under Nitrogen 13

Decarboxylation Measurements 13

Transamination Measurements 14

pH - Optimum 14

Temperature Optimum 15

Determination of Michaelis-Menten Constant and Activation Energy 15

Determination of the Relationship Between Glutamic Acid Decarboxylase Activity and Browning 18

Measurement of the Fluorescence 19

Determination of the Relationship of Percentage of Damaged Kernels to Glutamic Acid Decarboxylase Activity and Fluorescence 20

The Effect of Storage at Various Moisture Contents on Glutamic Acid Decarboxylase Activity 21

The Effect of Storage at Various Temperatures on Glutamic Acid Decarboxylase Activity 21

Determination of Various Enzyme Activities in Different Wheat Varieties 22

RESULTS 23

Standard Deviation of Warburg Determinations 23

The Effect of pH on the Decarboxylation of Glutamic Acid by Wheat Germ 26

Michaelis-Menten Constant 26

Activation Energy 26

The Effect of Nitrogen Atmosphere on the Decarboxylation of Glutamate 34

Relationship of Moisture-Induced Browning to Glutamic Acid Decarboxylase Activity 37

The Effect of Storage of Pawnee Wheat Conditioned to Different Moisture Levels at +25°C on Decarboxylase Activity 42

The Effect of Storage of Different Wheat Varieties in a "Dry" State at Various Temperatures 42

Study of Various Enzyme Activities in Different Wheat Varieties 45

DISCUSSION 51

Reaction Kinetics of the Decarboxylation of Glutamate by Wheat Germ 51

The Effect of Environment on Viability and Glutamic Acid Decarboxylase of Wheat 53

The Effect of Variety and Growth Conditions on Glutamic Acid Decarboxylase in Wheat Grains 56

Suggestions for Future Research 57

SUMMARY 59

ACKNOWLEDGEMENTS 61

LITERATURE CITED 62

INTRODUCTION

From the moment when grains are ready for harvest they are subject to physical, biological and biochemical changes. In severe cases deterioration may lead to loss of viability. Cycles of wetting and drying in the field caused by periodical changes in rain and sunshine prior to harvest may result in bleaching and weathering of kernels as well as in formation of internal fissures in the endosperm of vitreous wheats. Similar changes are intentionally produced by the tempering process in flour milling.

The most important environmental factors affecting the viability and storage life of grains are temperature and humidity. An increase in moisture accelerates respiration mechanisms, but the most obvious deleterious consequence of storing damp grain is the proliferation of certain saprophytic fungi which cause spontaneous heating, biochemical changes and loss of viability.

In the present study, attention has been focused on more subtle changes which appear in grain within moments after wetting, due to activation of enzymes. Linko and Milner (29) found recently that wetting activates glutamic acid decarboxylase in wheat embryos resulting in a significant carbon dioxide evolution characterized by elevated respiratory quotients (34). Specifically, this work was undertaken to clarify the biochemical mechanism which produces carbon dioxide immediately

upon wetting of embryos. It was apparent that this reaction might play an important role in the deterioration of grain in storage. The kinetics of glutamic acid decarboxylase, the relationship of the enzyme to environmental factors and non-enzymatic browning, as well as its distribution in different varieties of wheat, all were studied.

REVIEW OF THE LITERATURE

The Relationships Among Moisture Content, Mold Growth, Respiration, and Viability with Special Reference to Grain Stored at Elevated Moisture Levels

The respiration of sound dry grains is of an extremely low order in comparision with that of vegetative saprophytic fungi, whose spores occur on and frequently within the coat of grains. An increase in moisture content is followed by a gradual increase in respiration until a certain critical moisture level is reached (2, 3, 7, 27). This increase in respiration as well as the loss of viability in grain stored at elevated moisture levels was first attributed to the metabolism of the embryo itself (2, 3, 26). Barton (4, 5) and Hodnett (23) studied the effect of moisture and temperature on seed viability and found that in general the loss of viability during storage was correlated to the moisture content and temperature. However, it was recognised that the metabolic activity of the embryo is too low to account for

any heating or respiration of practical importance at moisture levels below 25 percent. Later, the importance of saprophytic fungi in the relationship between moisture content and respiratory activity of grains became well established (9, 13, 24, 40, 41, 43, 48), and the rapid loss in viability and biochemical quality suffered by grains stored under moisture conditions favorable to fungal growth has been related to toxic products and enzymes secreted by these fungi. Gilman and Barron (21) were the first to point out that a sharp increase in respiration occurs at the same moisture level at which fungal growth begins.

A more detailed study with wheat embryos and dicotyledenous seeds showed that significant carbon dioxide evolution and a less pronounced oxygen uptake occur immediately upon wetting, resulting in highly elevated respiratory quotient values (20, 34). This gas exchange recedes, however, several hours before the germination of fungal spores can affect the subsequent respiratory pattern. That this excessive carbon dioxide/oxygen ratio involves the metabolism of the seed itself rather than that of fungi is indicated by the fact that autoclave-sterilized seeds inoculated with pure cultures of fungi which normally cause spontaneous heating, produce the same heating trends without the sharp rise of R.Q. (42).

Linko and Milner (31) observed that the addition of pyruvate and α-ketoglutarate to wheat germ markedly increased

carbon dioxide evolution and thus R.Q. value. Later it was shown, however, that the carbon dioxide evolution from wetted wheat embryos is mainly due to decarboxylation of glutamate by an enzyme which is activated upon wetting (11, 29, 30). It was also found that the increase in moisture content initiates enzymatic breakdown of storage proteins (30). The resulting free amino acids could be expected to participate in non-enzymatic browning reactions so characteristic of "sick" wheat (34). Breakdown of proteins and loss of metabolic intermediates closely related to the tricarboxylic acid cycle are therefore believed to be initial causes of loss of viability in grains and seeds stored at elevated moisture levels. The later appearance of vegetative saprophytic fungi greatly aggravates the deteriorative process.

Enzymatic Decarboxylation of Glutamic Acid with Special Reference to Higher Plants

The enzyme group which catalyses the decarboxylation of amino acids in one step to yield carbon dioxide and an amine

(i) $R\text{-}CH(NH_2)\text{-}COOH \longrightarrow R\text{-}CH_2\text{-}NH_2 + CO_2$

(i) was first described in microorganisms by Gale (19). Since this pioneer discovery a multitude of amino acid decarboxylases has been detected (35). In general bacterial decarboxylases are adaptive enzymes, their formation being largely dependent on the presence of a specific substrate. However, D-diamino-

pimelic acid decarboxylase is known to be a "constitutive" enzyme always present in bacteria which normally synthesize L-lysine by the decarboxylation of meso-α,ε-diaminopimelic acid (17). All other known amino acid decarboxylases are specific for L-amino acids.

By far the most predominant amino acid decarboxylase in higher plants is glutamic acid decarboxylase. As early as 1913, Abderhalden et al. (1) noticed that microorganisms were able to form γ-aminobutyric acid directly from glutamate. It was not, however, until 1937, when Okunuki (47) described this enzyme in higher plants. His attempts to obtain solutions of the enzyme failed, thus leading to the conclusion that glutamic acid decarboxylase was bound to cellular structures. Later Schales et al. (55) discovered that glutamic acid decarboxylase could be obtained in clear solutions in good yields from carrots, but the enzyme was inactivated by dialysis. The activity could be restored by the addition of pyridoxal phosphate. The authors reported the presence of glutamic acid decarboxylase in 30 different plants, squash, avocado and green pepper being particularly rich sources.

The work of Schales was followed by the discoveries of the enzyme in barley (6, 45), alder (39), legumes (23), and Scopolia japonica (61). Glutamic acid decarboxylase has also been shown in brain (50, 67), in chlorella (65, 66), and in several microorganisms (8, 44). The reaction product γ-amino-butyric acid was first identified among the naturally-occuring

free amino acids in potato tuber by Steward et al. (60), but only very slight glutamic acid decarboxylase activity was found in these tissues (59, 60, 62). Thereafter γ-aminobutyric acid has been found to be widely present in higher plants, but its metabolic fate is still largely unknown (32, 58).

Very recently Rohrlich (51, 52, 53) has described glutamic acid decarboxylase and γ-aminobutyric acid in wheat. Following the course of the reaction by manometrically measuring the carbon dioxide, he was able to confirm the recent discovery of Naylor and Tolbert (45) that this enzyme is activated by light. He also found that the enzyme activity was lowered during long storage of grain. Király and Farkas (25) found that glutamic acid decarboxylase activity was considerably lowered in wheat infected by rust (Puccinia graminis). On the other hand, DeVay (18) was able to detect γ-aminobutyric acid in wheat only when it became moldy, at relatively high moisture content. However, Linko and Milner (30) recently reported the presence of this amino acid in sound wheat grains (1 to 2 μg/g dry wt.). The amount of γ-aminobutyric acid increases significantly following wetting due to the activation of glutamic acid decarboxylase (29).

All the amino acid decarboxylases that have been studied carefully are known to require pyridoxal phosphate as a cofactor. At least histidine decarboxylase requires also a metal ion, such as Fe^{3+} or Al^{3+} (22). From studies with non-enzymatic model systems (36) containing substrate, pyridoxal

phosphate and a metal ion, Snell and his associates (33) have concluded that Schiff's bases derived from amino acids and pyridoxal phosphate are stabilized by chelation. Subsequent intramolecular reaction, such as expulsion of H^+, CO_2, OH^- or SH^- coupled with a shift of electrons, would produce chelates which are spontaneously hydrolysed. That the amino group may be necessary for this type of decarboxylation, is shown by the fact that α-ketoglutaric acid is not directly decarboxylated by squash homogenates (54) in contrast to the effect of carboxylase (46, 68) on other α-keto acids. Schales and Schales found indeed that the initial step in the decarboxylation of α-keteglutaric acid was the transamination with α-alanine or aspartate to glutamate, which then is decarboxylated. That only aspartate and α-alanine are able to transaminate with α-ketoglutarate to any significant degree is also shown to be true in wheat grains (29). Cruickshank and Isherwood (16) have also reported the presence of glutamic-alanine and glutamic-aspartic transaminases in wheat germ. However, the keto-analogs of almost all of the natural free amino acids are now known in plants (64), and it has been suggested that these keto-compounds play an important role in transamination reactions leading to the biosynthesis of protein amino acids, and as a means to maintain balance between different metabolites.

The transamination of γ-aminobutyric acid with α-ketoglutaric acid has been demonstrated in alder (38).

The literature concerning glutamic acid decarboxylase and related transaminating enzymes in plant tissues in general and in wheat grains in particular, suggests that considerable investigation remains to be carried out to establish clearly the role of these enzymes in the biochemistry of storage changes in wheat.

MATERIALS AND METHODS

Materials

Fresh granular wheat germ was obtained from International Milling Co., Minneapolis, Minnesota. Samples of 8.9 and 9.2 percent moisture content were employed.

Most experiments with grains were performed using soft red winter wheat, variety Seneca, which was grown in Ohio. Moisture content of this material was 11.6 percent, total protein 9.9 to 10.4 percent, and germination above 95 percent. For special tests several other varieties of wheat were used (see pp. 21 to 23).

The granular wheat germ was used as such, without further grinding. Whole wheat kernels were ground for Warburg tests in a micro-Wiley mill to pass 20 mesh screen. All materials were stored in air-tight containers at +4°C.

All chemicals used were of purest obtainable grade.

Moisture Content

Moisture content was determined by drying granular wheat germ or grains ground to pass the No. 20 screen for one hour at 130°C (10).

Manometric Techniques

Assays were carried out in Warburg flasks according to the direct method as outline by Umbreit et al. (63). The method is based on the principle that at constant temperature and constant gas-volume any changes in the amount of gas can be measured by changes in its pressure. The apparatus consists of a removable flask equipped with one or more side-arms, attached to a manometer containing liquid of known density. One sidearm vessels were used for this study. Ethyl lactate colored with crystal violet was used as manometer liquid. The reaction chamber of clean dry Warburg vessels received the enzyme preparation (usually 100 mg of granular wheat germ or 500 mg of ground wheat grains). One milliliter of standard 0.067 M (M/15) phosphate buffer of pH 5.8 (63), or a solution of substrate in this buffer was pipetted into the sidearm. One flask-manometer combination was left without enzyme preparation in order to detect slight pressure and temperature changes independent from the enzyme activity. In addition to this so called thermobarometer a typical experimental setup consisted of five pairs of flask-manometer

combinations. One flask of a pair had a folded filter paper square soaked in 0.2 ml of 20 percent potassium hydroxide in the center well in order to trap the carbon dioxide evolved. This was necessary to correct the results for the oxygen uptake. The conditions in both flasks are considered to be practically equal with the exception that in one flask the tissue respires in the presence of carbon dioxide, and in the other one in its absence. Generally, though, carbon dioxide has very little effect on respiration.

