# TREATMENT OF WHEAT WITH IONIZING RADIATIONS
## V. Effect of Gamma Radiation on Some Enzyme Systems[1]

PEKKA LINKO AND MAX MILNER[2]


### ABSTRACT

Germination (wet filter paper), dehydrogenase (as indicated by TTC reduction), glutamic and pyruvic acid decarboxylase, alpha-amylase, and protease activities and maltose value, were determined in wheat samples after treatment with gamma radiation at levels of 0.05, 0.1, 0.5, 1.0, and 3.0 megarep. Germination was reduced even by the lowest radiation treatment (0.05 megarep), whereas the TTC test with whole kernels indicated no significant decrease before the 1.0 megarep treatment. Spectrophotometric determination of formazan produced from TTC by ground grains showed a regular but curvilinear decrease in relation to radiation applied, considerable formazan color remaining even in completely nonviable grain treated with 3.0 megarep. These data suggest that at least a part of the reduction of TTC to formazan is caused by irradiation-produced factors; browning reaction products are the most probable.

Glutamic and pyruvic acid decarboxylase activities both decreased to about half of the original value in grain treated with 3.0 megarep.

Maltose value showed a considerable rise with radiation level due to increased starch susceptibility, whereas alpha-amylase activitity was virtually unchanged even after 3.0 megarep. There was similarly no impairment of protease activity.


In the course of a study of the effects of gamma radiation on the respiratory, biochemical, and technological properties of wheat, it was noticed (13) that dosages between 125,000 and 625,000 rep eliminate fungal respiration in the grain, apparently without increasing its protein solubility or fluoresence value. The effect of irradiation on the enzymes of the grain itself becomes important in any evaluation of irradiation as a practical way to sterilize grain. Holmes (5) has recently written an extensive review of the biochemical effects of ionizing radiations, including the effects on various enzymes other than those in wheat. The purpose of the present work was to investigate the effects of gamma-rays on certain enzyme systems in wheat grains.

## Materials and Methods

*Material.* Wheat used in this study was a composite of several standard hard red winter varieties of good breadmaking quality, produced at various experimental fields throughout the U.S. hard red winter

[1] Manuscript received July 13, 1959. Contribution No. 324, Department of Flour and Feed Milling Industries, Kansas Agricultural Experiment Station, Manhattan. Supported by a grant from the Rockefeller Foundation.

[2] Respectively, Assistant Professor and Professor, Kansas State University.

wheat area in 1957 and 1958. The composite sample contained 13.4% protein (14% moisture basis) and 1.6% ash. Germination, as estimated with moist filter paper in Petri dishes, was 94–96%. The moisture content, as determined by drying ground (2 minutes by Waring Blendor) material 1 hour at 130°C. in a forced-draft air oven, was 9.0% (wet-weight basis). The grains used for enzyme experiments were ground by micro-Wiley mill to pass a 20-mesh sieve.

These wheats were sealed in No. 2 metal cans and sent to the Materials Testing Reactor of the U.S. Atomic Energy Commission, Idaho Falls, Idaho, for radiation treatment. Radiation dosages of 0.0, 0.05, 0.1, 0.5, 1.0, and 3.0 megarep were used. After their return, the irradiated cans were opened and each treatment group was mixed by a MacLellan batch mixer for 30 minutes prior to sampling for enzyme activity determinations.

*Germination Test.* One hundred kernels were surface-sterilized by soaking 2 minutes in 0.1% mercuric chloride solution, followed by a thorough rinsing in tapwater. The grains were then allowed to germinate between moist filter papers for 7 days at 25°C. Germinated grains were counted and removed every second day.

*Dehydrogenases.* Dehydrogenase activity was determined by two different methods. In the first method kernels were soaked overnight in distilled water, cut lengthwise, and again soaked overnight in a 1% solution of 2,3,5-triphenyltetrazolium chloride (TTC). The percentage of colored embryos was then estimated by visual examination.

The second method was a modification of that proposed by Sorger-Domenigg *et al.* (12). Five milliliters of 1% TTC solution buffered to pH 7.3 with 0.067*M* phosphate buffer were added to a test tube containing 1 g. of ground grains. After two vacuum infiltrations, the test tubes were incubated 1 hour at 38°C. Formazan produced was extracted with 25 ml. of acetone, and the transmittance was measured at 520 $m\mu$ with a Beckman Model DU spectrophotometer.

