AD

TECHNICAL REPORT
FD-27

A STUDY OF THE RADIATION-INDUCED SOFTENING OF PLANT TISSUES

by

Z. I. KERTESZ L. M. MASSEY, Jr.

New York State Agricultural Experiment Station
CORNELL UNIVERSITY
Geneva, New York

Contract No. DA 19-129-QM-1584

October 1965

U. S. Army Materiel Command
U. S. ARMY NATICK LABORATORIES
Natick, Massachusetts

AD

TECHNICAL REPORT
FD-27

A STUDY OF THE RADIATION-INDUCED SOFTENING OF PLANT TISSUES

by

Z. I. Kertesz L. M. Massey, Jr.

NEW YORK STATE AGRICULTURAL EXPERIMENT STATION
CORNELL UNIVERSITY
Geneva, New York

Contract No. DA 19-129-QM-1584

Project Reference:
7-84-01-002

October 1965

U. S. Army Materiel Command
U. S. ARMY NATICK LABORATORIES
Natick, Massachusetts

FOREWORD

The availability of shelf-stable, highly acceptable food items for use in military feeding systems is considered a necessity. Many currently available thermally processed items, because of texture changes caused by the process, do not fully meet requirements because of their limited utility, stability and acceptability. Radiation processing, or "cold" sterilization as it is frequently called, has the potentiality of yielding products that have good military utility, good storage stability, and good acceptability. Therefore, research to develop process criteria that can be used to produce irradiation sterilized meats is under way.

The work covered in the contract was performed by the New York State Agricultural Experiment Station, Cornell University, under Contract DA19-129-QM-1584, during the period June 1960 to August 1962. It represents an investigation on the biochemical and physiological aspects of radiation-induced changes in plant tissue.

Drs. Z. I. Kertesz and L. M. Massey, Jr. were the Project Officers and Official Investigators and Drs. D. F. Splettstoesser, R. M. Smock, L. Han, W. E. Payne and D. F. Tallman the collaborators for Cornell University.

The U. S. Army Natick Laboratories' Project Officer was Mr. M. Simon, and the Alternate Project Officer was Dr. F. Heiligman, both of the Food Division.

EDWARD S. JOSEPHSON, Ph.D.
Associate Director for Food Radiation
Food Division

APPROVED:

FERDINAND P. MEHRLICH, Ph.D.
Director, Food Division

DALE H. SIELING, Ph.D.
Scientific Director

W. W. VAUGHAN
Brigadier General, USA
Commanding

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The radiation-induced changes in a large variety of fresh fruits and vegetables were investigated, and the physiology and biochemistry of some changes, particularly softening, investigated in detail. Possible beneficial results with several commodities, particularly apples, were noted.

A STUDY OF THE RADIATION-INDUCED SOFTENING OF PLANT TISSUES

SUMMARY

Investigations conducted over the two year period of this contract are reported. Much of the research has centered around the basic aspect of irradiation-induced softening of plant tissues. The acquisition of a 4,000 curie cobalt-60 source designed specifically for food irradiation research has permitted both confirmatory and exploratory experiments involving intact samples of fresh fruits and vegetables in statistically significant numbers.

The results of the irradiation experiments with intact samples are summarized. Although the responses exhibit considerable variation from commodity to commodity, it is the finding of this investigation that microbial activity did not appear to be the primary factor affecting the shelf-life of most of the items treated with doses in the kilo-rad range. Some beneficial results were noted from certain dosages and storage temperatures for several commodities. The treatment for storage scald in apples appears to be most promising. A retardation of in-storage softening rate was noted for all varieties tested, particularly Rome Beauty.

The results of studies concerning the physiological and biochemical aspects of tissue softening have also been summarized. Certain aspects of respiratory activity elicited by gamma irradiation have been investigated. Carbon dioxide evolution stimulation from irradiated lettuce is found to be completely dose-rate dependent, an anomaly in apple tissue respiration stimulation related to maturity. A moisture relationship resulting in tissue softening and a "dry" appearance in the flesh of irradiated apple tissue has been demonstrated and related to tissue breakdown by freezing. It is suspected that radiation-induced changes in differential permeability of cell membranes is involved. The mechanism of radiation-induced pectin degradation has been further investigated, particularly in respect to moisture content and the protective effect of sugars. The results confirm previous observations that higher moisture contents (above 50%) enhance the pectin degrading action of radiation. An indication that the sugars provide some protective action in the moist samples was also obtained.

INTRODUCTION

The purpose of this project was to conduct studies to further define and explain the radiation induced changes which occur in plant tissues, particularly those changes associated with: (1) the softening which occurs in tissues exposed to radiation and the relationship which exists between this sfotening and concurrent changes which take place in the polysaccharide constituents of plants, and (2) changes in tissue physiology brought about by absorbed radiation. The main body of this work is in a sense a continuation of that completed for the Quartermaster Research and Engineering Command under a preceding Contract (1).

The radiation source used during part of this work was the approximately 200 curie Co-60 source at the University of Rochester Atomic Energy Commission Project, Rochester, N. Y. Unfortunately the geometry of this source and its activity are such that only small samples of tissue were utilized and relatively long exposure periods were required for significant dosages. During the progress of the contract, however, we placed into operation a 4,000 curie Co-60 source here at Geneva. The power of this source, together with the geometry of the exposure area, permitted considerably more flexibility for expanded research. For the first time, it was possible for us to irradiate intact samples of moderate to large sized items in essentially unlimited numbers.

Several years experience in study of the effects of ionizing radiations on several plant tissues has indicated that some tissues are more suitable for some aspects of this study than others. For example, for softening studies a tissue such as apple is more suitable whereas in some of the respiratory activity studies a rapidly respiring leaf tissue may be preferred. In other studies, such as the influence of gamma radiation upon the chemical constituents of living plant tissues, it may be expedient to use chemicals from still another plant origin. This diversification is justified on the basis that this study is aimed more at developing an understanding of the mechanisms of action of ionizing radiations upon plant tissues in general than in the development of new or modified procedures for specific end-item applications.

METHODS AND MATERIALS

Investigations covered by this contract fall into two general classifications: first, those pertaining to the during and post-irradiation behavior of intact samples of fresh fruits and vegetables treated in numbers sufficient to permit statistically significant observations, and second, those pertaining to the physiology and biochemistry of the changes observed.

I. Irradiation of fresh fruits and vegetables

As tests of this nature at this Station have only recently been made possible by the acquisition of our 4,000 curie Co-60 source, the principal purpose of this study was exploratory. All fruit and vegetables utilized for this study were grown and harvested at the Experiment Station. Not only the previous history of the items was well known but also the handling of the items prior to, during, and following irradiation was accomplished with utmost care. In all instances, except where noted, the items were picked at the height of their eating maturity and irradiated within minutes or a few hours of harvest, and immediately placed into storage. In the strawberry, cherry, pea, raspberry, bean and lettuce experiments, the items were packaged in cellophane bags immediately prior to irradiation, and not removed until the termination of the study. In this packaging, care was taken to maintain gas exchange to minimize oxygen depletion and carbon dioxide accumulation through tissue respiration, and yet minimize the chance of post-irradiation contamination with air-borne organisms. In the tomato and apple experiments, the fruit were not packaged. In most cases a preliminary experiment was conducted with each item to determine the general susceptibility of the tissues to obvious radiation damage. This experiment was followed by more detailed studies including post-irradiation storage surveillance at one or more temperatures.

The fruits and vegetables were harvested in a mature state from the Experiment Station plots on the same day the irradiations were performed unless otherwise noted. All irradiations were accomplished in air, usually over an 18 hour period. The dosages and post-irradiation storage temperatures are summarized in Table 1.

The observations made of the tissues thus treated were as follows:

(1) General observations as to the changes occurring during irradiation conducted immediately following exposure, as well as during subsequent storage.

(2) The measurement of microbiological reduction during initial radiation, and changes occurring during subsequent storage.