After connecting the flasks to the appropriate manometers they were immersed in a water bath of constant temperature (+30.0 ± 0.1°C, unless otherwise indicated). After a ten minutes incubation period with stopcocks open the contents of the sidearm were tipped into the reaction chamber, and the shaker was started (106/108 strokes per minute). The stopcocks were left open for five more minutes, after which the liquid in the right side manometer arm was adjusted to the reference point of 150 mm. After closing the stopcocks readings were taken at five minutes intervals by always adjusting the liquid on the closed arm of the manometer to the selected reference point. From these readings both the oxygen uptake and carbon dioxide evolution could be calculated.

The Warburg flasks and manometers were previously calibrated. Based on these values, the flask constants were calculated as shown below. The use of a flask constant enables one to calculate from the observed pressure changes the amount

(in mm^3 or μl at 0^oC and 760 mmHg pressure) of gas utilized or given off during a given time interval. The following formula was used for calculation of flask constants (63):

$$k = \frac{V_g \frac{273}{T} + V_f \alpha}{P_o} \qquad \text{(ii)}$$

where: k = flask constant.
V_g = volume of gas phase in flask including connecting tubes down to the reference point.
V_f = volume of fluid in vessel
T = temperature of bath in absolute degrees.
α = solubility in reaction liquid of gas involved (expressed as ml gas/ml liquid when gas is at a pressure of 760 mmHg at the temperature T).
P_o = standard pressure (760 mmHg) expressed as mm of manometer fluid, = 12,690.

Because the retention of carbon dioxide by buffer at pH 5.8 is negligible, one-sidearm vessels could be used. A correction for the retention of carbon dioxide was made by calculating the flask constants using so called effective value of α, α'. The values of α'/α as reported by Umbreit et al. (63) were employed.

The amounts of oxygen uptake and carbon dioxide evolution were calculated as follows:

$$x_{O_2} = h_{O_2} k_{O_2} \qquad \text{(iii)}$$

$$x_{CO_2} = (h_{O_2} - \frac{x_{O_2}}{k_{O_2}})\, k_{CO_2} \qquad \text{(iv)}$$

where: x_{O_2} = amount of oxygen uptake in microliters.
h_{O_2} = change in the manometer fluid level in the system with potassium hydroxide.
k_{O_2} = flask constant for oxygen uptake measurement.
x_{CO_2} = carbon dioxide evolution in microliters.
h_{CO_2} = change in the manometer fluid in the system without potassium hydroxide.
k_{CO_2} = flask constant for carbon dioxide evolution measurement.

For the convenience of calculations, the readings and results were tabulated according to the following example:

Flask with KOH					Flask without KOH				
R	D	CD	µl O_2	total O_2	R	D	CD	µl CO_2	total CO_2
150					150				
141	-9	-11	-11.07	-11.07	203	+53	+51	+78.46	+ 78.46
132	-9	-7	-8.61	-19.68	242	+39	+41	+62.77	+141.23
124	-8	-8	-9.84	-29.52	268	+26	+26	+44.41	+185.64
118	-6	-7	-8.61	-38.13	284	+16	+15	+28.71	+214.35
110	-8	-8	-9.84	-47.97	294	+10	+10	+23.45	+237.80
104	-6	-5	-6.15	-54.12	300	+ 6	+ 7	+15.64	+253.44

where: R = observed readings at five minutes intervals.
D = difference between two readings.
CD = corrected difference for thermobarometer readings.

Respiratory quotients (R.Q.) were calculated as the ratio of carbon dioxide produced/oxygen consumed.

The reproducibility of the results was determined by the method of least squares (v):

(v) standard deviation = $\pm\sqrt{\frac{f^2}{N}}$

where: f = deviation from the arithmetic mean value.
N = number of determinations.

Respiratory Measurements under Nitrogen

Flasks were prepared as previously described with the exception that all vessels were left without potassium hydroxide in the center well. The flasks were connected to the manometers and placed into the constant temperature water bath, as usual. The manometer fluid was raised to about 300 mm, and approximately one liter of pure Linde compressed nitrogen was passed through the system via the sidearm stopcock. After closing the sidearm and manometer stopcocks the liquid level in the closed manometer arm was adjusted to 150 mm, whereas the level in the left arm dropped down to about 0 mm. The shaker was started, contents of the sidearms were tipped at 1/2 minute intervals, and readings were taken at five minutes intervals, with the exception of the first five minutes period during which readings were taken at one minute intervals.

Decarboxylation Measurements

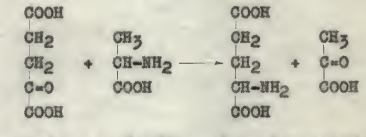
The enzyme source in these studies consisted of 100 mg of granular wheat germ or 500 mg of ground wheat grains which were placed in the main compartment of Warburg vessels. One milliliter of 0.1 molar buffered substrate solution (pH 5.8) was pipetted into the sidearm. The standard Warburg technique

as previously described was applied.

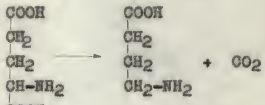
Transamination Measurements

Transamination of α-ketoglutaric acid with α-alanine (vi) was measured from the rate of decarboxylation of glutamate (vii) formed in the transamination reaction. Standard Warburg technique was used. One milliliter of 0.1 molar mixture of α-ketoglutarate and α-alanine was pipetted into the sidearm.

(vi)



α-keto-glutaric acid — α-alanine — glutamic acid — pyruvic acid



glutamic acid — γ-amino butyric acid

pH- Optimum

The effect of pH on glutamic acid decarboxylase was determined using 100 mg samples of wheat germ and 1 ml of standard M/15 phosphate buffers (64) of pH 5.0, 5.2, 5.4, 5.6, 5.8, 6.0, and 6.2.

Temperature Optimum

One hundred milligram samples of wheat germ were used. One milliliter of 0.05 M glutamate of pH 5.8 was used in the sidearm. The measurements were done at temperatures of 21°, 25°, 30°, 35°, 40°, 45°, and 50°C.

Determination of Michaelis-Menten Constant and Activation Energy

One of the earliest and most fundamental concepts for the interpretation of enzyme-catalyzed reactions was that developed by Michaelis and Menten (37). They found that on increasing the substrate concentration the rate of an enzyme-catalyzed reaction increases up to a maximum velocity, depending on the enzyme concentration and on a constant, which in turn depends on both of the nature of the enzyme-substrate system and on the physical conditions of the experiment. They assumed that enzyme (E) and substrate (S) form first a complex (ES), which then decomposes into enzyme and products (viii).

(viii) $$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k} E + \text{products}$$

The Michaelis-Menten constant (K_m) can be expressed as follows:

(ix) $$K_m = \frac{(E)(S)}{(ES)} = \frac{k_{-1} + k}{k_1}$$

It can be shown from the equations (viii) and (ix) that the reaction velocity (v) is:

(x) $$d(P)/dt = \frac{k(E)_o(S)}{K_m + (S)}$$

where $(E)_o$ is the total concentration of enzyme, free and combined. The maximum reaction velocity (V_{max}) is obtained when all of the enzyme is in combined form, hence (E) = (ES), and

(xi) $$v = V_{max} = k(E)_o$$

If this value is substituted into the Michaelis-Menton equation (x), one obtains:

(xii) $$v = \frac{V_{max}(S)}{K_m + (S)}$$

If one takes the reciprocal of each side of (xii), one gets:

(xiii) $$1/v = (K_m/V_{max})(1/S) + 1/V_{max}$$

which is equivalent to the expression (xiv):

(xiv) $$y = ax + b$$

From equations (xiii) and (xiv) on can see that K_m and V_{max} are normally easily determined by plotting the reciprocal of initial velocity (v) versus the reciprocal of substrate concentration (S), and evaluating V_{max} from extrapolation to $(S) = \infty$; hence $1/(S) = 0$; and K_m from extrapolation to $1/v = 0$.

For the determination of K_m, the carbon dioxide output by 50 mg of wheat germ from 1 ml of glutamate solution (pH 5.8) of different concentrations was determined. The following glutamate concentrations were used: 0.5, 0.4, 0.2, 0.1, 0.05,

0.025, 0.01, and 0.005 M.

Two methods were used for extrapolation of the initial reaction velocities: (a) the observed velocities x/t (µl CO_2/ 5 min) were plotted versus time t, and initial reaction velocity was evaluated by extrapolating to the value t = 0 (manometer stopcock closing time was taken as zero time), and also to the value t = -5 min (time of tipping of the substrate solution), and (b) the observed velocities x/t were plotted versus x, and initial reaction velocity was evaluated by extrapolating to the value x = 0, as suggested by Schales and Schales (56). In all cases the initial reaction velocities were corrected by subtracting the values obtained when wheat germ was wetted with buffer containing no substrate.

The Arrhenius equation (xv) was applied for the determination of the energy of activation.

(xv) $$\frac{d \ln k}{dt} = \frac{\beta}{T^2}$$

(xvi) $$\log k = -\frac{\beta}{2.303T} + \alpha$$

where: k = reaction velocity constant.
β = E/R; E = activation energy (cal); R = gas constant.
T = absolute temperature.
α = constant.

By integrating equation (xv) and changing to base 10 logarithms one obtains equation (xvi), which is equivalent to the expression (xiv). Hence, if log k is plotted versus the

reciprocal of the absolute temperature, activation energy E is obtained from (xvii).

(xvii) $$\text{slope} = -\frac{1}{2.303R} E$$

Reaction velocity constants based on $k = \frac{1}{t} \log \frac{a}{a-x}$ (a = initial concentration of carbon dioxide; x = amount of carbon dioxide evolved during time t) were determined at different temperatures for 100 mg of wheat germ and 1 ml of 0.05 M glutamate. Velocity constants were extrapolated to the time of tipping of the substrate solution (-5 min). Two sets of experiments were performed employing different temperatures, as follows:

I	II
21°C	21.0°C
25°C	22.1°C
30°C	23.2°C
35°C	24.1°C
40°C	26.0°C
45°C	27.0°C
50°C	28.0°C
	29.7°C
	35.0°C

Determination of the Relationship Between Glutamic Acid Decarboxylase Activity and Browning

Fifty grams samples of granular wheat germ of 8.9 percent moisture content were conditioned to various moisture levels. The amount of water to be added to raise the water

content to 13, 15, 18, and 25 percent, respectively, was calculated from the following formula:

(xviii) $$w = \frac{y - x}{1 - 0.01y}$$

where: w = g of water to be added to 100 g of germ.
y = desired moisture percent.
x = original moisture percent.

Calculated amount of water was pipetted into the germ, which was simultaneously being stirred by means of a heavy glass rod. The samples were placed into tightly closed sample flasks, and put in an air oven at 100°F (38°C) constant temperature. Small amounts of germ were taken out of the oven after 1, 2, 3, 4, 6, and 8 days. The moisture contents of these samples were estimated, and amounts equal to 91.1 mg dry weight (amount of dry material in 100 mg of original germ) were used in Warburg tests, and amounts equal to 911 mg dry weight for fluorescence measurements.

Standard Warburg technique was used with the exception that samples were ground in a mortar before weighing into the vessels. One milliliter of 0.1 M glutamate (pH 5.8) was used in the sidearm.

Measurement of the Fluorescence

A slightly modified procedure of Cole and Milner (14) was used. Exactly 25 ml of 0.2 M hydrochloric acid were pipetted into an erlenmeyer flask, into which an amount of browned germ equal to 911 mg dry material was previously

weighed. After swirling a few times by hand the flasks were allowed to stand over night at room temperature. Under these extraction conditions, no improvement in extraction efficiency was found if germ was ground prior to the extraction. To the contrary, it was subsequently difficult to obtain clear solutions for fluorometry, if ground material was used. The mixture was then filtered through Whatman No. 5 filter paper, and the clear filtrate was used as such for fluorescence determination.

Measurements were made with Coleman Photoelectric Fluorometer, using B_1S and PC-1 filters. Apparatus was balanced to zero with 0.2 M hydrochloric acid and standardized to read at 60 with 0.1 ppm sodium fluorescein solution.

