

# Cereal Chemistry

Vol. 50

January-February 1973

No. 1

## Effect of Wetting Temperature on Glutamic Acid Decarboxylase Activity in Wheat Embryos

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### ABSTRACT

The temperature at which dried-wheat embryos are wetted was found to have a considerable effect on glutamic acid decarboxylase activity. Activity levels at 24° and 30°C. were, respectively, 3.42 and 4.11 times higher than in an ice bath. Pyridoxal-5'-phosphate was found to restore activity at low temperatures, while not eliminating the temperature effect completely.

Extensive research work has been undertaken on the chemical and physical processes taking place in grains (especially in wheat) both during storage (1) and in the initial stage of germination (2). Of special interest were the primary reactions which eventually lead to deterioration of the grain; once these were known, means could be sought for slowing them down and prolonging storage life.

It was found that wetting of wheat grains, or separated embryos, results in instantaneous activation of the glutamic acid decarboxylase (GAD) enzyme, accompanied by release of CO<sub>2</sub> and an increase in free  $\gamma$ -amino butyric acid (3,4). The moisture level promoting this activation is lower than those needed for an active respiration system and for development of fungi. In other words, the above metabolite-producing enzymatic activity (hereafter referred to as GADA) already exists at a lower moisture level, but this activity is arrested (since the level is too low for complete germination), with loss of viability as a result (5).

Since GADA was found to be affected by storage conditions and in correlation with viability, it may serve as a useful quality index both for the storage conditions and for the pre-storage history of the wheat. A rapid manometric method for GADA determination in wheat was developed by Linko (6); it also proved satisfactory for rice (7) and corn (8).

Wheat subjected to consecutive drying and wetting was found to be more susceptible to deterioration than unwetted wheat at the same level of storage moisture. Apparently, a short wetting interval suffices to set in train a deterioration

process, through temporary activation of the enzyme GAD. Hence the practical significance of the moisture level at which the various enzymatic systems in the embryo are activated.

The object of the present study was to determine whether the temperature at which the dry embryos were wetted affects GADA.

#### MATERIALS AND METHODS

Wheat grains of the variety Florence, a known locally grown variety, were provided (from the latest harvest) by the Hazera Seed Cleaning Coop., Haifa. Their moisture content was 8.85% (wet-weight basis), and they were stored in moistureproof containers at  $-18^{\circ}\text{C}$ . The embryos were carefully separated from the endosperm with a razor blade, ground (trituated) in mortar, and kept at ambient temperature only for the time of trituration and subsequent weighing.

GADA was determined manometrically, as described in *Manometric Techniques* (9). Twenty-five milligrams of trituated embryos was placed in each of the main vessels of the Warburg apparatus. The vessels were held at different temperatures (crushed-ice bath, and in water baths of  $24^{\circ}$  and  $30^{\circ}\text{C}$ ., respectively). The vessels were held for 20 min., so that the dry trituated embryos could reach the temperatures mentioned.

Two milliliters of phosphate buffer at pH 5.3 ( $20^{\circ}\text{C}$ .) was added to the trituated embryos kept at the different temperatures, and 0.5 ml. of 0.2M glutamic acid (in M/15 phosphate buffer, pH 5.3) was introduced into the side arm, and the vessels were immediately inserted into the Warburg apparatus. Pyridoxal-5'-phosphate (PLP), when used, was added after the addition of the buffer.<sup>1</sup> All the experiments in the Warburg apparatus were carried out at  $30^{\circ}\text{C}$ .

After 10 min. of equilibration the stopcocks were closed, the substrate was tipped from the side arm into the vessels, and manometric readings were taken at 10-min. intervals for 60 min. Determinations were carried out in triplicates and results were reported as microliters of  $\text{CO}_2$  released.

#### RESULTS AND DISCUSSION

Figure 1 demonstrates the wide range of GADA levels obtained as a result of the effect of temperature of the dry trituated embryos at the moment of wetting. Embryos submerged in crushed-ice bath while wetting show a very low activity of GAD compared to the activity of the same batch of embryos at a wetting temperature of  $30^{\circ}\text{C}$ .

While calculating the relative GADA levels from the data presented in Fig. 1, it was found that the activity of the enzyme treated at  $24^{\circ}$  and  $30^{\circ}\text{C}$ . was 3.42 and 4.11 times more, respectively, than that treated at  $0^{\circ}\text{C}$ . (in crushed-ice bath). In fact, the temperature effect is so sensitive that closely controlled temperature conditions of the dry embryos when buffer is added are essential for significant and reproducible results to be obtained.

Figure 2 shows the effect of PLP added after the addition of the buffer solution to embryos submerged in the crushed-ice bath while wetting. The optimal dose to

<sup>1</sup>The appropriate amounts of pyridoxal-5'-phosphate, calculated per ml. liquid volume of each experiment, were added in 0.5 ml. of M/15 phosphate buffer solution (pH 5.3) to the main vessel. In this case, only 1.5 ml. buffer was used for the wetting, to make the total volume of liquids in the vessel 2.5 ml. (including 0.5 ml. glutamic acid, in the side arm).