(3) Physiological changes occurring in the tissues both during and subsequent to irradiation. These changes include: (a) respiratory activity acceleration; (b) softening measurements; and (c) changes in color, sugar content, and subsequent susceptibility to such specific post-harvest disorders as storage scald and brown-core in apples, etc.

(4) Samples of the irradiated items were prepared for the table according to accepted procedures for the individual fruit or vegetable under consideration, and, when results from (1) and (2) above indicated such a test advisable, were subjected to a preliminary objective taste-test.

II. Physiological and chemical changes occurring as a result of irradiation

Respirational activity phenomenon

Lettuce. One interesting phenomenon which came to light in studies conducted under the immediately preceding Contract (1) was that there appeared to be little significant difference in respiratory acceleration rate between a tissue receiving

a given dose-rate over a 9-hour exposure period, and that receiving the same dose-rate over an 18-hour exposure period, despite the fact that in the latter case the total dose administered to the tissue was twice that of the former. The apparent dependence of tissue response to dose-rate rather than to total dose absorbed warranted further investigation with lettuce tissue.

The preliminary experiments had been conducted over a multi-hour period with carbon dioxide production measured in a static system over the whole period. Polyethylene boxes of approximately 300 ml. capacity with closely fitting lids were employed for this purpose. A small plastic ring was fixed to the inside bottom of each box to support a 5 ml. Pyrex beaker containing 2 ml. of 1 N aqueous NaOH and a small filter paper wick. The tissue samples were weighed and placed in the boxes. Immediately prior to irradiation the small beakers were charged with alkali and the lids sealed onto the boxes. Following irradiation, the small beakers and contents were placed into rubber-stoppered, wide-mouthed Erlenmeyer flasks each containing 50 ml. of carbon dioxide-free water. Carbonate was then determined by the Hopkins modification of the barium precipitation method (2). Carbon dioxide evolution at ambient temperature (21-23°C, in most cases) was calculated in terms of mg. per kg. fresh weight per hour.

The above procedure was considered impractical for short-term studies and was modified as follows: The plastic boxes were adapted so that an air stream could be drawn continuously over and around the tissues throughout the duration of the irradiation. A modification of the carbon dioxide trapping method of Troug (3) was used in which a moist carbon dioxide-free air at a flow rate of 6.5 liters per hour was drawn through each of the plastic boxes containing the tissue as described above (but without the alkali-containing beaker) and hence into separate absorption towers containing 0.02 N NaOH. The rate of flow through the boxes was controlled by individual Indicating Variable Flowmeters. Following appropriate flushing and adjustments at the start of the experiment, the respiratory carbon dioxide was allowed to be trapped in the absorption towers for a given time interval (usually 1 hour). At the end of the absorption period, the towers were removed and fresh towers immediately introduced into the air stream for the next absorption period. The used towers were then washed out with successive portions of carbon dioxide-free distilled water, the carbonate precipitated with barium, and the sample titrated in the presence of ethanol with 0.02 N HCl.

In these experiments it was found convenient to combine the two methods by measuring short-term effects by the air stream method, followed by measuring the long-term effects by the static method by stopping the air stream and placing the alkali-containing beakers into the boxes followed by removal, precipitation and titration as before.

Apples. A relatively detailed study of the date-of-harvest and post-harvest storage relationships of apple tissues in respect to radiation-induced respiratory activity stimulation was conducted under the immediately preceding contract (ibid). During this study, tissue pieces of Rome Beauty apples harvested at various dates, were subjected to gamma radiations both immediately after harvest, and following various periods of storage, and respiratory activity changes as well as tissue firmness changes were measured. Briefly it appeared that: (1) The during-irradiation response in carbon dioxide evolution to radiations of kilorad intensity is negligible for fruit picked in a climacteric or post-climacteric condition. (2) The during-irradiation response in oxygen con-

sumption appears to be relatively uniform at the highest dosage throughout the harvest period. (3) Radiation-induced tissue softening was found to occur at all harvest dates. (4) Stimulation of carbon dioxide evolution, oxygen uptake, and softening rates as brought about by radiation tend to increase with length of storage.

The theoretical aspects of the rather marked change in carbon dioxide evolution rate response in the fruit treated immediately following harvest are of interest. It has long been known that the climacteric exhibited by the apple fruit signifies marked changes in the characteristics of respiratory activity, chemical composition and many other physiological and biochemical functions (4,5). The reason for the onset as well as the details of many of the changes has been relatively unknown, however. It appears that some biochemical or physiological factor, different in the pre-climacteric Rome Beauty fruit from the climacteric or post-climacteric fruit, may be responsible for the observed increase in carbon dioxide evolution rate in response to gamma radiation.

As McIntosh apples exhibit not only a high respiratory activity but also a clearly definable, sharp climacteric rise, it seemed of interest to confirm the results of Rome Beauty with McIntosh apples. A McIntosh apple tree in the Station orchards was therefore reserved and samples of fruit were picked from this tree at each of six consecutive harvest dates commencing August 22 and continuing until October 3, 1960. At the latter date the fruit was beginning to drop from the tree due to maturity. The remainder of the fruit was therefore picked at that time and placed in storage at 70°F for the duration of the experiment. Samples were removed from this temperature at each of three weekly intervals for further testing. Aliquot fruit from several of the harvests were placed at 40°F storage for subsequent testing immediately following picking.

Several of the fruit from each harvest were used immediately following picking in experiments to show the effect of irradiation upon the respiratory activity and softening during irradiation. From these, three lots (Sept. 19, Oct. 3, and Oct. 10) were selected as representing pre-climacteric, climacteric, and post-climacteric fruit, respectively, and were retested after 7 weeks' storage at 40°F.

In order to facilitate gas exchange from and throughout the tissue being irradiated, several small 10 mm x 18 mm cylinders of naked tissue were used. The irradiation was conducted as previously described, utilizing the polyethylene boxes over approximately an 18 hour period. An alkali trap and subsequent barium precipitation and titration were used for the carbon dioxide evolution rate determination by the "static" system described above. Oxygen uptake was determined by sampling the ambient air followed by direct analyses by means of the Van Slyke gas analysis apparatus. Tissue softening was determined by the use of the Chatillon pressure tester used directly upon the tissue cylinders immediately following the irradiation period.

Moisture relationships in irradiated tissues

It has been observed frequently by us that irradiated fruit and vegetable tissues possess a "dry" appearance when cut or broken. The mechanism of this response has been ascribed variously to certain phenomenon which are known or are

suspected to be occurring during the irradiation exposure. Chief among these possible mechanisms has been pectin degradation, desiccation resulting from increased evaporative rates brought about by the radiation, and changes in bound water occurring as a result of changes in molecular and/or colloidal dispersion.

Of these mechanisms, certainly the first mentioned is in operation in the apple tissues. Previous studies of our earlier contracts (1, 6, 7) have unquestionably correlated pectin degradation with irradiation dosage. The mechanism of this effect is that degradation thus incurred results in the "uncementing" of the tissues and allows the cells to pull apart. Thus the "cut" face of tissue so affected is composed of the exterior surfaces of many intact cell walls, rather than a true plane section through both intact and sectioned cells as occurs with "crisp" tissue.

Concerning the second, it is possible to demonstrate that tissues may become desiccated when high levels of radiation are employed under circumstances which favor water loss by diffusion or evaporation. However, we have demonstrated the development of a "dry" appearing surface during irradiation of intact samples with either no loss of or in fact an actual increase of total moisture of the sample.

