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EFFECT OF PHYSIOLOGICAL STRESS ON POTATO POLYPHENOL OXIDASE*

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Abstract—Potato tubers, varieties Netted Gem and Pontiac, were subjected to γ-irradiation, to mechanical injury and to controlled storage, and the effects of these factors on polyphenol oxidase were studied. The enzyme activity was increased, over corresponding control levels, by a γ-irradiation dosage of 2·0 krads, and by mechanical injury to the tissue. A decrease in activity resulted from irradiation dosages of 5·0 and 8·5 krads, and from prolonged storage. Disc electrophoresis on polyacrylamide gel of the extracts revealed isoenzyme patterns from which a mechanism may be deduced which could account for the differences in overall polyphenol oxidase activity.

INTRODUCTION

BROWNING reactions, initiated by the enzyme polyphenol oxidase (*o*-diphenol: O_2 oxidoreductase, E.C. 1.10.3.1), constitute a serious problem during the storage and processing of many fruits and vegetables. Rapid darkening occurs when tissues are bruised, cut, peeled, crushed or diseased, leading to wastage of raw material. It is therefore desirable to control the activity of this enzyme and to understand the mechanism responsible for changes in activity resulting from physiological stress.

The purpose of this investigation was to study the response of polyphenol oxidase in potato tubers of varieties Netted Gem and Pontiac to sprout-inhibiting levels of γ-irradiation,[1,2] to mechanical injury,[3–5] and to prolonged storage. Concurrent changes in the isoenzyme pattern were also studied, to attempt to elucidate a mechanism which could account for induced differences in overall polyphenol oxidase activity.

RESULTS

Initial Effect of γ-Irradiation

An irradiation dosage of 2·0 krads resulted in a stimulation of polyphenol oxidase activity in tubers of both varieties. A dosage of 5·0 krads did not significantly affect enzyme activity in Pontiac tubers. In other cases, irradiation dosages of 5·0 and of 8·5 krads lowered the activity of the enzyme compared with that occurring in untreated control tubers, with the greater decrease in activity resulting from the 8·5 krads treatment (Fig. 1).

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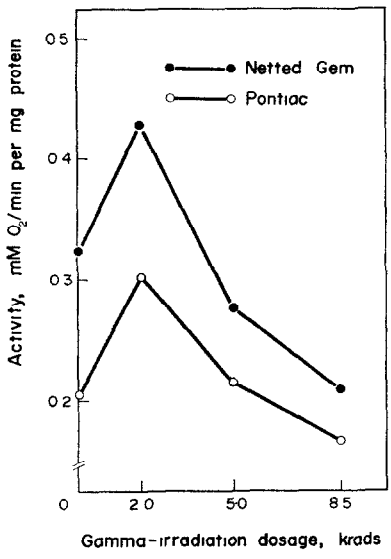
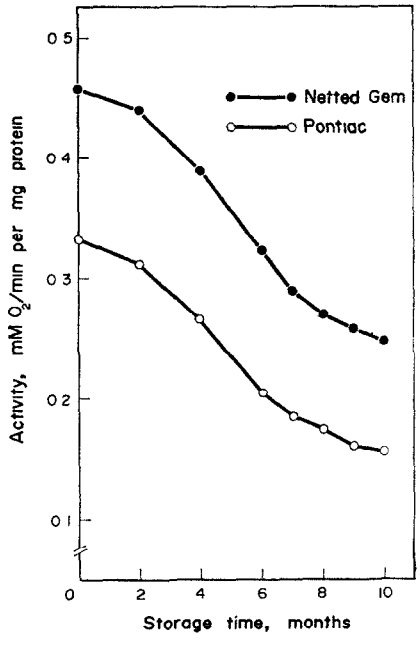


FIG. 1.



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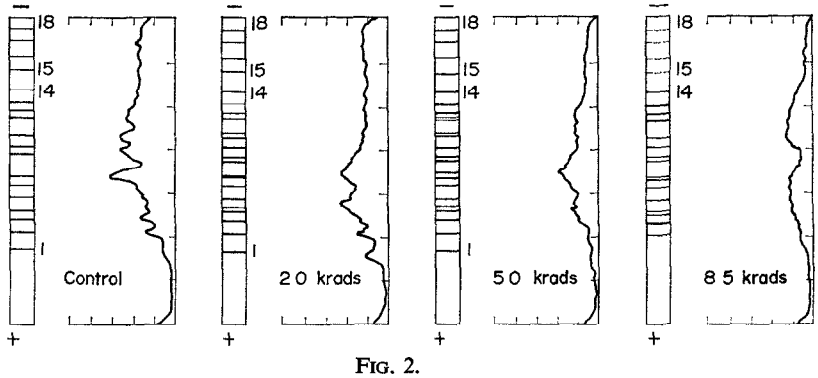

FIG. 2.