Determination of the Relationship of Percentage of Damaged Kernels to Glutamic Acid Decarboxylase Activity and Fluorescence

Several commercial samples of wheat grains containing various percentages of germ-damaged ("sick") kernels were obtained from the Grain Division, Agricultural Marketing Service, U. S. Department of Agriculture, Kansas City, Mo. Samples containing 4, 25, 50, 75, and 100 percent of damaged kernels were employed in the study. The embryos were carefully dissected off individual kernels by means of a razor blade; care was taken to keep contamination with endosperm to a minimum. The germ samples thus obtained were ground by micro-

Wiley mill to pass 20 mesh screen.

Glutamic acid decarboxylase activity was determined with 100 mg samples as previously described, using one milliliter of 0.1 M glutamate (pH 5.8) in the sidearm.

Fluorescence was measured from 1 g samples as described earlier with the exception that the 0.2 M hydrochloric acid extracts were diluted 1 to 100 before the actual measurements.

The Effect of Storage at Various Moisture Contents on Glutamic Acid Decarboxylase Activity

Samples of Pawnee variety hard red winter wheat were conditioned to 11.0, 14.5, 18.9, 27.0, and 36.0 percent moisture levels, and stored in air tight containers at +25°C for different periods of time. At various time intervals, the glutamic acid decarboxylase activities of the amounts of ground grains corresponding to 500 mg of the original wheat of 11.0 percent moisture content were determined, using one milliliter of 0.1 M glutamate solution in the sidearm.

The Effect of Storage at Various Temperatures on Glutamic Acid Decarboxylase Activity

Samples of "dry" wheat grains of Langdon, Seneca and Ponca varieties were stored in air tight containers at +4°, +27°, and +39°C. After a three month storage period, the glutamic acid decarboxylase activities of 500 mg of ground grains were determined, using one milliliter of 0.1 M glutamate

solution in the sidearm.

Determination of Various Enzyme Activities in Different Wheat Varieties

Following wheat varieties were employed in this study:

Hard red winter wheats:	Pawnee, Bison, Red Chief, Ponca, Triumph, Concho.
Hard red spring wheats:	Selkirk, Lee, Rescue.
Soft red winter wheats:	Vigo, Seneca.
Soft white wheats:	Genesee, Elmar.
Durum wheats:	Vernum, Langdon, Mindum.

Five hundred milligrams of ground grains of different varieties were weighed into the main chamber of Warburg vessels, and one milliliter of buffered substrate solution was pipetted into the sidearm. The following substrates were employed:

For glutamic acid decarboxylase activity:	0.1 M glutamate
For pyruvic acid carboxylase activity:	0.1 M pyruvate
For transaminase activity:	0.1 M mixture of α-keto-glutarate+ α-alanine
As control for transaminase activity:	0.1 M α-alanine

A study of the glutamic acid decarboxylase activity in eleven wheat varieties from the 1958 crops grown at twelve Kansas Agricultural Branch Experiment Stations was carried out. The following varieties were used: Kiowa, Bison, Pawnee, Wichita, Ponca, Concho, Turkey, CI 12804, CI 12871, CI 13015, and CI 13285. These varieties were obtained from the following

stations: St. John, Hayes, Tribune, Garden City (dry land), Garden City (irrigated), Mound Valley, Bellville, Mankato, Canton, Colby (dry land), Hutchinson, and Manhattan. Composites for each variety and station were made to eliminate the effects of stations and varieties, respectively. Fifty grams of each sample were weighed, mixed thoroughly, ground with micro-Wiley mill to pass 20 mesh screen, and mixed again. Five hundred milligrams were weighed for enzyme activity determinations, which were performed as previously described, using one milliliter of 0.1 M glutamate in sidearm.

RESULTS

Standard Deviation of Warburg Determinations

Several runs for glutamic acid decarboxylase activity were made using 100 mg samples of granular wheat germ for calculation of the standard deviation of Warburg determinations. Employing the method of least squares, the standard error was determined for oxygen uptake and carbon dioxide evolution both from values obtained from the same run (Table 1), and from values obtained from several different runs performed under identical conditions (Table 2). A relatively good picture about the variations due to differences in biological material was therefore obtained. If values from the same run were used, the standard deviation $\pm\sqrt{\Sigma r^2/n}$ for oxygen uptake was about ± 6 to 7 percent, and for carbon dioxide evolution only about ± 1 to 3 percent. However, if values from different

Table 1. Standard deviation of Warburg determinations as calculated from values obtained from the same run[1].

µl O_2/30 min	f	f^2	µl CO_2/30 min	f	f^2
One milliliter of 0.05 M glutamate					
-45.510	-4.059	16.475	+216.884	-1.521	2.313
-50.008	+0.439	0.193	+222.960	+4.555	20.748
-47.619	-1.950	3.803	+218.016	-0.389	0.151
-48.594	-0.975	0.950	+220.076	+1.671	2.792
-56.115	+6.586	43.375	+214.089	-4.316	18.628
sum -247.846		f^2 64.795	sum +1092.025		f^2 44.632
mean -49.569		f^2/N 12.959	mean +218.405		f^2/N 8.926
		f^2/N ±3.59			f^2/N ±3.11
One milliliter of 0.1 M glutamate					
-56.580	-1.113	1.239	+276.396	+1.447	2.094
-63.118	+5.425	29.431	+282.812	+7.863	61.827
-58.562	+0.869	0.755	+286.094	+11.145	124.211
-51.480	-6.213	38.601	+263.000	-11.949	142.779
-58.725	+1.032	1.065	+266.442	-8.507	72.369
sum -288.465		f^2 71.091	sum +1374.744		f^2 403.280
mean -57.693		f^2/N 14.218	mean +274.949		f^2/N 80.760
		f^2/N ±3.77			f^2/N ±8.97

1) f = deviation from the arithmetic mean value.
N = number of determinations.

runs performed with relatively long time intervals in between were combined, the error for both oxygen uptake and carbon dioxide evolution increased to about ±12 percent.

Table 2. Standard deviation of Warburg determinations as calculated from values obtained from different runs

µl O_2/30 min	f	f^2	µl CO_2/30 min	f	f^2
One milliliter of 0.1 M glutamate					
-58.725	-2.459	6.047	+202.259	-34.783	1029.857
-52.890	-8.294	68.790	+198.443	-38.599	1489.883
-63.960	+2.776	7.706	+271.655	+34.613	1198.060
-60.270	-0.914	0.835	+236.334	-0.708	0.501
-62.730	+1.546	2.390	+259.814	+22.772	518.564
-68.530	+7.346	53.964	+253.748	+16.706	279.090
sum -367.105		f^2 139.733	sum +1422.253		f^2 4515.955
mean -61.184		f^2/N 23.289	mean +237.042		f^2/N 752.659
		f^2/N ±7.41			f^2/N ±27.4
One milliliter of buffer without substrate					
-28.29	-0.26	0.068	+74.274	-2.648	7.012
-29.52	+0.97	0.941	+79.092	+2.170	4.709
-28.29	-0.26	0.068	+74.299	-2.623	6.880
-27.06	-1.49	2.220	+72.972	-3.950	15.603
-29.52	+0.97	0.941	+78.227	+1.305	1.730
-29.52	+0.97	0.941	+75.592	-1.330	1.769
-28.29	-0.26	0.068	+79.522	+2.600	6.760
-25.83	-2.72	7.398	+79.490	+2.568	6.595
-30.75	+2.20	4.840	+78.227	+1.305	1.730
-27.06	-1.49	2.220	+79.459	+2.537	6.436
-28.29	-0.26	0.068	+76.890	-0.032	0.001
sum -507.93		f^2 19.772	sum +1333.215		f^2 59.225
mean -28.55		f^2/N 1.797	mean +76.922		f^2/N 5.384
		f^2/N ±1.34			f^2/N ±2.32

The Effect of pH on the Decarboxylation of Glutamic Acid by Wheat Germ

As can be seen from Fig. 1 the pH optimum of glutamic acid decarboxylase in wheat germ is close to 5.8.

Michaelis-Menten Constant

In all cases the initial reaction velocities were corrected by subtracting the values obtained when wheat germ was wetted with buffer containing no substrate. As shown in Table 3, the velocities at time $t = 0$ did not markedly differ from each other in regard to the method used for extrapolation. As could be expected, there was, however, a slight variation in V_{max} depending on the method of extrapolation of the initial reaction velocity (Table 4). V_{max} varied from 11.8 to 13.4 µl/min/100 mg germ/1 ml 0.1 M glutamate. Michaelis-Menten constant K_m was always found to be 0.025 M. Figure 2 shows the effect of concentration on initial reaction velocity, and Fig. 3 the extrapolation of K_m and V_{max}, when initial velocities were determined by the method (a).

Activation Energy

Figure 4 shows the effect of temperature on the oxygen uptake and the carbon dioxide release by wheat embryos wetted with glutamate solution. The optimum temperature of the decarboxylation of glutamic acid appears to be about 30°C, whereas that of the oxygen uptake mechanism is around 40°C.

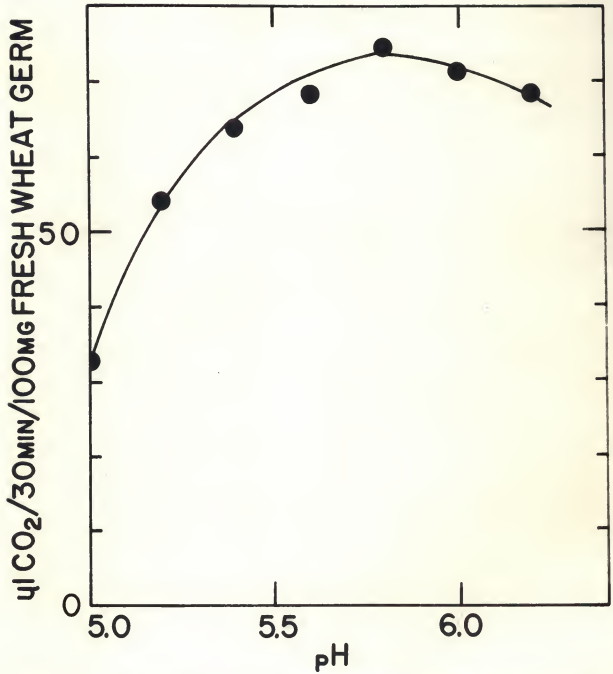


Fig. 1. The effect of pH on the decarboxylation of glutamic acid by wetted wheat germ. 100 mg germ and 1 ml of M/15 phosphate buffer.

Table 3. Velocity of decarboxylation of 1 ml of 0.1 M glutamic acid (pH 5.8) by 100 mg of wheat germ at time t = 0

Glutamate concentration M	Method (a) uncorr. µl/min	Method (a) corr. µl/min	Method (b) uncorr. µl/min	Method (b) corr. µl/min
0.5	12.5	10.6	12.9	11.1
0.4	12.5	10.6	12.9	11.1
0.2	12.0	10.1	12.3	10.5
0.1	11.0	9.1	11.4	9.6
0.05	10.1	8.2	10.5	8.7
0.025	8.2	6.3	7.3	5.5
0.010	5.1	3.2	5.4	3.6
0.005	3.8	1.9	4.3	2.5
0	1.9	0	1.8	0

Table 4. Effect of method of extrapolation on the maximum initial velocity of decarboxylation of glutamic acid by wheat embryos

Method of extrapolation of initial velocity	V_{max} (µl/min)
Method (a), extrapolated to t = 0 min	11.8
Method (a), extrapolated to t = -5 min	13.4
Method (b), extrapolated to x = 0 µl	12.5

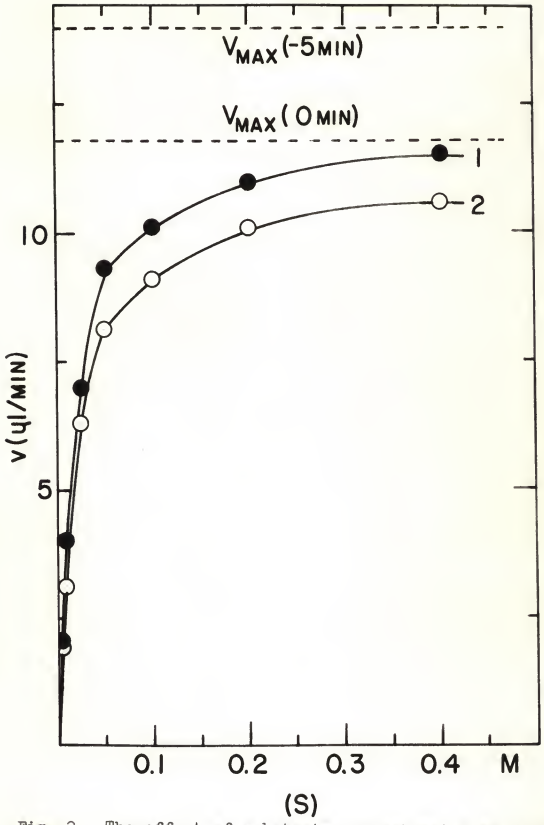


Fig. 2. The effect of substrate concentration on initial reaction velocity of the decarboxylation of glutamic acid by wetted wheat germ. 50 mg germ and 1 ml of substrate solution (pH 5.8 at 30°C). (1) t = -5 min (tipping time); (2) t = 0 min (stopcock closing time).