We have from time-to-time, conducted brief exploratory experiments aimed at discovering evidence implicating changes in bound water as the influencing factor in determining this response. Several experiments based on the measurement of water retention under numerous conditions have been conducted with no noteworthy results. Recently, however, irradiated apple tissue was subjected to moisture determination according to the toluene distillation method (8). Although there was found to be no significant difference between the total moisture content of irradiated and unirradiated tissues, it was noted that the rate at which the moisture distilled over was more rapid in the irradiated sample than in the non-irradiated sample. Initial efforts to repeat this observation met with mixed results. Therefore a study of the conditions necessary to bring out these various distillation rates was conducted. It was found necessary to control flask temperature and resulting rate of distillation to rather close limits. To facilitate temperature control, and to allow for simultaneous replicate determinations, a thermostated oil bath was found satisfactory. Cylinders of apple tissues 17 mm in diameter and approximately 22 mm in length were adopted as standard. Using these samples, it was found that a bath temperature of 132 to 135°F produced distillation rates which differentiated the response to a maximum extent.

Effect of moisture content and the presence of sugar upon pectin degradation

We have previously reported experimental results obtained upon the irradiation of apples (6) indicating that apples of different varieties and maturities show considerable similarity in the occurrence in the threshold dosages of radiation-induced softening and that the softening is accompanied by drastic changes in the pectic constituents. Similar results were reported by others (9). Various investigators have also noted that both the water content and the presence of protective materials like sugars will effect the extent of degradation caused by the irradiation of pectins (1, 10).

Thus it was a logical continuation of previous work to isolate from apples the portion containing all polysaccharides including pectins, and irradiate this fraction, the alcohol-insoluble solids (AIS) in the presence of controlled pro-

portions of sugars and moisture. The experiments reported below give some information on the role of sugars and water contents in the changes which occur in the pectins of irradiated AIS.

The AIS used in these experiments was a mixture obtained from seven varieties of apples of "commercial picking maturity". The AIS was prepared from the peeled apples. A weighed sample of tissue was added to a volume of boiling 95% ethanol to give a final alcohol concentration of 70%. After a few days, the mixture was ground in an electric blendor and soaked in several renewed portions of fresh 70% and then 95% ethanol. Finally, the ethanol was squeezed out and the AIS was dried first in air, then in slight vacuum over P_2O_5, and ground to pass a 30-mesh sieve.

Irradiations were performed in small screw-top glass vials at the University of Rochester source. The required amounts of water to attain the desired moisture contents were added to the vials and mixed with the solid constituents by the use of a small polished-end glass rod. The rod was then cut off in a manner to fit into the closed vial, and the samples in the closed vials were allowed to stand overnight to allow equalization of the moisture. It is interesting to note that, at approximately 85% moisture level, the proportion of sugar approached that found in a ripe apple.

The details of pectin extractions have been described elsewhere (6, 7). Briefly, the contents of the vial were washed with water into a centrifuge tube which then was put several times under slight vacuum to remove the air from the sample. After each of the three water extractions for 30 minutes at 30°C the mixture was centrifuged, the supernatant extract removed, and replaced with fresh water. After the completion of the water extractions the sample was transferred into a crucible with a fritted glass bottom and extracted three times for 30 minutes at 30°C with a solution containing Calgon and sodium chloride. These extracts were sucked through the fritted glass. Finally, in the same crucible, the residue was extracted at 80°C three times with 0.05 N HCl. The three separate extracts with the same solvent were combined and made up to volume in a manner that the final solutions contained 0.2% Calgon and 0.8% sodium chloride and had a pH of 6.0. Viscosity determinations were made on these solutions at 30°C in Cannon-Fenske-Ostwald viscometers. Uronic acid determinations on the same solutions were made by the method described by Owens et al. (11).

RESULTS AND DISCUSSION

I. Irradiation of fresh fruits and vegetables

The results of this extensive series of tests are most difficult to present in tabular form. Hence a brief description by commodity is presented as follows:

Strawberries: In the preliminary experiment, berries of the Catskill variety were irradiated with from 0.01 to 2.0 Mrad. The effect of various doses on the number of microbial contaminants is shown in Table 2. No color change was found at any dosage used, but softening and flavor loss became objectionable above 0.5 Mrad.

In the three subsequent storage experiments which included both the Catskill and Fulton varieties, dosages of from 0.05 to 0.5 Mrad were used. Berries were

stored at either 75° or 35°F following irradiation. At the higher storage temperature not only were all berries in all experiments spoiled by the fifth day, but no level of irradiation extended the storage life significantly. At the lower temperature, however, an increase in the storage life of berries of at least one week longer than the control was obtained with dosages of 0.1 and 0.2 Mrad with the 0.1 Mrad dose being slightly better. Treatment with this radiation dose extended the total storage life to three weeks or longer in some cases (See Table 3).

As one might expect, storage life is influenced by variety and state of maturity at harvest. Those berries that were slightly immature or barely mature appeared to benefit most from the dosages noted above. The late maturing variety, Fulton, a hardier berry than Catskill, showed more benefit from irradiation than did Catskill.

Microbiological studies on stored strawberries showed a lack of correlation between yeast and mold counts and the general acceptability of the product. Assuming that the important spoilage organisms were being enumerated (plated on potato dextrose agar - 5 day incubation at 21°C), these results indicate that factors in addition to microbial activity (i.e. physiological tissue breakdown) were having an important effect on the storage life of this fruit .

Respiratory activity data are difficult to interpret, due to unusually large between-sample variations. It is suspected that this is due to varying levels of microflora carried by the individual berries or to marked changes associated with maturity. Generally, however, the observations of the preceding paragraph are confirmed by these observations, in that the immature berries, as well as those of the more hardy Fulton variety, tended to be accelerated less than mature or overmature berries or berries of the Catskill variety.

Cherries: In the preliminary experiment, Schmidt cherries were irradiated from 0.01 to 2.0 Mrad. The effect of the various doses on the number of microflora contaminating the fruit is shown in Table 2. Softening, as measured by the Chatillon pressure tester, was found to occur at 0.036 Mrad and above, and to have a 50% value of about 0.4 Mrad. Softening was generally found to be objectionable above 50%. Loss of flavor and significant flesh bleaching was noted at 0.5 Mrad and above. No off-flavors were observed.

In the four post-irradiation storage experiments, Schmidt, Windsor and Napoleon cherries were irradiated with doses from 0.1 to 0.4 Mrad. Irradiations were carried out at 70°F ± 2°F or 45° ± 3°F. The cherries irradiated at the higher temperature were stored at 75°F and those irradiated at the lower temperature at 35°F.

The cherries stored at 75°F were of the varieties Windsor (1 experiment) and Napoleon (2 experiments), all of which were infected with brown rot prior to harvest. In general all samples stored at this temperature contained less than 20% sound cherries after 16 days storage due principally to the development of brown rot. In the experiments with the Napoleon variety, dosages of 0.1 Mrad in one experiment and dosages of 0.1 and 0.25 Mrad in the other did delay the onset of the brown rot from 7 to 10 days longer than the control. In the one Windsor experiment irradiation showed no beneficial effects. Before the start of spoilage, samples receiving dosages of 0.25 and 0.4 Mrad exhibited excessive softening and

were described as having less flavor than either the control or the 0.1 Mrad samples.

The cherries stored at 35°F were of the varieties Schmidt, Windsor, and Napoleon (2 experiments). Brown rot did not develop in any cherries stored at this temperature, the main cause of spoilage being mold. All cherries kept well for the first 50 days of storage, with generally less than 10% spoilage. After 50 days, the cherries began spoiling, in some cases quite rapidly. By 70 days, the 0.4 Mrad sample contained the fewest sound cherries (less than 25%). Cherries receiving other dosages had all incurred about the same (50%) spoilage. In respect to quality, the lowest dosage is the only one that consistently compared favorably with the unirradiated control. Dosages of 0.25 and 0.4 Mrad resulted in post-irradiation softening which frequently became objectionable and was usually accompanied by a loss in flavor and in the dark-fleshed Schmidt variety, a marked bleaching of the flesh.

Concerning the physiological response of cherries to irradiation, the findings of our work conducted under previous Contract (1) were largely substantiated. The overall response of Schmidt cherries to irradiation is indicated in Table 4. There appeared to be little or no varietal or date of harvest effect upon the magnitude of the response. From the relatively extensive degree of stimulation afforded by the doses in the order of 0.4 to 0.5 Mrad, it would be predicted that the storage longevity of samples so treated would be adversely affected.