FIG. 1. EFFECT OF γ-IRRADIATION ON POLYPHENOL OXIDASE ACTIVITY.
The reaction mixture composition was: catechol, 10 mM in sodium phosphate buffer, 0·05 M, pH 6·2, 3·0 ml; enzyme preparation, 0·05 ml. The substrate solution was oxygenated at 25° for 5 min, immediately prior to the addition of the enzyme. The rate of reaction was measured by following oxygen uptake at 25°, using an oxygen electrode.[13]

FIG. 2. EFFECT OF γ-IRRADIATION ON THE ISOENZYME PATTERN OF POLYPHENOL OXIDASE EXTRACTED FROM TUBERS OF THE PONTIAC VARIETY.
Electrophoresis was undertaken in an anionic gel system with 7·5% of polyacrylamide gel (pH 9·3, 4°). The current was maintained at 4·17 mA/tube, and the operative voltage was 100 V for the first 0·5 hr, followed by 200 V for the final 1·5 hr. The enzyme activity bands were developed in 1·5 mM DL-3,4-dihydroxyphenylalanine. The direction of migration was towards the anode. Densitometer tracing: abscissa = relative mobility; ordinate = percentage light transmission.[11]

FIG. 3. EFFECT OF PROLONGED STORAGE ON POLYPHENOL OXIDASE ACTIVITY.
The experimental conditions are described in the text and in the legend of Fig. 1.

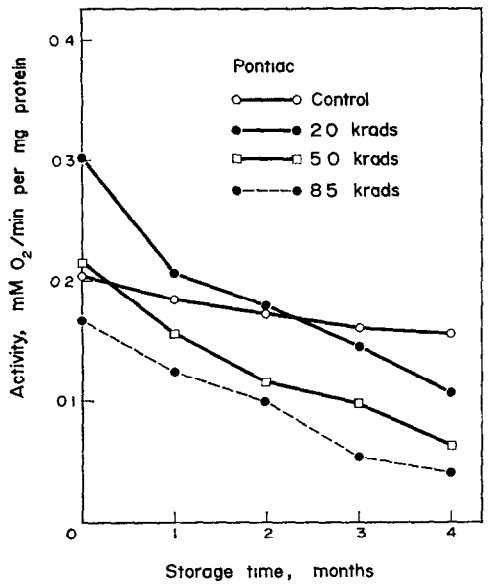

FIG. 4.

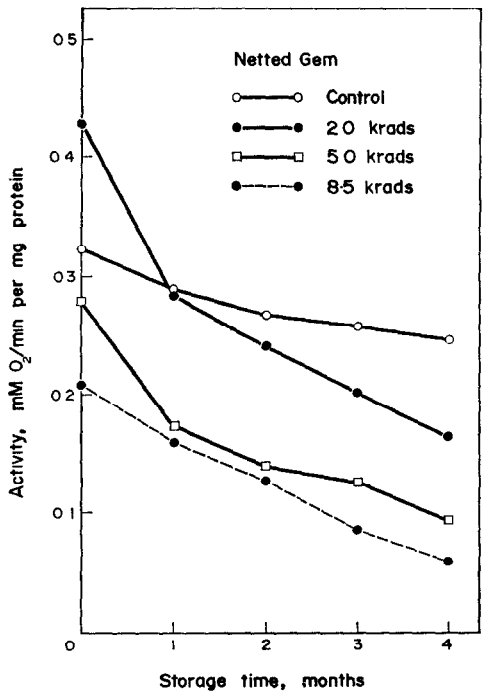

FIG. 5.

FIG. 4. EFFECT OF POST-IRRADIATION STORAGE ON POLYPHENOL OXIDASE ACTIVITY IN PONTIAC POTATOES.
The experimental conditions are described in the text and in the legend of Fig. 1.

FIG. 5. EFFECT OF POST-IRRADIATION STORAGE ON POLYPHENOL OXIDASE ACTIVITY IN NETTED GEM POTATOES.
The experimental conditions are described in the text and in the legend of Fig. 1.