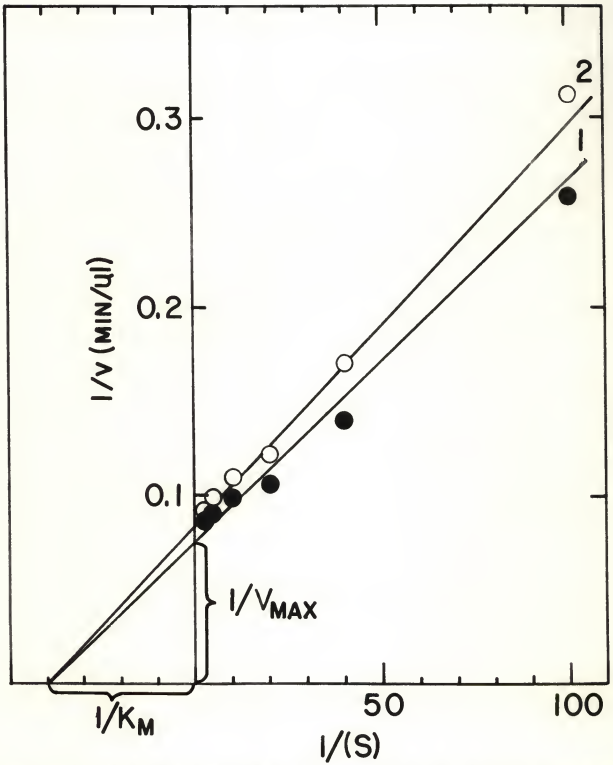


Fig. 3. Extrapolation of K_m and V_{max}. Initial velocities determined by method (a). 50 mg germ and 1 ml of substrate solution (pH 5.8 at 30°C). (1) t = -5 min (tipping time); (2) t = 0 min (stopcock closing time).

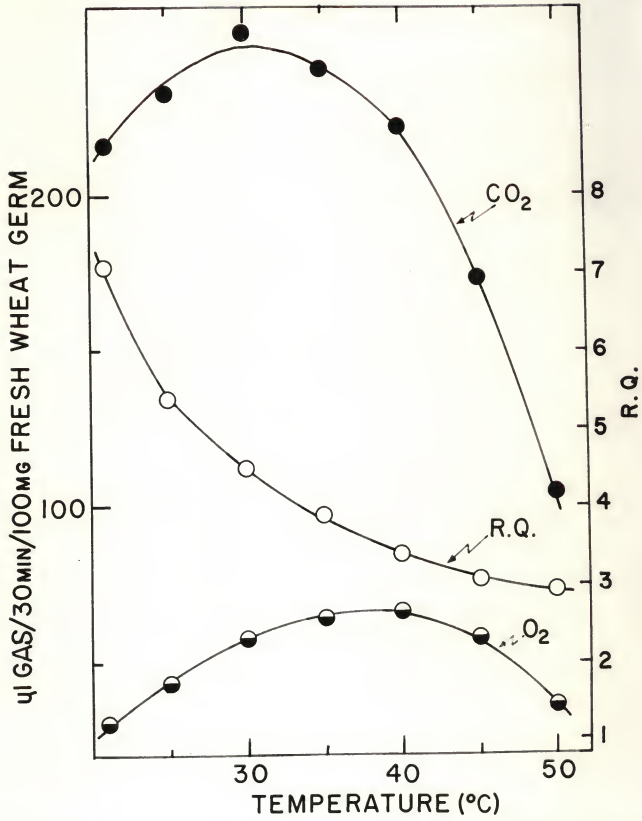


Fig. 4. The effect of temperature on carbon dioxide evolution, oxygen uptake, and R.Q. by wetted wheat germ. 100 mg germ and 1 ml 0.05 M glutamate at pH 5.8.

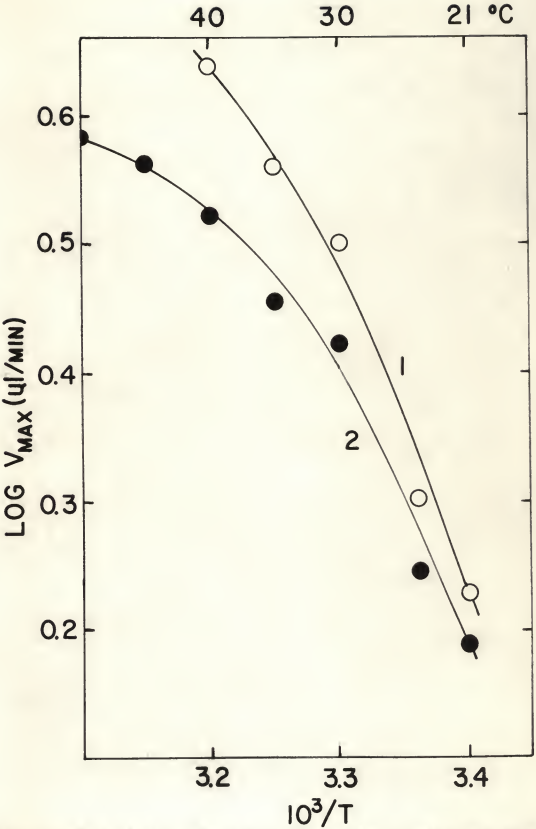


Fig. 5. The effect of temperature on the maximum rate of decarboxylation of glutamate by wetted wheat germ. 100 mg germ and 1 ml 0.05 M glutamate at pH 5.8. (1) t = -5 min (tipping time); (2) t = 0 min (stopcock closing time).

An interesting observation is that R.Q. decreases continuously while the temperature increases from 21° to 50°C.

Reaction velocities were determined at various temperatures both at t = 0, and at t = -5 min. Log V_{max} (µl/min) was plotted versus the reciprocal of absolute temperature 1/T. As can be seen from Fig. 5 the correlation was reasonably linear only from 21° to 30°C. At higher temperatures the enzyme was seemingly partially inactivated. Hence only this temperature range was used for the determination of the energy of activation.

When velocity constants $k = \frac{1}{t} \log \frac{a}{a-x}$ (where a = theoretical amount of carbon dioxide based on the amount of glutamate supplied = nRT/P = 0.815T), determined at various temperatures, where plotted against time, straight lines were again obtained but only within the temperature range of 21°C to 30°C. However, when the values of k were extrapolated to the time t = -5 min, it was evident that k_{-5min} increased up to 50°C, whereafter the values of k rapidly decreased with time.

When 100 mg of wheat germ and one milliliter of 0.05 M glutamate were used, an activation energy of E = 9100 cal was obtained. The correction for the carbon dioxide evolution caused by wetting the germ with buffer containing no substrate had no effect on the slope = E/(2.303R), and hence on the activation energy.

The Effect of Nitrogen Atmosphere on the Decarboxylation of Glutamate

Figure 6 shows that in the presence of nitrogen there is a significant increase in pressure within the first minute after tipping the solution from the sidearm. A careful study showed that this unusual increase in pressure continued for about five minutes. To find out how much of this immediate pressure increase may have been due to the wetting directly because of some physical phenomenon rather than due to enzymatic decarboxylation of glutamate, some measurements were done after inactivation of the enzymes. For this purpose 100 mg samples of granular wheat germ were heated at 150°C for periods of 10, 30, 60, and 180 minutes, after which the rate of carbon dioxide evolution was determined as usual. Buffer (pH 5.8) with no substrate was used in the sidearm. Other studies were done by inactivating the enzymes by means of 70 percent ethanol.

It was first noticed that only ten minutes of heating at 150°C caused almost total loss of decarboxylase activity. The results are summarized in Table 5. It can be seen that if the enzymes were denaturated with 70 percent ethanol, after which the germ was air dried at room temperature (25°C), no sudden pressure increase took place. In fact, there was a moderate decrease in pressure. However, if the ethanol treated material was dried at 150°C for ten minutes, an immediate "gas" production within the first minute after

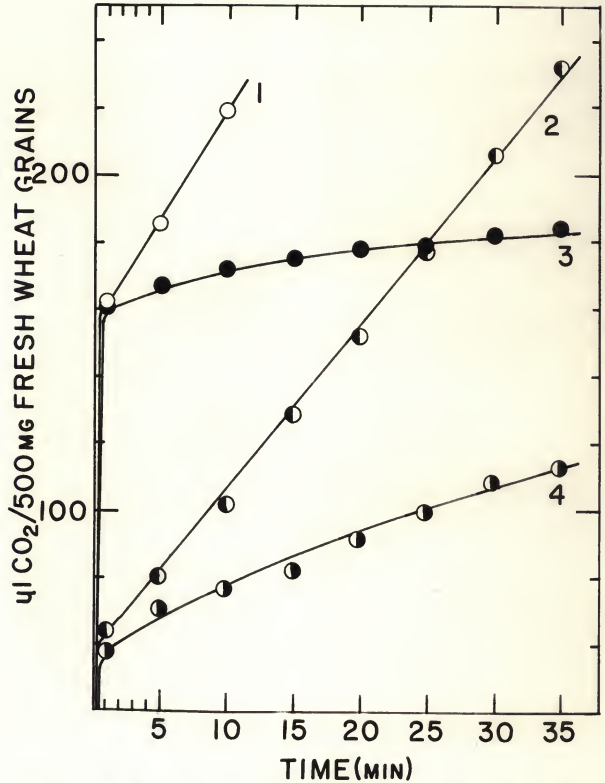


Fig. 6. The effect of wetting of wheat germ with buffer (3,4) and with buffered 0.1 M glutamate solution (1,2) (pH 5.8 at 30°C) on decarboxylation under air (2,4) and nitrogen (1,3).

Table 5. The effect of denaturation of enzymes on the initial pressure increase observed in Warburg manometer due to wetting of germ

Time, min	Stopcock closed before tipping				Stopcock closed 5 min after tipping		
	no treatment	10 min at 150°C	5 min with 70% EtOH, dried in air	5 min with 70% EtOH, dried at 150°C	no treatment	10 min at 150°C	5 min with 70% EtOH, dried in air
	µl CO_2						
1	+92.09	+88.29	-31.63	+103.65	-	-	-
5	+94.81	+86.95	-39.17	+103.65	+16.93	+5.19	+14.43
30	+163.55	+94.87	-3.91	+120.49	+83.42	+19.48	+68.97

wetting could again be noticed. It may be noted that closing stopcocks prior to the wetting caused a considerable immediate pressure increase also in the flasks containing potassium hydroxide in the center well. This pressure increase was, however, of a much smaller order of magnitude than in the vessels containing no potassium hydroxide.

Figure 6 shows also that after the significant initial pressure increase during the first minute after wetting, carbon dioxide evolution remains greater under anaerobic conditions. It can also be seen that the presence or absence of glutamate does not affect the initial pressure increase.

Relationship of Moisture-Induced Browning to Glutamic Acid Decarboxylase Activity

Samples of granular wheat germ (8.9% moisture content) conditioned to 13, 15, 18, and 25 percent moisture levels, were stored at 100°F for different periods of time. No mold growth could be noticed in any of these samples during the eight day experimental period. The moisture contents in the upper layer of these samples after different experimental storage periods are shown in Table 6.