Peas. In the preliminary experiment it was observed that peas receiving from 0 to 0.1 Mrad at 70°F had soured during the irradiation period (Table 2). Color was unchanged by up to 2.0 Mrad and softening easily detected at 1.0 Mrad. No off-odors (except the sour odor in the control and low dosages) were observed.

In the following experiments both unblanched and blanched shelled peas were irradiated with 0.1 to 0.5 Mrad. Those irradiated and stored at 75°F were spoiled within 5 days regardless of dosage. In the samples stored at 35°F all three irradiation dosages about doubled the 10 day storage life of the unirradiated control. Flavor was very poor in all samples (including control) throughout storage.

Peas in pods were irradiated with up to 1.5 Mrad and stored both at 75°F and 35°F. At 75°F storage all samples spoiled within 5 days, with the samples receiving 0.1 Mrad retaining the most unspoiled pods longest. At 35°F storage, the control was 15% spoiled at 13 days and completely spoiled at 21 days. Irradiated peas at all levels up to 0.5 Mrad extended the storage life about one week. Irradiation at the 0.1 Mrad level produced the best results, peas being still 100% unspoiled at 21 days storage and still possessing a fair fresh pea flavor. Irradiation at 1.5 Mrad level turned pods and peas a dull gray and produced a "hay-like" odor.

As with strawberries, microbial activity did not appear to be the primary factor affecting shelf-life. In one trial, for example, peas subjected to 0.5 Mrad were unacceptable after 21 days storage at 35°F. The standard plate count at this time yielded a figure of only 1×10^4 organisms per g.

It is difficult to make a definitive statement concerning the physiological aspect of pea irradiation at this time. The respiratory activity of shelled peas is extraordinarily high (3,000 to 6,000 mg. O_2 per kg. fresh weight per hour).

Although some acceleration of this rate was indicated at all of the irradiation levels, it is probable that diffusion rates and limiting factors not existing in tissues exhibiting a more moderate rate of respiratory activity (i.e. 40 to 50 mg. CO_2 per kg. fresh weight per hour, for cherries), as well as the extensive period of time during which these measurements were taken, complicate interpretation of the results. Considerably more study must be given to the time-course of this response, as well as to the general respiratory mechanisms and controlling factors in peas before continuing the study into the irradiation field with this commodity.

Raspberries. Red, purple and black raspberries were each irradiated with dosages of up to 1.0 Mrad and stored at 35°F without preliminary exploratory experiments.

Red raspberries were evaluated after a one day storage. Irradiation caused softening which increased with increasing dosage. At the highest dose, the berries had shrunk to one-half of their original volume and were very juicy. Whereas the highest dose caused a severe bleaching of color, the color of the 0.5 Mrad sample was as good as that of the control. All radiation levels brought about inferior flavors, the highest level producing a disagreeable off-odor as well. Mold had appeared in all storage samples except those receiving 0.5 and 1.0 Mrad by 14 days. Mold appeared in the latter two dosages by 17 days, and all samples were spoiled by 20 days.

Purple raspberries responded in a manner identical to that of the red raspberries and will not be discussed here.

The color of black raspberries was unaffected by doses up to 1.0 Mrad. Noticeable softening and shrinking occurred only in the 1.0 Mrad sample. No off-odors or loss in odor was observed at any dosage. In flavor, all irradiated dosages except the 1.0 Mrad was preferred over the control, particularly the sample receiving 0.05 Mrad. All berries lasted 10-11 days before the appearance of mold. Irradiation with up to 1.0 Mrad did not prevent the initiation of mold growth, but it progressed more slowly than in the control in all but the 1.0 Mrad sample.

Physiological studies of the irradiated berries did not reveal anything particularly noteworthy concerning the susceptibility to irradiation. Although respiratory activity was stimulated by radiation in a manner previously described for other tissues, and although this respiratory activity stimulation appeared to be slightly more for the red and purple raspberries than with the black, differences were small and considered to be insignificant in view of the small number of trials conducted.

Green beans. Fresh, unsnipped green beans were irradiated with up to 2.0 Mrad, and immediately evaluated for quality. Beans receiving the higher irradiation dosages were not soft when eaten, but had lost the usual "snap". Color was unaffected at any dosage used and flavor was impaired only at the highest dose. The odor of the sample receiving the highest level was described as being "aromatic" and not the natural bean odor.

In a subsequent storage experiment beans were irradiated with from 0.01 to 0.5 Mrad at 70°F and stored at 35°F. When tasted after 2 days storage, the sample receiving the highest dose was liked least with no consistent preference

among the others. All samples were 95% sound after 10 days but had developed rust-colored surface spots which in some cases almost covered the surface of the bean. By 17 days all samples were evaluated as over 50% spoiled.

Although the results of the physiological investigations are very clear, corresponding in the nature of the response to that obtained with other tissues (See Table 5), the interpretation is somewhat difficult. Green beans are of a heterogeneous nature, anatomically speaking. They are composed of pod tissue of leaf-like nature, structure and respiratory activity, and seed tissue usually associated with high respiratory rates at immaturity. As might be expected, the respiratory activity stimulation was intermediate in its response between that of leaf tissue (See 1) and that of peas (See above). On the basis of previous experience, it would be anticipated that differences in the radiation sensitivity of the bean seed and pod would exist. It is significant to note that the bulk of the general observations concerning the keeping quality of beans were conducted on the external (pod) response.

Tomatoes. An experiment was performed to test the effect of gamma radiation upon the rate of ripening of tomatoes harvested as immature fruit. Towards this end, tomatoes of the variety Fireball were harvested at the four stages of immaturity designated as "green", "turn", "pink", and "light red". "Green" is defined as the state in which the fruit shows no color but green, and "turn" the state in which the fruit show a fading of the green and the development of a yellow color, but show no pink. "Pink" and "light red" are self explanatory. Samples of tomatoes from each maturity classification were irradiated with doses of 0.001, 0.01, and 0.05 Mrad over a 2 hour period, and 0.01 Mrad over an 18 hour period. Subsequent to the irradiation exposure, the samples were placed at 70°F to ripen, and regraded for maturity periodically to determine the rate of ripening.

Results indicate that if the tomatoes were irradiated before the appearance of the red color in the fruit (e.g. "green or turn" stage of maturity), a significant decrease in the rate of ripening was obtained (See Table 6). This decrease is generally proportional to the amount of radiation. When 90% of the control fruit was ripe, only 65% of the fruit receiving the highest dose was ripe. The sample receiving the 0.01 Mrad dose over the 18 hour period ripened at a rate intermediate between that of the sample receiving the 0.01 Mrad dose over a 2 hour period and that of the sample receiving the 18 hour dose rate but only over a 2 hour period (0.001 Mrad). After pink or red color had appeared, however, irradiation did not appear to affect the rate of ripening significantly.

The above results indicate two aspects of physiological response to gamma radiation which warrant further attention. First, there appears to be a stage in maturity which may be sensitive to gamma radiation, as well as a stage which may not be sensitive. This is indicated by the fact that tomatoes which did not show the appearance of red coloration were inhibited in their ripening rate whereas tomatoes which did show red coloration were not. Second, there is a definite indication that the rate at which the radiation is delivered to the tissue as well as the total dosage may govern the response. This dose-rate dependency has been noted before in the physiological aspects of this (and past) work.

In another experiment involving tomatoes, ripe fruit of the Red Jacket variety were irradiated, both packaged and unpackaged, at dosages between 0.05 and 2.0 Mrad, and subsequently stored at 35°F. Observations made immediately

following irradiation indicated that color was unaffected by even the highest dose used, but softening could be detected at 0.5 Mrad and was quite severe at 1.0 and 2.0 Mrad. No off-odors were produced, but all irradiated samples had a more "ripe tomato" odor than did the control, the intensity of which increased with increasing dosage.