*Decarboxylases.* Glutamic and pyruvic acid decarboxylase activities were determined manometrically as previously described by Cheng *et al.* (2,3). The activities were reported as microliters of carbon dioxide produced during 30 minutes from 1 ml. of 0.1*M* glutamate (pH 5.8) by 500 mg. of ground grain.

*Alpha-Amylase.* Alpha-amylase activity measurements were based on methods developed by Sandstedt *et al.* (11) and Redfern (10). The procedure was modified slightly because of the low enzyme activities encountered. Ground grains (2.5 g.) were extracted with 50 ml. of 0.2% calcium chloride solution. Ten milliliters of this solution were used without further dilution for the alpha-amylase determination. Twenty

milliliters of soluble starch substrate solution were used (equivalent of 0.01 g. of starch).

*Maltose Value.* Maltose value was determined according to the procedure described in *Cereal Laboratory Methods* (1).

*Protease.* Protease activity was determined according to Miller and Johnson (8), except that 1.3334 g. of ground kernels were used, and the final results were accordingly multiplied by 3.

## Results and Discussion

Figure 1 shows that results of viability determinations vary greatly with the method used. The ordinary germination test between filter papers indicates viability to decrease rapidly with increasing radiation dosage; 0.5 megarep retards root growth completely. However, when kernels cut lengthwise were soaked in TTC solution, the coloring of the embryos remained virtually the same up to 0.5 megarep dosage. The

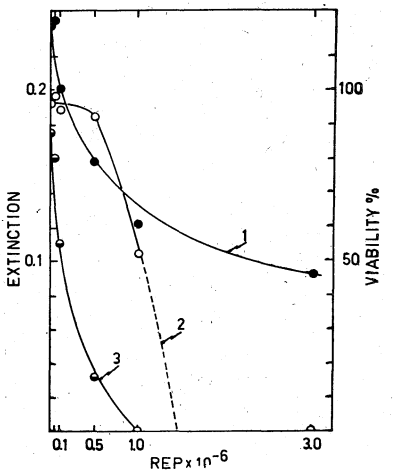


Fig. 1. The effect of gamma-irradiation of wheat grains on viability. 1: 2,3,5-triphenyltetrazolium chloride test with ground grains; 2: the same with lengthwise-cut kernels (visual examination); 3: germination test. (Curve 1 is related to extinction scale; 2 and 3 to the viability percent scale.)

first significant decrease in viability as observed by a color change occurred at 1.0-megarep dosage. No coloring remained after 3.0-megarep irradiation. On the other hand, when dehydrogenase activity was determined spectrophotometrically in ground material, it was found that even after irradiation treatment at 3.0 megarep the formazan color intensity decreased only about 60% from the control. This result might suggest that dehydrogenase activity, which produces formazan, is rela-

tively resistant to radiation. In general, many enzymes have been found to be quite radioresistant. Thus, Green (4), for example, found that 2.0 megarep, which was sufficient to kill microorganisms, caused only a 29% reduction in the proteolytic activity of calf heart. In the case of TTC, however, it is possible that the reducing compounds which may be formed during irradiation can nonenzymatically reduce TTC to formazan. Thus, if prior to the TTC treatment the enzymes of the ground material were inactivated by heating for 15 minutes at 150°C., the formazan color intensity increased with increasing radiation from almost nil approximately to the same level found with normal determination at 3.0 megarep dosage. Since high radiation levels stimulate a Maillard reaction in wheat (9,13), it is probable that reductones formed during the browning reaction may partially account for nonenzymatic production of formazan from TTC.

Figure 2 shows that 3.0-megarep irradiation of wheat kernels causes approximately 50% decrease in glutamic acid and pyruvic acid decarboxylase activities.

Alpha-amylase activity is normally very low in intact wheat grains (11). Table I shows that alpha-amylase activity remains virtually unaffected by irradiation up to 3.0 megarep, as is also the case with protease activity. Maltose value increases greatly with increasing radiation dosage, which agrees well with earlier results (7,9). Lee (7) found no increase in the saccharifying power in papain extract of flour from irradiation, suggesting that no actual activation of beta-amylase takes place. Hence, the increase in maltose value, as indicated in previous publications of this series, is due to a sharp increase in the susceptibility of the starch fraction to beta-amylase attack.