As can be seen from Fig. 7 fluorescence increased very little below 18 percent moisture levels during the experimental heating period. However, at higher moisture levels a rapid increase in browning and fluorescence was observed. At 25 percent moisture level the fluorescence reading increased five-

Table 6. Moisture contents of wetted wheat germ samples after various periods of heating at 100°F (from duplicates)

Original moisture (%)	Moisture content (%) after various time periods (days) in oven at 100°F					
	1	2	3	4	6	8
8.9	8.26	8.14	7.66	7.60	7.18	6.81
13	12.08	11.64	11.92	11.50	10.82	11.56
15	13.92	14.14	13.23	13.00	12.79	12.51
18	16.83	16.74	16.56	16.85	17.59	16.97
25	22.97	24.36	24.47	25.19	25.89	25.92

fold within eight days. Correspondingly (Fig. 8), there was only a slight decrease in the rate of decarboxylation of glutamic acid at moisture levels below 18 percent. But at 18 percent moisture level the decrease in decarboxylase activity was significant, and at 25 percent moisture level decarboxylase activity virtually disappeared within three days.

When germ ends of commercial "sick" wheat kernels containing different percentages of damaged embryos were cut off, and the fluorescence and glutamic acid decarboxylase activity were determined, a good inverse correlation between the degree of fluorescence and glutamic acid decarboxylase activity was found (Fig. 9). However, slight decarboxylation was observed even with the sample classified as 100 percent damaged. The relative fluorescence in these samples was much higher than that developed in artificially wetted commercial germ.

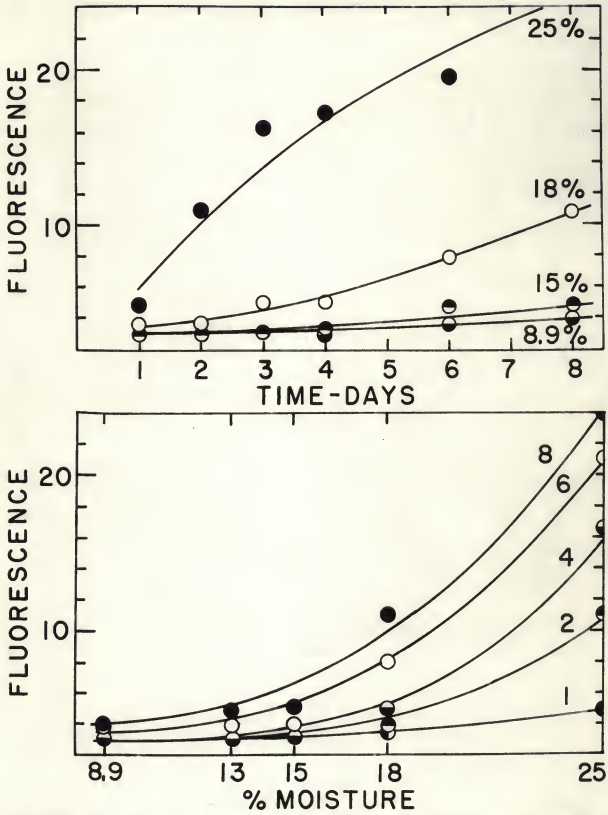


Fig. 7. (upper) The effect of time on the fluorescence of wheat germ stored at various moisture levels at 100°F. (lower) The effect of moisture on the fluorescence of wheat stored various periods of time at 100°F.

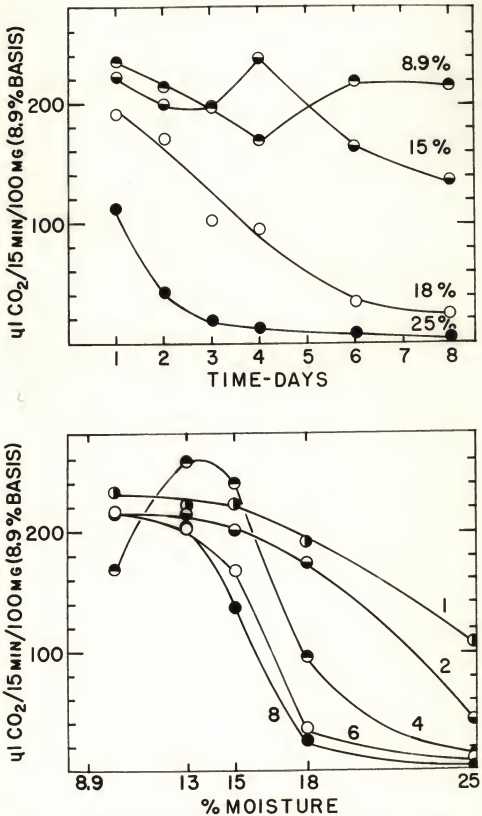


Fig. 8. (upper) The effect of time on the decarboxylation of glutamate by wheat germ stored at various moisture levels at 100°F. (lower) The effect of moisture on the decarboxylation of glutamate by wheat germ stored various periods of time at 100°F.

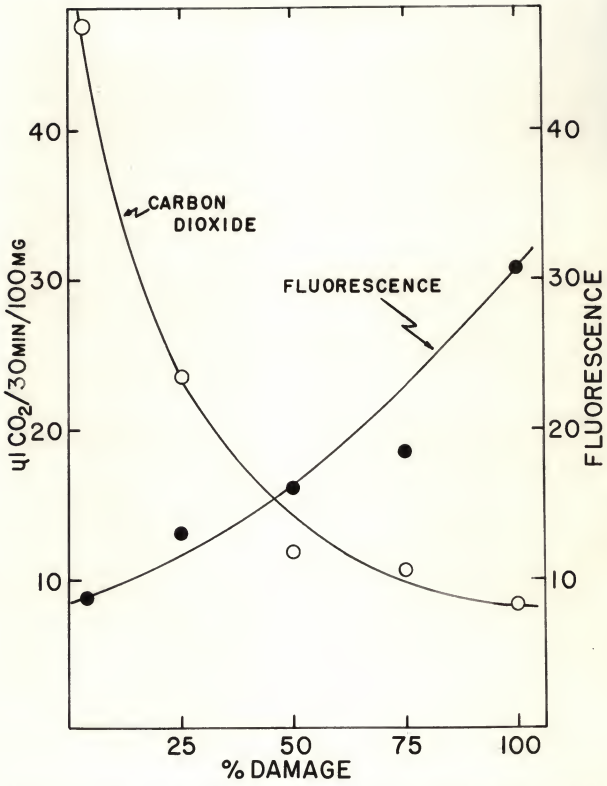


Fig. 9. The relationship between percent of damaged embryos in commercial "sick" wheat to decarboxylation of glutamic acid, and fluorescence.

The Effect of Storage of Pawnee Wheat Conditioned to Different Moisture Levels at +25°C on Decarboxylase Activity

Samples of Pawnee wheat conditioned to 11.0, 14.5, and 18.9 percent moisture levels remained relatively free from mold growth during the experimental period of five weeks. However, several weeks later, the 18.9 percent sample gradually molded heavily, the two lower moisture samples still remaining seemingly mold free. Grain which contained 27.0 and 36.0 percent moisture, respectively, molded heavily within a few days. However, with further storage in sealed containers this mold growth disappeared rapidly due to anaerobic conditions caused by increased carbon dioxide evolution. These samples then remained mold free for several months.

As can bee seen from Fig. 10, glutamic acid decarboxylase activity decreased in all samples during the course of time. This decrease was very significant at moisture levels higher than 18.9 percent, the activity virtually disappearing within a couple of weeks. However, it may be seen from Fig. 10 that the enzyme activity even in the 18.9 percent sample had decreased to about half from the original value within 43 days.

The Effect of Storage of Different Wheat Varieties in a "Dry" State at Various Temperatures

As can be seen from Fig. 11, the glutamic acid decarboxylase activity of Langdon, Seneca, and Ponca grains stored in

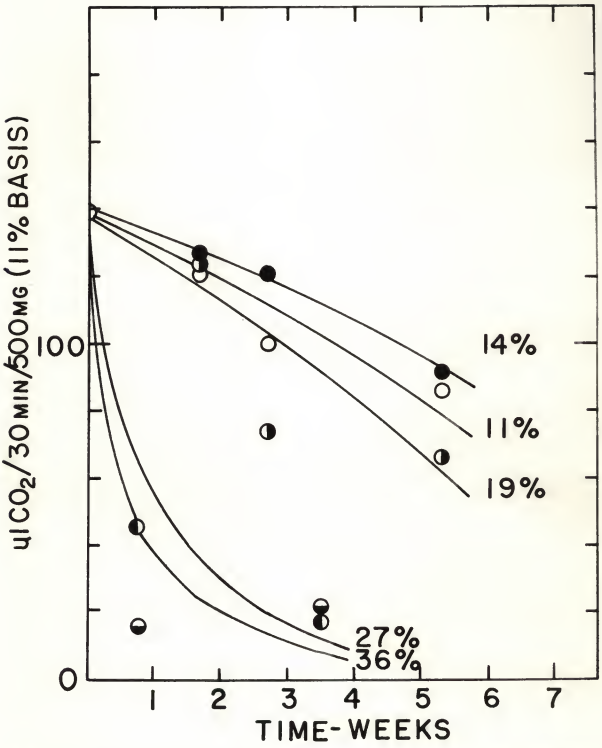


Fig. 10. The effect of storage of Pawnee wheat conditioned to different moisture levels, at +25°C, on glutamic acid decarboxylase activity.

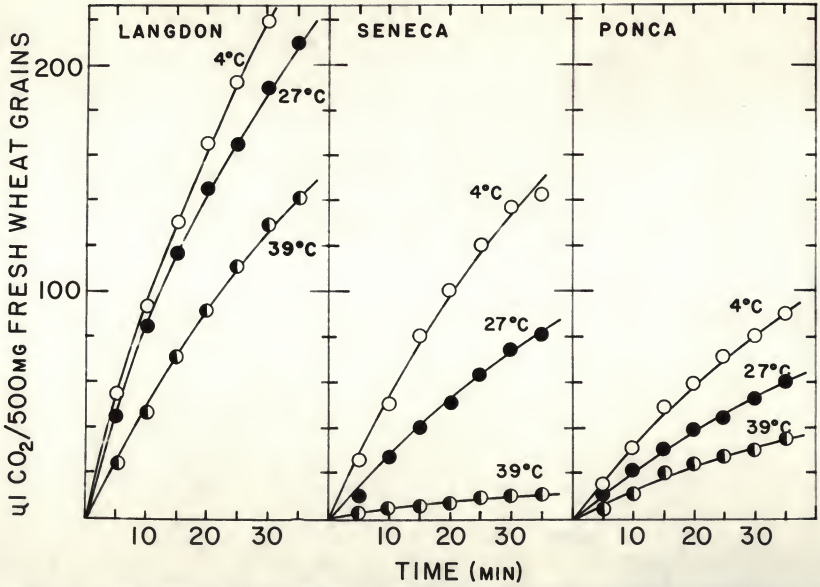


Fig. 11. The effect of storage at various temperatures of different varieties of wheat in a dry state on glutamic acid decarboxylase activity.

airtight containers at +4°, +27°, and +39°C decreased at the two higher storage temperatures as compared to the enzyme activity retained at +4°C. Moisture content of the samples in these experiments was about 11 percent. Langdon variety seemed to be more resistant to loss in enzyme activity than the other two. Seneca was most sensitive. Within three months of storage at room temperature (+27°C), carbon dioxide evolution from glutamate decreased to about half of that in grain stored at + 4°C. Three months storage at +39°C virtually inactivated this enzyme system.

Study of Various Enzyme Activities in Different Wheat Varieties

Several wheat varieties were studied for their glutamic acid-alanine transaminase, and glutamate, pyruvate, and alanine decarboxylase activities. The results are presented in Figs. 12 to 15. From these figures it is apparent that marked differences occur among classes and varieties of wheat. However, no correlation could be found in these enzyme activities to the class. For example, Pawnee wheat exhibited one of the highest glutamic decarboxylase activities in contrast to the weak activity in Ponca, yet both are hard red winter wheats.

When determinations were made using composites from different stations, and different varieties of 1958 crop, much smaller variations in enzyme activities were found (Table 7). However, Ponca variety again showed the lowest activity.