During subsequent storage, softening became more pronounced in all irradiated samples except 0.05 Mrad which was about as firm as control. Spoilage occurred in both controls and irradiated samples at a similar rate and to a similar extent so that by 40 days storage all samples were over 50% spoiled.

No physiological or microbiological measurements were performed with these fruit.

Apples. Two-bushel lots of hand-graded 2-3/4-inch apples of the varieties McIntosh, Cortland, and Rome Beauty were each subjected to radiation doses of 0.05 and 0.1 Mrads over a 6-hour exposure period to gamma radiation from our 4,000 curie Co-60 source under ambient temperatures of approximately 70°F. The fruit was picked at early, medium and late maturity harvest dates, the medium date being approximately that of commercial maturity. In one of the McIntosh pickings, and in two of the Rome Beauty pickings, a third treatment of 0.1 Mrad over a 12 hour exposure period was utilized for a dose-rate study. An attempt was made to measure respiratory activity of the fruit during irradiation by trapping the CO_2 from the ambient air stream.

Immediately following irradiation, the fruit were graded as to firmness (Magness Taylor) and percent soluble solids and respiratory activity of a representative sample taken at ambient (70°F) temperature during the subsequent 7-9 day period. The fruit were then placed in commercial cold storage where they were maintained for several months. Upon removal from storage, they were assessed for quality and the incidence of certain manifestations of physiological deterioration known to be characteristic of the variety in question.

The results of the during-irradiation measurements were disappointing. It was not possible, with the experimental setup utilized for this experiment to obtain reliable carbon dioxide evolution figures with intact apples via ambient air-stream analysis. The results of these analyses have been discarded as unreliable, and will not be discussed.

The results of the respiratory activity measurements immediately following irradiation are of interest, however, and are presented in Figures 1 and 2. The following conclusions may be drawn from analysis of these data:

(1) Radiation appears to stimulate oxygen uptake of apples at each maturity tested. In contradiction to the results previously obtained with tissue cylinders, however, with intact fruit this stimulation appears to be of much longer duration. Although a part of this prolonged stimulation may be more apparent than real, due to the retardation of diffusion through the flesh and the surrounding skin, the prolonged period of this effect may not be wholly explained by this artifact.

(2) Radiation doses of 0.05 and 0.1 Mrads appeared to hasten the onset of the climacteric rise in both varieties tested. This effect is particularly apparent in the early harvest of McIntosh.

The results of the analysis of apples at harvest (but immediately following irradiation) and after storage, as indicated in Table 7 are also of interest. The following conclusions may be drawn from these data:

(1) It will be noted that the irradiated fruit softened at a slower rate during subsequent storage of up to 5 months under refrigeration than did the non-irradiated controls. Thus, despite the softening accomplished by the period itself, several of the irradiated samples (particularly Rome Beauty) were significantly firmer upon removal from storage than were the unirradiated samples. To our knowledge, this is the first time this has been shown.

(2) The control of storage scald by irradiation was excellent. Apples from our 1961 season were highly susceptible to this physiological disorder. Other experiments involving control chemicals had little effect this year on either the incidence or degree of severity of this disorder. It is probable that the degree of control brought about by irradiation in this instance is of commercial interest.

(3) The control of brown-core in early and medium picked McIntosh was significant. As immature fruit of this variety are particularly susceptible to this disorder, it is possible that this might also be of commercial interest. In our opinion, however, this effect is of secondary interest to that of scald control.

(4) Breakdown of the McIntosh apples was noted, which may be possibly related to the radiation treatment. Although this effect was not noted in either the Cortland or the Rome Beauty fruit, its severity in the McIntosh fruit was sufficient to warrant further investigation.

(5) As noted in the data for the Cortland apples, irradiation was detrimental to flavor. The principal response appears to be a diminution of the "bouquet" in apple flavor, rather than the production of an "off" flavor although such was noted in one sample. Although not tabulated, this was also noted with the other two varieties tested. This effect was rated in an informal manner by the experimenters, rather than a result of an appropriately designed taste panel. Hence it is not known at this time precisely how important this effect may be to the acceptability of the product.

It is concluded, therefore, that storage longevity extension of apples by means of ionizing radiations holds enough promise to warrant further attention in terms of: (1) inhibition of storage scald, (2) reduction of the incidence of brown-core, and (3) possible increase of fruit firmness following extended storage periods. The incidence of tissue breakdown and the detrimental influence of flavor upon the fruit should be further investigated as to their possible detrimental influence on the process.

Lettuce. Duplicate heads of lettuce were exposed to each of two radiation dosages (0.1 Mrad and 0.3 Mrad) at ambient temperatures over a 28-hour period, and then stored, along with duplicate unirradiated control samples, at each of two storage temperatures (75° and 40°F). Surveillance was conducted by making observations upon the visible characteristics of the lettuce at frequent intervals, and taking samples from the outer leaves during the first week of storage for bacterial counts. The results of this surveillance are indicated in Table 8. None of the irradiated lettuce kept as well as did the unirradiated controls.

This effect was most marked at the higher temperature. The results of the bacterial counts are of interest. They indicate not only a fairly high degree of pasteurization was accomplished at the higher dose, (low temperature counts) but also a loss of resistance of the lettuce to bacterial invasion which was manifested during storage at the higher temperatures.

On the basis of these preliminary storage longevity tests, it is concluded that pasteurization may be obtained in lettuce tissues with relatively low doses of ionizing radiation delivered over a relatively long period of time, and that the principal factor contributing to degradation in storage of irradiated tissues is of physiological origin. This factor is expressed directly either during or immediately subsequent to irradiation or indirectly through lowered resistance to microbial infestation.

One of the symptoms of irradiated lettuce noted is the development of yellow, sunken, lesions along the under midrib of the lettuce leaf. These spots may occur in non-irradiated lettuce which has been in storage for extensive periods, and can markedly detract from the attractiveness of the product. The lesions are apparently of physiological, rather than microbial,origin. We have noted lesion development in lettuce either during or immediately following irradiation in air. Several experiments involving irradiation of lettuce leaves in a nitrogen atmosphere (less than 0.02 percent oxygen) have been performed. It has been observed that the exclusion of air from the lettuce leaf results in the total absence of lesions. In a few of the experiments performed, some tissue collapse has been noted during 18 hours of anaerobiasis at 25°C. It has been found, however, that the tissues do not collapse when so exposed under refrigerated (4°C) conditions, but that refrigeration itself is apparently not effective in eliminating the lesions. The influence of gamma irradiation upon the respiratory activity of lettuce tissue under the influence of nitrogen atmosphere and of low temperatures is presented in Table 9. It is of interest to note that whereas the percent response of tissues irradiated in nitrogen is only approximately 50 percent that of tissues irradiated in air, tissues irradiated at both the high and low temperatures responded approximately to the same extent.

The effect of the irradiation atmosphere on the contaminating microorganisms was studied. Several lettuce leaves of a similar size were placed in each of four desiccators. Two of the desiccators were evacuated and flushed repeatedly with nitrogen gas while the other two were left open to the air. Following a treatment of approximately 0.2 Mrad, 25 g. samples were removed and plated for viable counts. The results (Table 10) indicate that radiation-pasteurization of lettuce is as effective in an atmosphere of nitrogen as in air.

II. Physiological and chemical changes occurring as a result of irradiation.

Respirational activity phenomenon

Lettuce. Several experiments concerning the dose/dose-rate acceleration of lettuce tissue respiration were conducted, the results of which may be illustrated by the following two examples.

(1) Samples of lettuce tissue were aerated until a steady state of

carbon dioxide evolution rate was obtained. Duplicate samples were then irradiated at dose rates of approximately 0.014 and 0.004 Mrad per hour for the following 60 minutes. The resulting carbon dioxide evolution rates both during the irradiation period and during subsequent non-irradiated periods were compared to appropriate non-irradiated control samples. The samples were subjected to continuous irradiation at the above rates for 16.5 hours and the total carbon dioxide evolution rate over this entire period determined. The results of this experiment, expressed as the percentage of the unirradiated control are indicated in Figure 3. It will be noted that not only is the rate of carbon dioxide production proportional to the irradiation dosage, but also the maximum level obtained as a result of the 1-hour irradiation is nearly identical to the average rate obtained during the subsequent 16.5 hour irradiation. An approximately 2-hour delay in carbon dioxide evolution rate peak will be noted.