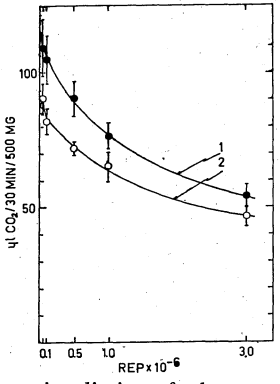


Fig. 2. The effect of gamma-irradiation of wheat grains on 1) glutamic acid decarboxylase, and 2) pyruvic acid decarboxylase activities. The stems on the points indicate standard deviations with each sample as calculated from five replicate determinations.

TABLE I

THE EFFECT OF GAMMA-IRRADIATION OF WHEAT GRAINS ON ALPHA-AMYLASE AND PROTEASE ACTIVITIES, AND ON MALTOSE VALUE

| IRRADIATION DOSAGE | ALPHA-AMYLASE ACTIVITY | PROTEASE ACTIVITY | MALTOSE VALUE |
|---|---|---|---|
| *megareps* | *SKB-units/g* | *Hb-units* | *maltose/10 g* |
| 0.00 | 0.027 | 12.4 | 153 |
| 0.05 | 0.028 | 12.4 | 156 |
| 0.10 | 0.026 | 12.4 | 179 |
| 0.50 | 0.026 | 12.2 | 216 |
| 1.0 | 0.024 | 12.1 | 244 |
| 3.0 | 0.020 | 11.9 | 315 |

All these results indicate that high irradiation dosages damage several enzyme systems considerably. However, at dosages sufficient only to kill insects in grain (about 0.05 megarep), the damage may not be great although seed viability will be affected. The long-term effects of deinfestation dosage levels on the technological properties of the grain as affected by storage remain to be determined.

**Acknowledgment**

The authors are greatly indebted to James R. Fleming for amylase and protease determinations.

**Literature Cited**

1. AMERICAN ASSOCIATION OF CEREAL CHEMISTS. Cereal laboratory methods (6th ed.). The Association: St. Paul, Minnesota (1957).
2. CHENG, YU-YEN. The kinetics and occurrence of wheat glutamic acid decarboxylase. M. S. Thesis, Kansas State University (1959).
3. CHENG, Y.-Y., LINKO, P., and MILNER, M. Glutamic acid decarboxylase in wheat grains. (A preliminary report.) Suomen Kemistilehti **31B:** 333–335 (1958).
4. GREEN, D. E. Report of Project No. 7-84-01-002, Quartermaster Food and Container Inst. for the Armed Forces, Chicago (1955).
5. HOLMES, BARBARA E. Biochemical effects of ionizing radiation. Ann. Rev. Nuclear Sci. **7:** 89–134 (1957).
6. KNEEN, E., MILLER, B. S., and SANDSTEDT, R. M. The influence of temperature on the development of amylase in germinating wheat. Cereal Chem. **19:** 11–27 (1942).
7. LEE, C. C. The baking quality and maltose value of flour irradiated with $Co^{60}$ gamma rays. Cereal Chem. **36:** 70–77 (1959).
8. MILLER, B. S., and JOHNSON, J. A. A simple linear relationship and definition of a unit for proteinase activity. Arch. Biochem. Biophys. **32:** 200–206 (1951).
9. MILNER, M., and YEN, Y.-C. Treatment of wheat with ionizing radiations. III. The effect on breadmaking and related properties. Food Technol. **10:** 528–531 (1956).
10. REDFERN, S. Methods for determination of alpha-amylase. IV. A glass end point color standard for use in the dextrinizing method; effect of temperature and starch lot on this method. Cereal Chem. **24:** 259–268 (1947).
11. SANDSTEDT, R. M., KNEEN, E., and BLISH, M. J. A standardized Wohlgemuth procedure for alpha-amylase activity. Cereal Chem. **16:** 712–723 (1939).
12. SORGER-DOMENIGG, H., CUENDET, L. S., and GEDDES, W. F. Grain storage studies. XX. Relation between viability, fat acidity, germ damage, fluorescence value, and formazan value of commercial wheat samples. Cereal Chem. **32:** 499–506 (1955).
13. YEN, Y.-C., MILNER, M., and WARD, H. T. Treatment of wheat with ionizing radiations. II. Effect on respiration and other indices of storage deterioration. Food Technol. **10:** 411–415 (1956).