Table 7. Glutamic acid decarboxylase activity of variety and station composites

Variety or station	Moisture %	µl CO_2/30 min/500 mg dry wt.
Variety composites (from 12 stations)		
Kiowa	11.3	109.2
Bison	10.7	102.2
Pawnee	9.8	103.0
Wichita	9.7	92.4
Ponca	10.0	62.2
Concho	10.0	119.2
Turkey	10.3	114.8
CI 12904	10.7	127.9
CI 12871	10.2	106.1
CI 13015	10.0	102.3
CI 13285	10.4	80.3
Station composites (of 11 varieties)		
St. John	12.3	97.4
Hayes	13.0	105.2
Tribune	12.2	101.8
Garden C. (dry)	13.1	112.0
" (irrigated)	13.2	106.0
Mound Valley	12.5	97.8
Bellville	12.8	93.0
Mankato	13.0	78.0
Canton	9.8	94.2
Hutchinson	10.8	103.2
Colby (dry)	12.3	107.9
Rowhattan	10.9	122.2

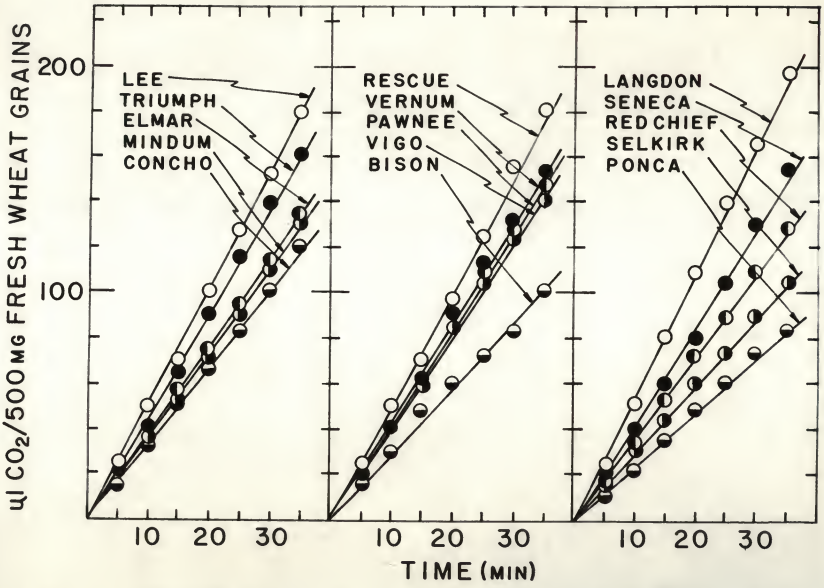


Fig. 12. Transamination of α-ketoglutaric acid with α-alanine by different wheat varieties.

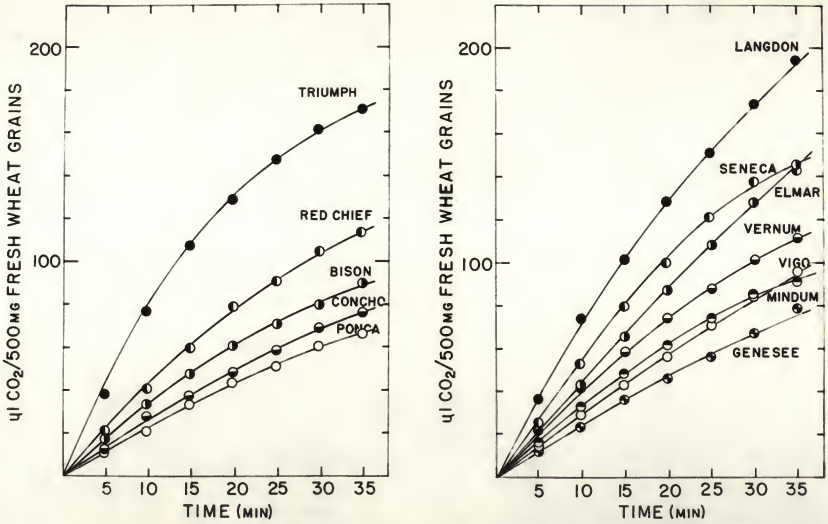


Fig. 13. Decarboxylation of glutamic acid by different wheat varieties.

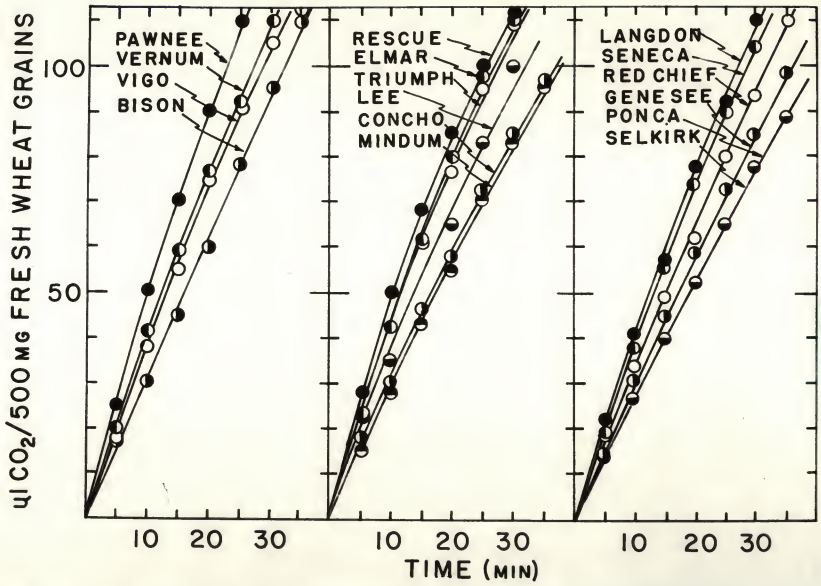


Fig. 14. Decarboxylation of pyruvic acid by different wheat varieties.

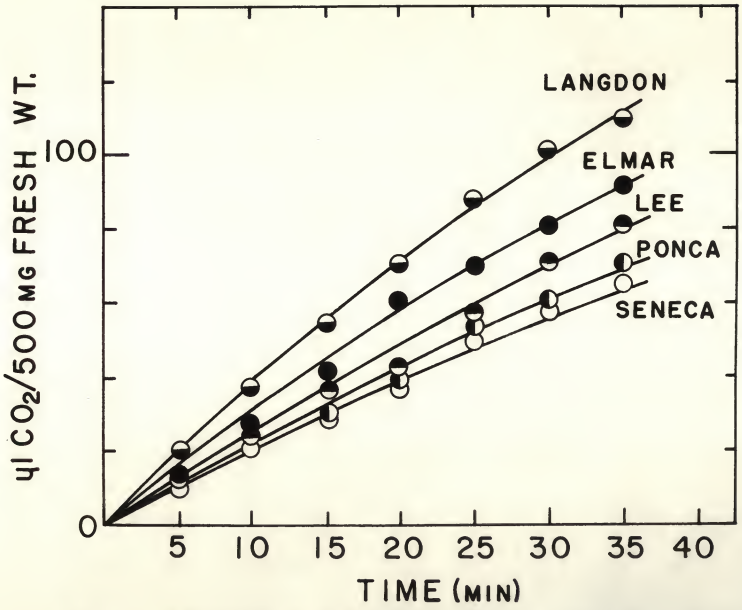


Fig. 15. Decarboxylation of alanine by different wheat varieties.

DISCUSSION

Reaction Kinetics of the Decarboxylation of Glutamate by Wheat Germ

Initial reaction velocities of enzymatic reactions are normally determined by plotting the observed velocities x/t versus time t, and extrapolating to the value t = 0. However, Schales and Schales (56) found that the enzymatic decarboxylation of glutamic acid does not truly follow a first order reaction, the values of velocity constants decreasing during the time. He also noticed that this decrease in reaction velocity seemingly depended on the amount of substrate present at any given time. Therefore reasonably straight lines are obtained by plotting the velocities x/t versus x. Initial reaction velocity can then be obtained by extrapolating to the value x = 0. However, because manometer stopcocks were normally closed five minutes after tipping the substrate solution into the reaction chamber, it was necessary to extrapolate the velocities to the value t = -5 min. But the method of Schales gives velocities only at time t = 0, hence it was found necessary to compare results obtained by both methods.

In all cases, the results were corrected by subtracting the values obtained when wheat germ was wetted with buffer solution containing no substrate. As could be expected, no significant differences for the initial and maximum reaction velocities at stopcock closing time were obtained comparing

the two methods.

The value 0.025 M found for the Michaelis-Menten constant is in good agreement with earlier results obtained with glutamic acid decarboxylase from various other sources. Gale (19) reported for cell-free bacterial glutamic acid decarboxylase a K_m = 0.027 M, and for intact cells, 0.005 M. This difference shows that the enzyme undergoes significant damage during isolation. According to Beevers (6), isolated barley glutamic acid decarboxylase rapidly loses its activity when redissolved, even at 0°C. He found a K_m = 0.0096 M for barley. Schales and Schales (56) obtained a K_m = 0.0036 ± 0.0004 for carrots, and Wingo and Awapara (67) a K_m = 0.021 for brain tissues.

The optimum temperature of 30°C for wheat glutamic acid decarboxylase is considerably lower than that of most other enzyme reactions. The majority of previous research with this enzyme has been done at about 37°C, and it seems quite evident that thermal inactivation begins to be significant at this temperature. The results of Rohrlich (51) for optimum temperature of wheat glutamic acid decarboxylase are even lower, about 27°C.

The fact that R.Q. value decreases continuously when temperature increases from 21° to 50°C indicates a relatively faster thermal inactivation of glutamic acid decarboxylase than of oxygen uptake mechanism. In this connection it is of interest to note that calculation of R.Q. values in biological material easily may lead to wrong conclusions in regard to the respiration itself, since the enzymatic decarboxylation of glutamate

naturally present in tissues can be carried out anaerobically (11, 45, 58) thus leading to very high R.Q. values, practically independent of oxygen uptake.

The correction for the carbon dioxide evolution caused by wetting the germ with plain buffer had no effect on the slope -E/(2.303R), and hence on the activation energy. This indicates that apparently much of the immediate carbon dioxide evolution from wetted germ is due to decarboxylation of the glutamate naturally present (30), rather than to carbon dioxide released from other sources.

The effect of pH on glutamic acid decarboxylase has been studied by several workers. The optimum pH of 4.0 to 4.5 for bacterial enzyme was reported by Gale (19). On the other hand, a relatively high optimum pH of 6.8 was found by Wingo and Awapara (67) for brain tissues. The values obtained for higher plants have been relatively constant regardless of the source of enzyme: carrots 5.60 to 5.80 (56), barley 5.0 to 5.5 (6), and wheat 5.5 (51). The optimum pH 5.8 found in this study is in good agreement with these earlier observations.

The Effect of Environment on Viability and Glutamic Acid Decarboxylase of Wheat

The importance of moisture and temperature as factors influencing the viability of grain during storage has been stressed earlier in this paper. At moisture levels as low as 15 to 18 percent several enzyme systems including glutamic acid decarboxylase are activated (29, 30).

In the present study it was shown that moisture contents above 25 percent result in a rapid mold growth at +25°C, which however, recedes completely within a few days in sealed containers due to the anaerobic conditiones developed by an enormous carbon dioxide evolution. However, at an 18.9 percent moisture level carbon dioxide production was not high enough to prevent mold growth, hence the grain stayed moldy for a period of several months. Despite these facts, glutamic acid decarboxylase activity decreased faster at moisture levels of 27.0 and 36.0 percent, indicating that the major contributor would be the effect of moisture on the grain itself, rather than the fungal growth induced by increased moisture content.

Considerable browning, as observed visually and by means of a fluorometer, also developed at +37°C, under conditions not favorable to fungal growth. These results confirm earlier observations by McDonald and Milner (34), who found that browning accompanied by increased fluorescence is promoted by elevated temperatures and humidities, and invariably preceded respiratory increases indicative of mold growth. It now appears that the decrease in glutamic acid decarboxylase activity due to elevated moisture levels clearly follows the increase in browning. This correlation was confirmed in commercial wheat samples with varying percentages of damaged ("sick") embryos.

Temperature increase alone has a pronounced effect on the retention of glutamic acid decarboxylase. Dry grains with

about 11 percent moisture content, when stored at +27°C for three months in air-tight containers showed a significant decrease in glutamic acid decarboxylase activity. This was not surprising in view of the fact that nonenzymatic browning is known to occur in a "dry" state (49). It should also be pointed out that different wheat varieties responded differently to this treatment.

It was recently noticed in this laboratory (30) that if wheat germ was kept under anaerobic conditiones after wetting, glutamate, aspartate, and the corresponding keto acids vanished much faster than if aerobic conditions were employed. This was thought to indicate that glutamate would decarboxylate faster under anaerobic than aerobic conditions. The significant carbon dioxide evolution within first minute after wetting was not affected by added glutamate suggesting that this immediate carbon dioxide production would be due to glutamic acid naturally present in grains. This naturally-occuring glutamate is more easily susceptible to decarboxylation than is added substrate, which has to penetrate the germ prior to the reaction. Carbon dioxide evolution can, however, explain only part of this sudden significant pressure increase. Part of it may be attributed to a release of loosely bound carbon dioxide or other gases. It is also very likely that the structure, and hence size of water-imbibing macromolecules changes with water absorption so that an apparent increase in volume occurs. This trend later slowly recedes.