(2) Samples of lettuce tissue were treated precisely as before except that instead of a single exposure to radiation, several successive hour exposures (separated by the approximate 4 minute interval required to effect changes in absorption towers) were used, again followed by the subsequent 16.5 hour continuous exposure. The results of this experiment are indicated in Figure 4. Similar conclusions to that of the first example may be derived.

Even from these relatively crude experiments it is obvious that the respiratory activity stimulation brought about by gamma radiation is much more nearly proportional to the radiation intensity than to the total dosage absorbed. The results of the second experiment (Figure 4) suggest a slight falling off of the respiratory activity for both irradiated samples towards the end of the intermittent irradiation periods. This result is believed to be spurious.

Apples. The results of the experiments conducted with post- and pre-climacteric apple tissues are summarized in Tables 11 and 12. Not only was the respiratory activity of McIntosh apple fruit pieces considerably higher than that previously measured with Rome Beauty, but also the climacteric rise was much sharper and more definable. There is a difference in the response of pre- and post-climacteric apples to carbon dioxide evolution rate acceleration by radiation. The same conclusions may be drawn from these experiments as those previously cited for the Rome Beauty experiments.

Moisture relationship of irradiated tissues.

We have found it expedient to use both whole and sectioned apple fruit for these studies. In the former case, apples were irradiated before the preparation of the test cylinder, and in the latter, the prepared cylinder itself was irradiated. In the latter instance, care was taken to prevent desiccation by placing the cylinder in a closed atmosphere (glass jar) in the presence of liquid water. The results of a typical moisture determination are presented in Figure 5. Here we have plotted the amount of water (in percent fresh weight) distilled from the samples at 5 minute intervals. It will be noted that the 1 Mrad sample is at a rate nearly equal to that of a comparable weight of free water.

A brief investigation as to the applicability of this method to the determination of moisture availability in irradiated lettuce leaf tissue was disappointing. No significant difference between irradiated and control samples could be found. We believe that, with leaf tissue, the relatively large surface-to-volume ratio results in the ready accessibility of moisture and therefore the

relatively small differences being measured in this test were not apparent.

A summary of dose-response curve is indicated in Figure 6. For this purpose, the percent response was calculated from the difference in moisture content of the irradiated samples at 20 minutes from that of the control sample on a fresh weight basis. At present it is not known if the break in linearity between 0.001 and 0.01 Mrads is real.

The influence of a number of variables on the response of apple tissue to irradiation has been studied. Several varieties of apples were tested for the magnitude of this response to irradiation, and no significant differences were found. The response of Rome Beauty apples of various harvest dates was also tested. These fruit had been at low-temperature storage for approximately 2 to 4 months. No significant difference of harvest date was found. Also, irradiation of tissues between the temperature limits of 6°C and 24°C was found to exhibit no influence on the magnitude of the response elicited.

One significant difference between the degradative response of biological chemicals (e.g. pectin, dextran, etc.) and that of such physiological processes as respiratory activity stimulation has been shown to be the response to differences in dose-rate. The extent of degradation brought about by irradiating pectin is proportional to total dose regardless of the rate at which that dose was delivered. On the other hand, the during-irradiation stimulation of carbon dioxide evolution is found to be directly proportional to dose-rate.

In order to investigate this aspect of the moisture-loss response in apple tissue, radiation varying in dose-rate by a factor of 10 was applied to apple tissue, and the subsequent rate of moisture loss in toluene distillation determined. The results are presented in Table 13. It is apparent that there is little significant difference between the moisture availability in the samples irradiated with 1 Mrad at each of the two time intervals, whereas there is a significant difference between that of the 1 Mrad samples and the 0.1 Mrad samples. The response is therefore more dependent upon total dose than upon dose-rate.

Thus it appears that we have demonstrated an irradiation response of apple tissue resulting in the increased availability of tissue moisture proportional to the amount of irradiation absorbed and not influenced by irradiation temperature between the range of 6°C to 24°C, nor influenced by relatively wide differences in dose-rate. In an effort to correlate this response with others of more clearly defined origin, loss of moisture by the toluene distillation technique from tissues which had been subjected to freezing and subsequent thawing was determined. The results obtained are presented in Figure 7. The similarity between this series of curves and those presented in Figure 5 is of interest.

Whether this response is an expression of changes in the bound water of the cell, or increased permeability of the cell to water resulting from one or more of several factors, or other factors is not known at present. It also remains to be seen how much, if any, of the softening brought about by gamma irradiation of plant tissues may be explained in terms of the loss of turgor of the tissue cells, and how much by the mechanism of pectin degradation and subsequent softening *per se*.

Effect of moisture content and the presence of sugar upon pectin degradation.

Table 14 shows an increase upon irradiation in the total uronides extracted from the AIS samples. We have observed this increase in every sample of irradiated apple AIS and even with irradiated apples. The increase depends on the radiation dosage and was more substantial upon 0.56 Mrad gamma irradiation (Table 14) than when only 0.27 Mrad was applied (Table 15). It is believed that this increase is the result of the enhanced solubility of all pectic constituents (see below) and not of the formation of uronides from sugars as observed by Wolfrom et al. (12), at high radiation dosages. Tests which we made with sugars irradiated at the dosages used in the present work did not show the formation of even traces of uronides.

Irradiation and increasing moisture contents at the time of irradiation consistently increased the proportion of water-soluble pectic substances (W). There was a corresponding decrease in the acid-hydrolyzable pectin fraction (H) representing "protopectin". The Calgon-soluble fraction (C) first increased slightly and then decreased but it is not clear whether these changes were significant or resulted from incidental variations.

The situation is quite different with regards to the viscosities of the extracted pectins shown in the right side of Table 14. These values, indicating the relative viscosities of the solutions on the anhydrogalacturonic acid (AUA) basis, are roughly proportional to the intrinsic viscosities of pectin samples and thus also to their weight-average molecular weights. The results indicate a gradual decrease in these values with irradiation and irradiation at increasing moisture contents. As expected from previous work, the difference caused by irradiation was substantial but increasing the moisture content of the samples from zero to 50% caused only a limited additional decrease in the viscosity values in the water (W) and Calgon (C) extracts and none in the acid-extracted pectins (H). This can be interpreted in terms of the water-effect theory that at 50% moisture content the AIS reacts "as if it were dry" just as we have found that pectin moistened to this extent acted in the same manner (1). It is also clear from these viscosity data that the highly-hydrated and more water-soluble pectins reacted more to the irradiation at increased moisture levels than did the insoluble pectic constituents represented by the acid-hydrolyzable (H) fraction. However, it is only fair to note that while the general trends bear out the above statements, the numerical values shown are not as regular as one might desire.

The experimental results in the left side of Table 15 show a general similarity to those in Table 14 although the trends are not quite as clear due to the lower radiation dosage and the several steps of small moisture content differences used. (In order to compensate for any possible differences caused in the extraction of the water-soluble pectins from the sugar-containing samples, the same sugar mixture was added before extraction to the samples irradiated without sugars).

In the dry mixture of AIS and sugar the presence of sugars caused no differences. In fact, the two samples (without and with sugars and irradiated dry) show as close agreement as can be obtained between duplicate extractions. In the presence of increasing proportions of water and added sugars the total uronide extraction showed some increase above that observed by irradiation with high moisture content but without sugars. While the distribution of uronide

fractions did not show startling differences between the with and without sugar series, there is some indication that the progressive transformation from the "protopectin" fraction (H) to the other two fractions (C and W) was less pronounced in the presence of sugars. Particularly interesting is the fact that at this radiation dosage the C fraction ("pectates") increased in the samples irradiated at high moisture contents and that this fraction increased somewhat more in the presence of sugar. We cannot explain this observation.