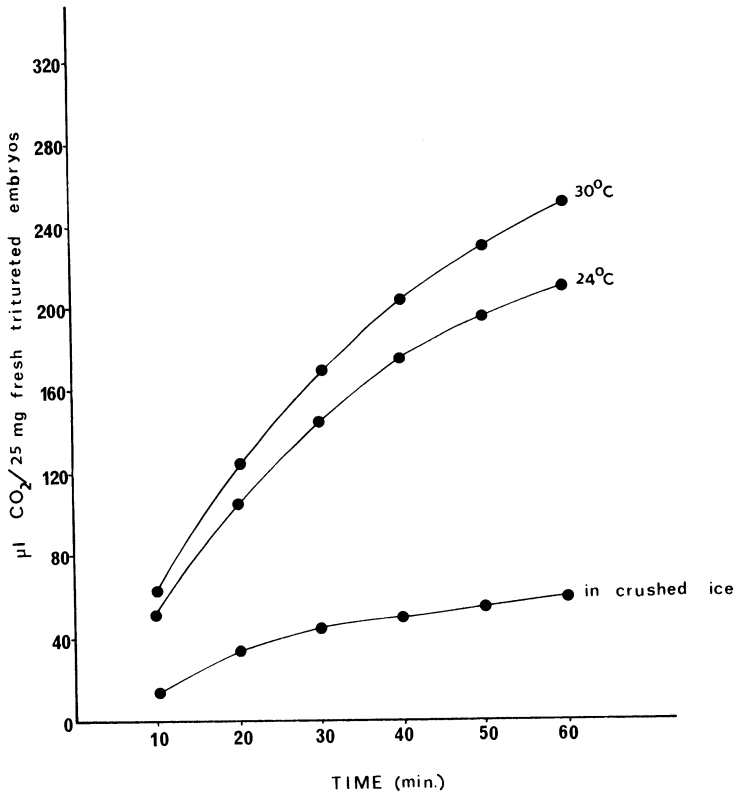


Fig. 1. GADA in wheat embryos vs. wetting temperature.

restore activity was 3  $\gamma$ . With larger doses no further improvement could be observed.

Figure 3 represents the effect of 1  $\gamma$  PLP on the activity of GAD at different wetting temperatures. The PLP failed to eliminate the temperature effect completely, and differences in activity could still be observed. While calculating the relative GADA levels from the data presented in Fig. 2 (with 1  $\gamma$  PLP), it was found that the activity of the enzyme treated at 24° and 30°C. was 1.42 and 1.76 times more, respectively, than that treated at 0°C. (in crushed-ice bath).

All experiments were repeated at least ten times and reproducible results were obtained. From the data cited above, it is demonstrated that the temperature of the dry embryos on the very moment of wetting has a marked influence on the activity of the enzyme GAD in wheat embryos.

The temperature at which the dry triturerated wheat embryos were held for 3 to 5 hr. (37°C., -18°C., or ambient) before attemperation had no effect whatsoever on the experimental results. Only the temperature at which the actual wetting has been performed had the effect mentioned above.

The lower CO<sub>2</sub> yield under the ice-bath conditions, represented in Fig. 1, may be due to irreversible fixation of the natural PLP in the embryos, rendering it

incapable of forming Schiff base and activating the enzyme. The addition of PLP renders the restoration of activity (see Figs. 2 and 3), but does not eliminate the temperature effect completely. It seems, therefore, that the enzyme protein has more than one site of binding with PLP, and that the cold wetting condition causes an irreversible linkage with the enzyme protein.

According to Tsai (10), the PLP and the enzyme may react together, either through formation of Schiff base with the amino group (a weak bond of catalytic character) or through fixation of the co-enzyme on the enzyme protein, a process in which the role of the co-enzyme is structural, namely, preserving the appropriate configuration of the enzyme molecule. However, Kretovich et al. (11) found that GAD in 5- to 6-days old pea sprout has allosteric properties.