Disc electrophoresis on polyacrylamide gel of the different extracts revealed marked differences among the isoenzyme patterns. Dosages of 2·0 krads and of 5·0 krads resulted in an increase in the number of activity bands with molecular weights below 280,000 (corresponding to Band 14), while at 8·5 krads the number of these bands decreased. The monomeric units (corresponding to Band 1) greatly increased on application of 2·0 krads, but decreased at 5·0 krads and were not detectable at 8·5 krads. Bands 15–18 (representing polymeric forms with a molecular weight greater than 280,000) decreased at 5·0 krads and appeared in trace amounts after a dosage of 8·5 krads. Figure 2 shows the changes observed in extracts from the Pontiac variety but similar changes occurred in Netted Gem.

Effect of Mechanical Injury

After the 48-hr storage period, intense bruises were readily observed on the tuber surface. The activity of polyphenol oxidase was increased in damaged tissue over that in control tissue for both varieties as shown in Table 1. Mechanical injury was also found to affect the isoenzyme patterns. In both varieties, the number and quantity of isoenzymes increased in damaged tissues, especially the monomeric forms and higher polymers.

TABLE 1. THE EFFECT OF MECHANICAL INJURY ON POLYPHENOL OXIDASE ACTIVITY IN TUBERS OF THE PONTIAC AND NETTED GEM VARIETIES

Variety	Treatment	Polyphenol oxidase activity (mM O_2/min/g protein)
Pontiac	Control	218
	Bruised	277
Netted Gem	Control	300
	Bruised	451

The potato tissue was bruised with a pressure of $3{\cdot}76 \times 10^7$ dynes/cm^2 and allowed to stand at 24° for 48 hr under moistened conditions. Enzyme extraction and estimation were carried out as described in the text and in the legend of Fig. 1.

Effect of Prolonged Storage

Polyphenol oxidase was estimated periodically from samples of tubers of both varieties stored for 10 months. Isoenzyme patterns were obtained at the commencement of the storage period and compared with those occurring after storage for 10 months. The activity of polyphenol oxidase decreased steadily over the entire storage period, with the greatest rate of decrease occurring between the fourth and sixth month of storage (Fig. 3). The isoenzyme patterns for both potato varieties showed aggregation towards the higher polymers after 10 months of storage, while there was a decrease in the quantity of smaller units.

Tubers irradiated at 2·0, 5·0 and 8·5 krads showed a continued decrease in polyphenol oxidase activity, after the initial effect of irradiation, throughout the post-irradiation storage period. In general, the rate of decrease in activity was greatest within the first month after irradiation. Tubers subjected to 8·5 krads showed the greatest percentage decrease in enzyme activity among all the irradiation treatments (Figs. 4 and 5).

DISCUSSION

The results obtained in this study demonstrated that potato polyphenol oxidase activity was increased, over corresponding control levels, by a γ-irradiation dosage of 2·0 krads, and by mechanical injury to the tissue. In general, a decrease in enzyme activity was shown upon the application of 5·0 and of 8·5 krads, and in the stated conditions of storage. We have also demonstrated concurrent changes in the isoenzyme patterns, from which it may be possible to speculate on a mechanism accounting for changes in overall activity.

The effect of γ-irradiation on enzyme activity depends upon several factors. Results obtained from studies on the effect of γ-irradiation on potato polyphenol oxidase do not appear to be very consistent.[6–10] Much depends upon such factors as the potato varieties employed, the irradiation dosages used, the physiological condition of the tubers, the extractability of the enzyme, and the method of estimation of activity. It is therefore

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difficult to make any definitive statement on the general effect of γ-irradiation on this particular enzyme.

During the development of the isoenzyme patterns, we had previously observed that the bands with the faster mobilities appeared before the bands with the slower mobilities, suggesting that the former bands represent the more active enzyme forms.[11] At 2·0 krads there are increases both in overall activity and in the number of bands, especially the more active monomers. At higher irradiation dosages, there is a decrease in overall enzyme activity and also in the monomers and higher polymers. The comparatively low activity at 8·5 krads may reflect partial destruction of all the activity bands. Such changes in the isoenzyme pattern may reflect corresponding changes in overall activity, although this does not rule out the possibility that irradiation-induced differences in charge rather than in molecular size may explain some of the observed changes in isoenzyme migration.