Turning now to the viscosity data in Table 15, it is quite clear that whereas there is little difference between the C and H fractions of the samples irradiated with or without sugar except that the C values in the presence of high moisture content and sugars might indicate a slight degree of protection. On the other hand, such a protective action is unmistakable in the W column representing the AIS irradiated with added sugars.

It is well known that aqueous solutions of polysaccharides are much more extensively degraded than are dry polysaccharides exposed to the same radiation dosage. The question of both theoretical and practical significance is the moisture content at which this transition to higher radiation degradation takes place. The present experiments confirmed the previous findings on pectin irradiated at different moisture contents that the moisture content has to be raised above 50% before the presence of water makes an important difference in the radiation effectiveness. The results of AIS irradiation experiments with added sugars are not sufficiently clear to allow the drawing of firm conclusions. The reason might be the comparatively low radiation dosage used in this experiment.

Literature Cited

1. Contract DA-19-129-QM-1164, (1960).

2. Hopkins, E. F. Bot. Gaz. 78, 311 (1924).

3. Troug, E. J. Ind. Eng. Chem. 7, 1045 (1915).

4. Kidd, F. and C. West. Plant Physiology 20, 467 (1945).

5. Smock, R. M. Apples and Apple Products, p. 138, Interscience Publishers, Inc., New York.

6. Contract DA-19-129-QM-727, (1956-57).

7. Contract DA-19-129-QM-328, (1955-56).

8. Official Methods of Analysis of the A. O. A. C., 9th Edition, p. 283, (22.003-22.005), Association of Official Agricultural Chemists, Washington 4, D. C.

9. McArdle, F. J. and J. W. Nehemias. Food Technology 10, 599 (1956).

10. Kertesz, Z. I., B. H. Morgan, L. W. Tuttle, and M. Lavin. Radiation Research 5, 372 (1956).

11. Owens, H. S., et al. Western Regional Research Lab. U. S. D. A. Mimeo June 1952.

12. Wolfrom, M. L. Radiation Research 10, 37 (1959).

Table 1. Summary of irradiation and post-irradiation storage conditions of various fresh fruits and vegetables being studied.

Exp. No.	Item	Variety	Irradiation temp. (°F)	Irradiation dose (Mrad)	Post-irradiation storage temp. (°F)
1	Strawberries	Catskill	70	0.01, 0.05, 0.20 0.50, 1.0, 2.0	None
2		Catskill	70	0.05, 0.20, 0.50	35, 75
3		Fulton	70	0.10, 0.25	35
4		Fulton	45-50	0.10, 0.50	35
5	Cherries (Sweet)	Schmidt	70	0.01, 0.05, 0.20 0.50, 1.0, 2.0	None
6		Schmidt	45-50	0.10, 0.25, 0.40	35
7		Napoleon	70	0.10, 0.25, 0.40	35, 75
8		Windsor	45-50, 70	0.10, 0.25, 0.40	35, 75
9		Napoleon	45-50, 70	0.10, 0.25, 0.40	35, 75
10	Peas Raw, (shelled)	Mixture	70	0.01, 0.05, 0.20 0.50, 1.0, 2.0	None
11		Geneva 51	45-50	0.10, 0.20, 0.50	35
12	(Raw, in pod)	Early Perfection 326	70	0.05, 0.10, 0.25, 0.50, 1.50	35, 75
13	(Blanched)	Mixture	70	0.10, 0.25, 0.50	35, 75
14	Raspberries (Black)	---	45-50, 70	0.05, 0.25, 0.50, 1.0	35
15	(Red)	---	45-50, 70	0.05, 0.25, 0.50 1.0	35
16	(Purple)	---	45-50, 70	0.05, 0.25, 0.50 1.0	35

Table 1. Summary of irradiation and post-irradiation storage conditions of various fresh fruits and vegetables being studied. (continued)

Exp. No.	Item	Variety	Irradiation temp. (°F)	Irradiation dose (Mrad)	Post-irradiation storage temp. (°F)
17	Bean, (Green)	Tendergreen	70	0.01, 0.10, 0.20 0.50, 1.0, 2.0	None
18		Tendergreen	70	0.01, 0.10, 0.20	35, 75
19	Tomatoes (unripe)	Fireball	70	0.001, 0.01, 0.05	70
20	(ripe)	Red Jacket	70	0.05, 0.10, 0.50 1.0, 2.0	35
21	Apples	McIntosh	70	0.05, 0.10	35
22		Cortland	70	0.05, 0.10	35
23		Rome Beauty	70	0.05, 0.10	35
24	Lettuce	Iceburg Type	70	0.1, 0.3	34, 75

Table 2. The effect of various irradiation dosages on the microflora contaminating fruits and vegetables.

Product	Microorganisms per gram (X 10^3)[a] at doses (Mrad)			
	0	0.2	0.5	1.0
Strawberries	210	25	19	12
Cherries	8.3	2.2	1.1	0.4
Peas[b]	13 X 10^5	32	5.6	0.03
Red raspberries	95	---	42	---
Black raspberries	100	---	34	---
Green beans	2100	70	0.8	0.1

[a] Vegetables plated on Difco Standard Plate Count agar; incubated 2 days at 32°C. Fruit samples plated on potato dextrose agar (Difco); incubated 5 days at 21°C.

[b] Significant growth of microorganisms occurred during the 18 hour irradiation period. Because varying amounts of growth probably occurred in all control and low-dosage samples, the data do not permit death rate calculations such as D values.

Table 3. Storage longevity of strawberries following radiation treatment

Variety	Irradiation date	Days storage at 35°F	% sound at dose (Mrad)					
			0	0.05	0.1	0.2	0.25	0.5
Catskill (slightly overmature)	3-4 July	3	100	100	---	100	---	100
		6	35	50	---	53	---	30
		8	9	44	---	42	---	21
Fulton (firm, mature)	13-14 July	8	80	---	90	---	92	---
		13	70	---	90	---	92	---
		19	15	---	90	---	80	---
		22	13	---	90	---	65	---
		26	10	---	50	---	30	---
Fulton (overmature)	18-19 July	6	87	---	77	---	63	---
		10	85	---	70	---	45	---
		15	30	---	50	---	36	---
		20	15	---	40	---	27	---

Table 4. The effect of gamma irradiation upon the respiratory activity of Schmidt cherries.

Radiation dose (Mrad)	Respiratory activity[a]	% Response[b]
None	52.0	---
0.01	55.5	+6.7
0.05	51.3	-1.3
0.2	62.5	+20.2
0.5	74.5	+43.3
1.0	100	+92.3
2.0	155	+197

[a] Average mg. CO_2 per kg. fresh weight per hour at 70°F, over the entire 18 hour irradiation period.

[b] Expressed as percent deviation from unirradiated control.

Table 5. The effect of gamma irradiation upon the respiratory activity of Tendergreen green beans.

Radiation dose (Mrad)	Respiratory activity[a]	% Response[b]
None	150	---
0.01	188	+25.5
0.10	217	+45.3
0.50	261	+74.6
1.0	362	+142.0

[a] Average mg. CO_2 per kg. fresh weight per hour at 70°F, over the entire 18 hour irradiation period.

[b] Expressed as percent deviation from unirradiated control.

Table 6. Effect of gamma radiation on the ripening rate of immature Fireball tomatoes

Maturity Classification	Irradiation date	Observation date	% ripe at doses (Mrad)				
			0	0.001	0.01[a]	0.01[b]	0.05
Green	28 August	5 September	10	40	30	0	10
		7 September	30	50	30	0	20
		11 September	70	60	40	10	30
		14 September	80	80	70	40	50
		19 September	100	90	100	60	100
Turn	31 August	5 September	40	10	0	20	0
		7 September	40	10	10	30	20
		11 September	100	60	80	50	70
		14 September	100	90	80	100	80
		19 September	100	100	100	100	100
Pink	3 September	5 September	30	10	50	20	40
		7 September	40	50	50	20	50
		11 September	90	70	100	50	80
		14 September	100	80	100	100	100
		19 September	100	100	100	100	100
Light Red	5 September	7 September	90	90	90	90	100
		11 September	100	100	100	100	100

[a] 5,000 rad/hour dose rate.