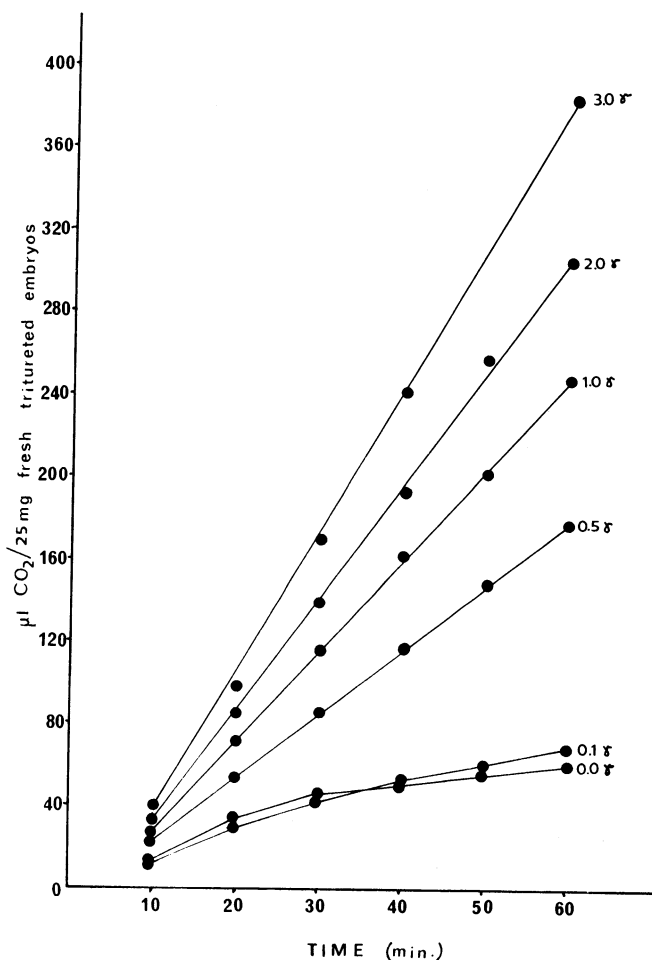


Fig. 2. Effect of various concentrations of PLP on GADA of wheat embryos wetted while submerged in a crushed-ice bath.

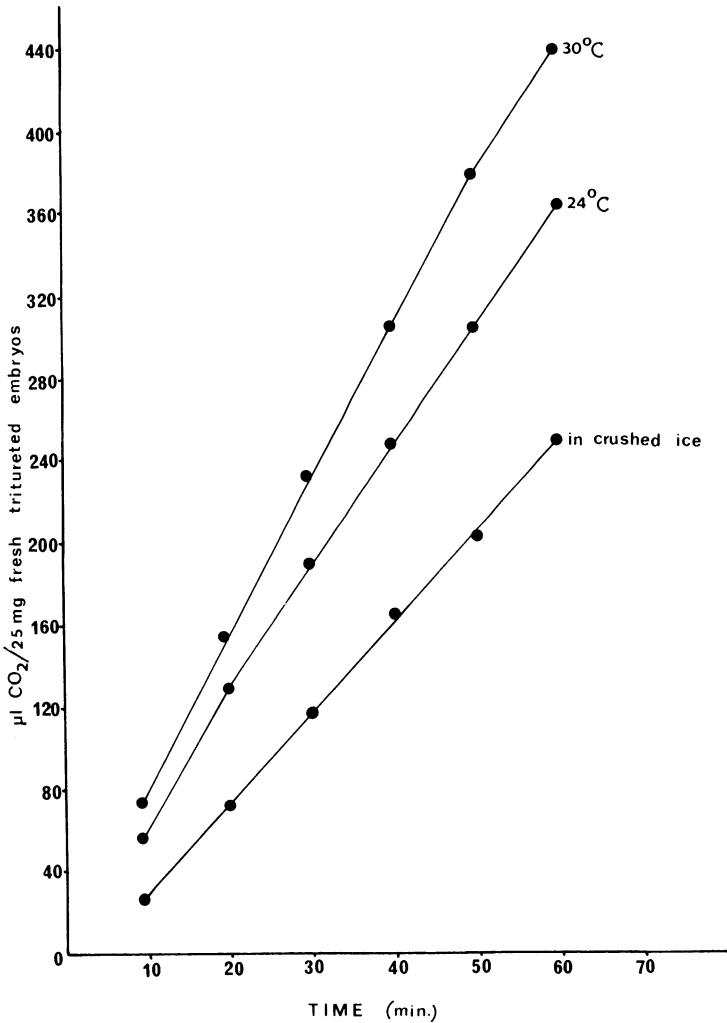


Fig. 3. Effect of  $1 \gamma$  PLP on GADA in wheat embryos at different wetting temperatures.

GADA levels obviously differ between wheat varieties according to their enzyme and PLP contents. Linko et al. (1) noted that in freshly harvested wheat with a low rate of damage and high rate of germination, the correlation between GADA and viability was insignificant. In another context, Linko (6) pointed out considerable differences in initial GADA between fresh crops of two wheat varieties, the levels remaining unaffected on addition of 0.01% PLP to the substrate solution.

From the above discussion it might be assumed that if GADA is to be used as an index of viability or damage, closely controlled wetting temperatures are essential for reproducible results to be obtained. Better understanding of the reaction mechanism between the PLP and the enzyme protein should be achieved; further

investigation might lead to a precise knowledge of the binding of PLP to the enzyme protein in new crop wheats, enabling the detection and followup of early changes associated with the loss of viability in storage.

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[Received February 28, 1972. Accepted July 11, 1972]