Mechanical injury was found, in both varieties, to increase the overall activity of polyphenol oxidase in damaged tissues compared to that in control tissues, as has been described elsewhere.[3–5] The observed changes in the isoenzyme patterns compare with results obtained by Hyodo and Uritani[5] who demonstrated by polyacrylamide gel electrophoresis at pH 9·3 the presence of three components of the enzyme in healthy sweet potato tissue, and six components in corresponding cut tissue. A net synthesis of enzyme protein seems most likely to account for our observed changes in isoenzyme patterns following mechanical injury to the tissue.[3,4]

On prolonged storage, polyphenol oxidase activity decreased steadily over the entire storage period, in contrast with the work of Mondy *et al.*[12] These workers showed that the enzyme activity in tubers of the Pontiac and Ontario varieties decreased markedly during the first month of storage at 4·4°, and remained relatively low and steady during the remainder of the 6-month storage period. Decrease in total enzyme activity during storage could be reflected in the isoenzyme pattern, since the higher polymeric forms of the enzyme appear to represent the less active forms.

The results from this study have a two-fold application. Firstly, they may lead to a greater understanding of the cellular mechanism of control of polyphenol oxidase activity, since it is possible to visualize some relationship between overall activity and the isoenzyme pattern. Also, there is a need to understand the effects of sprout-inhibiting levels of γ-irradiation on the overall physiology of the tuber. Secondly, they may have a practical application in food technology. Irradiation may represent an alternative method, for the control of enzymic browning, to the more commonly-used methods such as heat application or the use of sulphur dioxide. Our findings indicate that tubers may be irradiated at 8·5 krads in order to prolong their storage life and to improve their subsequent processing quality.

EXPERIMENTAL

Source material. Manitoba-grown Netted Gem and Pontiac potatoes were harvested and stored at 4° and 75–80% relative humidity. Sacks of potatoes were chosen randomly for sampling. Tubers were lined up into three rows of 100 potatoes each, the first, 101st and 201st potatoes contributing the first sample, and so on.

Enzyme preparation and assay. Polyphenol oxidase was partially purified[11] by ammonium sulphate fractionation, pigment removal by PVP, and centrifugation at 100,000 *g*, and its activity determined by the method of Yamaguchi *et al.*[13] Protein was determined by the biuret method,[14] using crystalline bovine serum

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albumin (British Drug Houses, fraction V) as the standard. Disc electrophoresis on polyacrylamide gel was carried out with an anionic gel system (7·5% polyacrylamide gel, running pH 9·3) described in the Polyanalyst Electrophoresis Apparatus manual published by Buchler Instruments, Inc., Fort Lee, New Jersey, U.S.A. Electrophoresis was at 4° at 4·17 mA/tube for 2 hr. The gels were modified by doubling the concentrations of riboflavin and of ammonium persulphate.[11]

The substrates employed were catechol (10 mM) for assay of the total enzyme activity, and DL-3,4-dihydroxyphenylalanine (1·5 mM) for the development of the isoenzyme patterns.

Initial effect of γ-irradiation. Tubers were irradiated at 0, 2·0, 5·0 and 8·5 krads at room temperature in a Gammacell 220 irradiation unit (Atomic Energy of Canada Ltd.). The γ-rays were emitted from a ^{60}Co source, giving a dosage rate of 1·6 Mrads/hr, with a decay factor of 0·604. The irradiation time was in the order of seconds, so the consequent temperature rise was neglected. The enzyme was extracted from the samples and estimated immediately after irradiation.

Mechanical injury. Bruising of the tubers was carried out quantitatively by use of a simple device similar to that described by Maas.[15] The bruising force applied to each tuber was $3·76 \times 10^7$ dynes/cm^2. After bruising, the potatoes were allowed to stand at room temp. (about 24°) for 48 hr, covered by moistened paper towels. The bruised area and a control area on the same tuber were removed separately with a cork borer of internal diameter 15 mm. Tissue plugs were taken up to approximately 6 mm under the periderm, and the enzyme extracted and estimated in the usual way.

Storage. Tubers were stored for 10 months following harvest, and their polyphenol oxidase activities were estimated periodically. Tubers were irradiated, after 6 months' storage, at 2·0, 5·0 and 8·5 krads, and were subsequently stored in similar conditions. Their polyphenol oxidase activities were estimated at monthly intervals over the post-irradiation storage period of 4 months.

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Key Word Index—Solanum tuberosum; Solanaceae potato; phenolase; physiological stress; γ-irradiation.