The fact that glutamate apparently decarboxylates faster under anaerobic than aerobic conditions is particularly interesting, since attempts have been made to store grains under anaerobic conditions in order to prevent mold growth. The present study suggests that grains seem to be more susceptible to moisture induced loss of glutamate under anaerobic conditions maintained by nitrogen, than when stored in air.

The Effect of Variety and Growth Conditions on Glutamic Acid Decarboxylase in Wheat Grains

As shown in Figs. 12 to 15 significant differences in glutamic acid decarboxylase activity exist between different varieties of wheat. However, no influence could be found due to class of wheat. Therefore it was assumed that variations in storage conditions and time would be major reasons for the observed differences, especially because only a few months storage at room temperature significantly lowers decarboxylase activity. When determinations were made using variety and station composites from wheats of 1958 crop, indeed smaller differences were encountered. However, in both cases Ponca variety exhibited the lowest activity suggesting that purely varietal changes may be possible.

Almost no differences could be found among varieties in regard to pyruvic carboxylase activity. This is in good agreement with other results from this laboratory which show that these two enzyme reactions are catalyzed by different enzyme systems. However, it is interesting to point out that

Ponca variety again showed the lowest activity. The differences in the decarboxylation of α-alanine as compared to that of pyruvate indicate that α-alanine may be directly decarboxylated rather than via transamination to pyruvate. Transamination is known not to be the intermediary step in decarboxylation of aspartate by Nocardia globerula (15). The contrary is true of α-ketoglutarate decarboxylation in certain higher plants (54).

Suggestions for Future Research

The kinetic studies confirm earlier observations that wetting of wheat germ immediately activates glutamic acid decarboxylase, which results in highly elevated R.Q. values. However, the relationship of this phenomenon to the loss of viability and hence to germination, remains largely unresolved. The fact that R.Q. decreases when temperature increases from 21° to 50°C suggests that there has to be a certain lower temperature exhibiting a maximum R.Q. value, because evidently when temperature approaches 0°C R.Q. approaches value 1. Apparently there will be a sharp decrease in R.Q. immediately prior to the maximum value. It would be of interest to obtain kinetic data at temperatures lower than +21°C, because the temperature at which R.Q. reaches the apparent sharp maximum could be important from the storage point of view.

Additional data should be obtained about the nonidentity of the enzyme systems decarboxylating glutamate and pyruvate. Therefore Michaelis-Menten constant and energy of activation for the decarboxylation of pyruvate by wheat germ should be

determined.

What is the real reason for the sharp increase in pressure in Warburg vessels within the first five minutes after wetting? The pressure increase during the first few minutes should be compared to the amount of glutamate naturally present in germ.

It would be of interest to determine in greater detail the effect of moisture and temperature on glutamic acid decarboxylase activity, as well as on browning and viability, because the correlation now observed between decarboxylase activity and browning indicates a close relationship of this enzyme or the endproduct γ-aminobutyric acid to the viability of grains.

Is there any correlation between succinic dehydrogenase activity and glutamic acid decarboxylase activity, in terms of dependence on moisture and temperature? If there is, could the rate of carbon dioxide evolution from wetted grains either with or without added glutamate be used to indicate the degree of viability? As succinic dehydrogenase now is involved in the viability testing using triphenyltetrazolium chloride, it would also be of great interest to find out if correlation exists between high glutamic acid decarboxylase activity and susceptibility to moisture-induced storage damage and loss of viability. If such a correlation exists, the determination of glutamic acid decarboxylase activity might aid plant breeders to develop varieties more resistant to storage damage. The answer to these questions will be the task of future research.

SUMMARY

The reaction kinetics of enzymatic decarboxylation of glutamic acid in wheat germ were studied by means of Warburg manometric techniques.

Initial reaction velocities were determined by (a) plotting the observed velocities x/t versus time t, and extrapolating to the value t = -5 min (reaction begins), and to the value t = 0 min (manometer stopcock closed); and (b) plotting the velocities x/t versus x and extrapolating to the value x = 0. Despite the fact that the reaction does not truly follow a first order reaction, in all cases a Michaelis-Menten constant K_m = 0.025 M was obtained indicating that the method of extrapolation of the initial velocities is not critical.

An activation energy of 9100 cal in the temperature range of 21° to 30°C was found when the reaction system involved 100 mg of granular wheat germ and one milliliter of 0.05 M glutamate (pH 5.8). The optimum temperature for glutamic acid decarboxylase was 30°C, whereas that of the oxygen uptake mechanism was about 40°C. Respiratory quotient decreased constantly with temperature increase.

The pH optimum of glutamic acid decarboxylase was 5.8.

The carbon dioxide evolution from wetted wheat germ due mainly to decarboxylation of glutamic acid proceeds faster in a nitrogen atmosphere than in air. A significant pressure increase in Warburg vessels occurs immediately upon wetting and continues for about five minutes, whereafter it recedes

a little. The nature of this pressure increase was studied and it was found that nearly half of it apparently is due to carbon dioxide evolution and the rest to some physical phenomenon probably associated with a apparent volume change of water-imbibing macromolecules.

Damp wheat germ heated for an extended period at 37°C showed a decrease in the decarboxylation of glutamate, accompanied by a simultaneous increase in browning. These changes were especially significant at moisture levels above 18 percent. Browning in commercial wheat samples, as indicated by fluorescence, increased when the percentage of "sick" wheat increased, and in this material also a simultaneous decrease in glutamic acid decarboxylase activity occured.

Glutamic acid decarboxylase activity decreased in damp Pawnee wheat stored in a moisture range of 11.0 to 36.0 percent. The decrease in enzyme activity was very pronounced at moisture levels above 18.9 percent.

Samples of Seneca, Ponca, and Langdon wheat varieties stored for three months in dry state at +4°, +27°, and +39°C showed a decrease in glutamic acid decarboxylase activity with increasing temperature. Langdon variety was most resistant towards loss in enzyme activity.

The activities of glutamic acid, α-alanine, and pyruvic acid decarboxylases, and glutamic acid-alanine transaminase in 16 wheat varieties including soft and hard winter types, as well as spring varieties including Durum types showed pronounced differences. No correlation between enzyme activity

and wheat class was observed, but among individual varieties Ponca exhibited the lowest activity. Differences in glutamic acid decarboxylase activity were generally small among eleven hard winter wheat varieties from comparable environmental background, but Ponca showed consistently less activity than other varieties. This led to the conclusion that varietal differences may occur.

ACKNOWLEDGMENTS

The writer is glad to have the opportunity of expressing sincere gratitude to her major instructor, Professor Max Milner, for his never-failing interest, invaluable advice and encouragement throughout the course of this investigation.

She is also indebted to Professor John A. Shellenberger, Head of the Department of Flour and Feed Milling Industries for providing excellent research facilities, and to Asst. Professor Pekka Linko for his advice and help during the course of this work and in preparation of this report.

Furthermore, she is very grateful to Cargill, Inc. for financial support.

LITERATURE CITED

(1) Abderhalden, E., G. Fromme and P. Hirsch.
Die Bildung von β-Aminobuttersäure aus l-Glutaminsäure unter dem Einfluss von Mikro-organismen. Hoppe-Seyler's Z. Physiol. Chem. 85, 131-135 (1913).

(2) Bailey, C. H.
Respiration of shelled corn. Univ. Minn. Agr. Expt. Sta. Tech. Bul. 2, 34 pp. (1921).

(3) Bailey, C. H. and A. M. Gurjar.
Respiration of stored wheat. Jour. Agr. Res. 12, 685-710 (1918).

(4) Barton, L. V.
Effect of moisture fluctuations on the viability of seeds in storage. Contrib. Boyce Thompson Inst. 13(2), 35-46 (1943).

(5) Barton, L. V.
Seed storage and viability. Contrib. Boyce Thompson Inst. 17(2), 87-103 (1953).

(6) Beevers, H.
An L-glutamic acid decarboxylase from barley. Biochem. Jour. 48, 132-137 (1951).

(7) Blish, M. J. and B. D. Hites.
A rapid and simple carbide method for estimating moisture in flour. Cereal Chem. 7, 99-107 (1930).

(8) Camien, M. N., L. E. McClure, A. Lepp and M. S. Dunn.
The preparation of β-aminobutyric acid by enzymatic decarboxylation of L-glutamic acid. Arch. Biochem. Biophys. 43, 378-380 (1953).

(9) Carter, E. P. and G. Y. Young.
Role of fungi in the heating of moist wheat. U. S. Dept. Agr. Cir. 838 (1950).

(10) Cereal Laboratory Methods, 6th ed., Publ. by American Assoc. of Cereal Chem., St. Paul, Minn. (1957).

(11) Cheng, Yu-Yen, P. Linko and M. Milner.
Glutamic acid decarboxylase in wheat grains. Suomen Kemistilehti B31, 333-335 (1958).

(12) Cheng, Yu-Yen, P. Linko and M. Milner.
On the nature of glutamic acid decarboxylase in wheat. Plant Physiol. (to be published).

(13) Christensen, C. M.
Deterioration of stored grains by fungi. Botan. Rev. 22, 108-134 (1957).

(14) Cole, E. W. and M. Milner.
Colorimetric and fluorometric properties of wheat in relation to germ damage. Cereal Chem. 30, 378-391 (1953).

(15) Crawford, L. W.
Studies on the aspartic acid decarboxylase of Nocardia globerula. Biochem. Jour. 68, 221-225 (1958).

(16) Cruickshank, D. H. and F. A. Isherwood.
Glutamic-alanine and glutamic-aspartic transaminase of wheat germ. Biochem. Jour. 69, 189-195 (1958).

(17) Denman, R. F., D. S. Hoare and E. Work.
Diaminopimelic acid decarboxylase in pyridoxine-deficient Escherichia coli. Biochim. Biophys. Acta 16, 442-443 (1958).

(18) DeVay, J. E.
A note on the effect of mold growth and increased moisture content on the free amino acids in hard red spring wheat. Cereal Chem. 29, 309-311 (1952).

(19) Gale, E. F.
The bacterial amino acid decarboxylases. Adv. in Enzymol. 6, 1-32 (1948).

(20) Gane, R.
The respiration and water content of seeds. Dept. Sci. Ind. Research (Great Britain), Rept. of Food Invest. Bd. 135-137 (1935).

(21) Gilman, J. C. and D. H. Barron.
Effects of molds on temperature of stored grains. Plant Physiol. 5, 565-573 (1930).

(22) Guirard, B. M. and E. E. Snell.
Pyridoxal phosphate and metal ions as cofactors for histidine decarboxylase. Jour. Am. Chem. Soc. 76, 4745-4747 (1955).

(23) Hodnett, G. E.
The effect of temperature and moisture during storage on the viability of grass seeds. Trop. Agriculture (Trinidad) 35, 208-212 (1958).

(24) Hyde, M. B.
Respiration of mature grains in relation to moisture content and fungal contamination. Congr. Intern. Botan. Rapps. et Communs. 8, Sect. 11/12, 106-107 (1954).

(25) Király, Z. and G. L. Farkas.
Infektions bedingte Änderung der Glutaminsäuredecarboxylase Aktivität beim rostfallenen Weizen. Naturwiss. 44, 353 (1957).

(26) Kolkwitz, R.
Über die Athmung ruhender Samen. Ber. deut. botan. Ges. 19, 285-287 (1901).

(27) Kretovich, V. L. and E. N. Ushakova.
Über die kritische Feuchtigkeit und den Athmungswechsel des Kornes beim Aufbewahren. Compt. rend. acad. Sci. URSS 29, 115-119 (1940); in Chem. Abstr. 35, 2564 (1941).

(28) Kulakarni, L. and K. Sohonie.
Glutamic acid decarboxylase in legumes. Nature 178, 925 (1956).

(29) Linko, P. and M. Milner.
Enzyme activation in wheat grains in relation to water content. Glutamic acid-alanine transaminase and glutamic acid decarboxylase. Plant Physiol. (1959), in press.

(30) Linko, P. and M. Milner.
Free amino and keto acids of wheat grains and embryos in relation to water content and germination. Cereal Chem. (1959), in press.

(31) Linko, P. and M. Milner.
Gas exchange induced in dry wheat embryos by wetting. Cereal Chem. (1959), in press.

(32) Linko, P. and A. I. Virtanen.
On the biosynthesis of some recently discovered derivatives of glutamic acid in plants. Acta Chem. Scand. 12, 68-78 (1958).

(33) Longenecker, J. B. and E. E. Snell.
A possible mechanism for kynureninase action. Jour. Biol. Chem. 213, 229-235 (1955).

(34) McDonald, C. E. and M. Milner.
The browning reaction in wheat germ in relation to "sick" wheat. Cereal Chem. 31, 279-295 (1954).

(35) Meister, A.
Biochemistry of the Amino Acids. Academic Pres, Inc., New York, N. Y. (1957).

(36) Metzler, D., M. Ikawa and E. E. Snell.
A general mechanism for vitamin-B_6 catalyzed reactions. Jour. Am. Chem. Soc. 76, 648-652 (1954).

(37) Michaelis, L. and M. Menten.
Biochem. Z. 49, 333 (1913); in J. B. Sumner and G. F. Somers Chemistry and Methods of Enzymes, 3rd ed., Academic Press, Inc., New York, N. Y. (1953).

(38) Miettinen, J. K. and A. I. Virtanen.
Nitrogen metabolism of pea and alder. Transamination of γ-aminobutyric acid and L(+)-citrulline with α-ketoglutaric acid. Acta Chem. Scand. 7, 1243-1246 (1953).

(39) Miettinen, J. K. and A. I. Virtanen.
Nitrogen metabolism of the alder (Alnus). The absence of arginase and presence of glutamic acid decarboxylase. Acta Chem. Scand. 7, 289-296 (1953).

(40) Milner, M., C. M. Christensen and W. F. Geddes.
Grain storage studies. V. Chemical and microbiological studies on "sick" wheat. Cereal Chem. 24, 23-38 (1947).

(41) Milner, M., C. M. Christensen and W. F. Geddes.
Grain storage studies. VI. Respiration of wheat in relation to moisture content, mold growth, chemical deterioration, and heating. Cereal Chem. 24, 182-199 (1947).

(42) Milner, M. and W. F. Geddes.
Grain storage studies. IV. Biological and chemical factors involved in the spontaneous heating of soybeans. Cereal Chem. 23, 449-470 (1946).

(43) Milner, M. and W. F. Geddes.
Respiration and Heating. Chapter IV in Storage of Cereal Grains and Their Products. Ed. by J. A. Anderson and A. W. Alcock. American Assoc. of Cereal Chemists, St. Paul, Minnesota (1954).

(44) Najjar, V. A. and J. Fischer.
Studies on glutamic acid decarboxylase from Escherichia coli. Jour. Biol. Chem. 206, 215-219 (1954).

(45) Naylor, A. W. and N. E. Tolbert.
Physiol. Plantarum 9, 220 (1956); in R. P. Smith "Glutamic acid decarboxylase and 4-aminobutyric acid in some biological systems." Ph.D. dissertation, Purdue University (1956).

(46) Neuberg, C. and J. Kerb.
Sugar free yeast fermentation. IX. Fermentation of keto acids by wine yeast. Biochem. Z. 47, 405-412 (1912).

(47) Okunuki, K.
Bot. Mag. (Tokyo) 51, 270; in O. Hoffmann-Ostenhof Enzymologie, Wien, Springer Verlag (1954).

(48) Ramstad, P. E. and W. F. Geddes.
The respiration and storage behavior of soybeans. Univ. Minn. Agr. Expt. Sta. Tech. Bul. 156 (1942).

(49) Richards, E. L.
Nonenzymatic browning: the reaction between D-glucose and glycine in the "dry" state. Biochem. Jour. 64, 639-644 (1956).

(50) Roberts, E. and S. Fraenkel.
γ-Aminobutyric acid in brain: its formation from glutamic acid. Jour. Biol. Chem. 187, 55-63 (1950).

(51) Rohrlich, M.
Glutaminsäuredecarboxylase im Getreide. Getreide u. Mehl 7, 89-91 (1957).

(52) Rohrlich, M.
Untersuchungen über die Glutaminsäuredecarboxylase im Getreide. II. Manometrische Messung der Glutaminsäuredecarboxylaseaktivität. Z. Lebensm.-Unters. u.-Forsch. 105, 189 (1957).

(53) Rohrlich, M. and R. Rasmus.
Untersuchungen über die Glutaminsäuredecarboxylase im Getreide. I. Papierchromatographischer Nachweis der Bildung von γ-Aminobuttersäure. Z. Lebensm.-Unters. u. -Forsch. 104, 313 (1956).

(54) Schales, O. and S. S. Schales.
Decarboxylations and transaminations in extracts of higher plants. Arch. Biochem. Biophys. 69, 378-389 (1957).

(55) Schales, O., V. Mims and S. S. Schales.
Glutamic acid decarboxylase of higher plants. I. Distribution; preparation of clear solutions; nature of prosthetic group. Arch. Biochem. 10, 455-465 (1946).

(56) Schales, O. and S. S. Schales.
Glutamic acid decarboxylation in higher plants. II. pH-Activity curve, reaction kinetics, inhibition by hydroxylamine. Arch. Biochem. 11, 155-166 (1946).

(57) Smith, R. P.
Glutamic acid decarboxylase and 4-aminobutyric acid in some biological systems. Ph.D. dissertation, Purdue University (1956).

(58) Steward, F. C. and R. G. S. Bidwell.
Protein metabolism, respiration and growth. A synthesis of results from the use of C^{14}-labelled substrates and tissue cultures. Nature 178, 734-738 (1956).

(59) Steward, F. C. and J. F. Thompson.
The nitrogenous constituents of plants with special reference to chromatographic methods. Ann. Rev. Plant Physiol. 1, 233-264 (1950).

(60) Steward, F. C., J. F. Thompson and C. E. Dent.
γ-Aminobutyric acid: a constituent of the potato tuber? Science 110, 439-440 (1949).

(61) Suzuki, Y. and N. Takakuwa.
Decarboxylation of L-glutamic acid in Scopolia japonica. Naturwiss. 44, 353-354 (1957).

(62) Thompson, J. F., J. K. Pollard and F. C. Steward.
Investigations of nitrogen compounds and nitrogen metabolism in plants. III. γ-Aminobutyric acid in plants, with special reference to the potato tuber and a new procedure for isolating amino acids other than α-amino acids. Plant Physiol. 28, 401-414 (1953).

(63) Umbreit, W. W., R. H. Burris and J. F. Stauffer.
Manometric Techniques, 3rd ed., Burgess Publ. Co., Minneapolis, Minnesota (1957).

(64) Virtanen, A. I.
Über neue Aminosäuren und andere physiologisch bedeutungsvolle Stickstoffverbindungen in höheren Pflanzen. Festschrift Arthur Stoll, 555-581 (1957), Sandoz A.G., Basel.

(65) Warburg, O., H. Klotsch and G. Krippahl.
Glutaminsäure-decarboxylase in Chlorella. Naturwiss. 44, 235 (1957).

(66) Warburg, O. and G. Krippahl.
Beweis der Notwendigkeit der Glutaminsäure für die Photosynthese. Z. für Naturforsch. 13b, 63-65 (1958).

(67) Wingo, J. G. and J. Awapara.
Decarboxylation of glutamic acid in brain. Jour. Biol. Chem. 187, 267-271 (1950).

(68) Zaleski, W. and E. Marx.
The role of carboxylase in plants. Biochem. Z. 48, 175-180 (1913).

THE KINETICS AND OCCURENCE OF WHEAT GLUTAMIC ACID DECARBOXYLASE

by

YU YEN CHENG

B.S., National Taiwan University, Taipei, Taiwan, 1956

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Flour and Feed Milling Industries

KANSAS STATE UNIVERSITY
OF AGRICULTURE AND APPLIED SCIENCE

1959

Introduction. From the moment when seeds or grains are placed in storage they are subjected to damage and deterioration. In severe cases this may lead to loss of viability. It has been generally accepted for a long period of time that the most important environmental factors affecting the viability of stored grains are temperature and humidity. Increase in moisture produces strong carbon dioxide evolution characterized by very high respiratory quotient values. In this laboratory it was noticed that wetting activates glutamic acid decarboxylase in wheat embryos, and it seemed very apparent that this reaction might be responsible for the strong carbon dioxide evolution from wetted germ, and therefore play an important role in the deterioration of grain in storage. The purpose of this study was to characterize the reaction by studying its kinetics, the relationship of glutamic acid decarboxylase to environmental conditions and browning, and the possible variations in the enzyme among different varieties of wheat.

Procedure. Warburg manometric technique was the major procedure employed. Because the retention of carbon dioxide by buffer at pH 5.8 is negligible, one-sidearm vessels were used, and the results were corrected for a small retention of carbon dioxide. The standard deviation was determined by the method of least squares.

Initial reaction velocities were determined by (a) plotting the observed velocities x/t versus time t, and extrapolating both to the time when substrate and enzyme were combined (-5 min) and to the time when manometer stopcocks

were closed (0 min); and (b) plotting the velocities x/t versus x and extrapolating to the value x = 0. Michaelis-Menten constant and the maximum initial velocity were obtained by plotting the reciprocal of initial velocity versus the reciprocal of substrate concentration, and evaluating K_m from extrapolation to 1/v = 0, and V_{max} from extrapolation to 1/(S) = 0.

The activation energy was obtained using the Arrhenius equation from the slope -E/(2.303R) of the plot of logarithms (base 10) of first order reaction velocity constants versus the reciprocal of absolute temperature.

Experimental results. Despite the fact that the decarboxylation of glutamate by wheat germ does not truly follow a first order reaction rate, in all cases a Michaelis-Menten constant K_m = 0.025 M was obtained indicating that the method of extrapolation of the initial velocities is not critical. This value was in good agreement with earlier results obtained with glutamic acid decarboxylase from various other sources.

An activation energy of 9100 cal in the temperature range of 21° to 30°C was found when the reaction system involved 100 mg of granular wheat germ and one milliliter of 0.05 M glutamate (pH 5.8). The correction for the carbon dioxide evolution caused by wetting the germ with buffer containing no substrate had no effect on the slope -E/(2.303R), and hence on the activation energy. This suggested that apparently much of the immediate carbon dioxide evolution from wetted germ is due to decarboxylation of glutamate originally present in wheat germ, rather than to carbon dioxide released from other sources.

The optimum temperature for glutamic acid decarboxylase was 30°C, whereas that of the oxygen uptake mechanism was about 40°C. Respiratory quotient decreased constantly with temperature increase from 21° to 50°C. The optimum pH of glutamic acid decarboxylase was 5.8.

The carbon dioxide evolution from wetted wheat germ proceeded faster in a nitrogen atmosphere than in air. A marked pressure increase in Warburg vessels occured immediately upon wetting and continued for about five minutes, whereafter it receded a little. When the nature of this apparent pressure increase was studied it was found that about 40 to 50 percent seemed to be due to carbon dioxide evolution, and the rest to some physical phenomenon probably associated with the change in volume of lyophilic macromolecules.

Damp wheat germ heated at 37°C showed a decrease in the decarboxylation of glutamate, accompanied by a simultaneous increase in browning. These changes were especially significant at moisture levels above 18 percent. In commercial wheat samples, browning, as indicated by fluorescence, increased when the percentage of "sick" wheat increased, and in this material also a simultaneous decrease in glutamic acid decarboxylase activity occured.

Glutamic acid decarboxylase activity decreased in damp Pawnee wheat stored in a moisture range of 11.0 to 36.0 percent. The decrease was very pronounced at moisture levels above 18.9 percent.

Samples of Seneca, Ponca, and Langdon wheat varieties stored for three months in dry state (about 11 percent moisture) at +4°, +27°, and 39°C showed a decrease in glutamic acid decarboxylase activity with increasing temperature. Langdon variety was most resistant towards loss in enzyme activity.

The activities of glutamic acid, α-alanine, and pyruvic acid decarboxylases, and glutamic acid-alanine transaminase in 16 wheat varieties including soft and hard winter, and spring varieties (including Durum types) showed pronounced differences. No correlation between enzyme activity and wheat class was observed, but for individual varieties Ponca exhibited the lowest activity.

Differences in glutamic acid decarboxylase activity were generally small among eleven hard winter wheat varieties from comparative environmental background, but Ponca showed consistently less activity than other varieties. This led to the conclusion that varietal differences may occur.