[b] 555 rad/hour dose rate.

Table 7. Analysis of apples at harvest (but immediately following irradiation) and after storage.

A. McIntosh		At Harvest and Irrad.		After Storage (3/21/62)						
Picking date	Treatment (Mrad)	Firmness (lbs)	Sol. Solids (%)	Firmness (lbs)	Scald (%) Slight	Severe	Total	Brown core (%)	Break-down (%)	Decay (%)
9/21/61	Control	15.2	10.3	12.1	39	52	91	71	1	0
	0.05	14.4	10.4	12.4	6	5	11	11	0	1
	0.1	13.4	10.3	12.1	0	0	0	22	2	1
9/29/61	Control	15.0	10.8	12.0	27	43	70	58	0	1
	0.05	13.3	10.3	11.8	0	0	0	7	0	1
	0.1	12.3	10.6	11.4	0	0	0	10	11	0
10/9/61	Control	12.9	10.8	10.1	25	8	33	33	31	2
	0.05	11.3	10.3	8.6	0	0	0	31	42	1
	0.1	10.5	10.8	8.2	1	0	1	37	45	3
	0.1-12	11.3	10.4	8.8	1	0	1	35	18	2

B. Cortland		At Harvest and Irrad.		After Storage (2/2/62)					
Picking date	Treatment (Mrad)	Firmness (lbs)	Sol. Solids %	Firmness (lbs)	Scald (%) Slight	Severe	Total	Breakdown %	Flavor
9/26/61	Control	15.9	10.6	10.9	0	100	100	0	OK
	0.05	14.5	11.1	12.5	13	9	22	0	flat
	0.1	13.3	10.8	11.9	3	3	6	1	flat
10/6/61	Control	15.1	11.0	11.0	11	85	96	0	OK
	0.05	14.0	11.5	12.7	28	10	38	1	flat
	0.1	13.5	11.3	10.6	9	5	14	0	flat
10/17/62	Control	14.7	11.4	11.3	27	6	33	0	OK
	0.05	13.7	12.1	11.7	1	4	5	1	OK
	0.1	13.1	11.9	11.6	1	1	2	0	off

Table 7 (continued)

C. Rome Beauty		At Harvest and Irrad.		After Storage (3/21/62)			
					Scald (%)		
Picking date	Treatment (Mrad)	Firmness (lbs)	Sol. Solids (%)	Firmness (lbs)	Slight	Severe	Total
10/13/61	Control	22.0	11.2	15.5	1	99	100
	0.05	19.5	11.2	17.8	51	34	85
	0.1	18.0	11.2	18.0	41	13	54
10/24/61	Control	20.2	12.0	15.0	6	93	99
	0.05	18.4	12.0	16.2	25	8	33
	0.1	16.5	12.8	16.5	14	1	15
	0.1-12	16.3	12.3	16.9	5	0	5
11/3/61	Control	20.2	12.0	15.9	25	21	46
	0.05	18.2	12.0	16.4	13	3	16
	0.1	15.9	11.9	16.3	4	5	9
	0.1-12	15.5	12.3	15.2	2	0	2

Table 8. Characteristics of Iceberg-type commercial lettuce heads stored at various temperatures for various periods of time following irradiation.

Irradiation dose (Mrad)	Storage temperature 75°F.			Storage temperature 34°F.					
	Storage period (weeks)			Storage period (weeks)					
	1	2	3	1	2	3	4	5	6
None	A-1	A-2 E-1 G-2	A-3 E-2 G-3 H	A-1	A-1	A-1 C-1	A-2 B-1 C-1 E-1	A-2 B-1 C-2 E-1	A-2 B-1 C-2 E-2 H
	(1.6×10^6)*			(6.3×10^6)*					
0.1	A-2 B-1 D-1	A-3 B-1 G-3 H	-	A-1 B-2	A-1 B-2 E-1	A-2 B-2 E-1	A-2 B-2 C-1 E-1	A-2 B-3 C-1 E-2	A-3 B-3 C-2 E-2 H
	(2.3×10^8)*			(2.0×10^5)*					
0.3	A-2 B-3 D-1 E-1	A-3 B-3 E-2 G-3 H	-	A-2 B-3	A-2 B-3 E-1	A-2 B-3 C-1 E-1	A-2 B-3 C-1 E-1	A-2 B-3 C-2 E-1 F-1	A-3 B-3 C-2 E-2 F-2 H
	(8.6×10^8)*			(4.6×10^4)*					

Key

A-1 Condition good
A-2 Condition fair
A-3 Condition poor
B-1 Few lesions
B-2 Moderate lesions
B-3 Many lesions
C-1 Slightly desiccated
C-2 Moderately desiccated
D-1 Ooze, yellow
E-1 Slight yellowing
E-2 Moderate yellowing
F-1 Trace of mold visible
F-2 Moderate mold visible
G-1 Slight tissue breakdown
G-2 Moderate tissue breakdown
G-3 Extensive tissue breakdown
H Terminated

* Bacterial counts/gram of external tissue conducted 6 days after the end of irradiation.

Table 9. Influence of atmospheric composition and temperature upon respiratory response of lettuce to gamma irradiation.

Atmospheric Composition	Temperature (°F.)	Irradiation dose (Mrad)	Respiratory Response	
			mg CO_2/kg/hr	% Response
Experiment I				
Air	70	None	117	-
Air	70	0.2	212	80
Air	57	None	64	-
Air	57	0.2	119	84
Experiment II				
Air	70	None	110	-
Air	70	0.2	198	82
Nitrogen	70	None	48	-
Nitrogen	70	0.2	69	45

Table 10. The effect of atmospheric composition on the reduction of the number of microorganisms contaminating Bibb lettuce by means of gamma irradiation.

Irradiation (Mrad)	Atmosphere	Viable count per gram
None	Air	2.4×10^7
None	N_2	2.0×10^7
0.2	Air	1.8×10^4
0.2	N_2	1.8×10^4

Table 11. Effect of gamma radiation on CO_2 production, O_2 consumption, and softening of McIntosh apple tissues. Mg. gas per Kg. fresh weight per hour corrected to 25°C. Firmness limit value in g. with Chatillon pressure tester, needle #2 of 0.83 mm. diameter.

Date of Harvest	Control			0.02 Mrad			0.07 Mrad			0.26 Mrad		
	Firmness	CO_2	O_2	Firmness	CO_2	O_2	Firmness	CO_2	O_2	Firmness	CO_2	O_2
Aug. 22	330	82	39	319	87	36	324	81	45	230	77	48
Aug. 29	318	82	41	327	82	40	300	82	43	215	81	49
Sept. 12	303	82	41	261	79	42	279	78	46	209	86	64
Sept. 19	251	76	38	272	71	37	249	68	40	200	75	53
Sept. 26	259	84	40	255	89	44	249	88	43	194	90	52
Oct. 3	204	96	44	205	97	48	182	99	55	149	106	63
Oct. 10*	176	84	39	169	82	41	150	85	44	156	91	51
Oct. 17	128	69	28	126	67	28	111	69	38	103	71	34
Oct. 24*	126	72	30	125	71	29	106	75	38	95	80	49

* These apples were harvested on Oct. 3 and stored at 70°F. until tested.

Table 12. The effect of gamma radiation on the softening and respiratory activity of tissues from McIntosh apples following cold storage. Mg. gas per Kg. fresh weight per hour corrected to 25°C. Firmness limit value in g. with Chatillon pressure tester, needle #2 of 0.83 mm. diameter.

	Radiation dose (Mrad)	FIRMNESS AND RESPIRATORY ACTIVITY					
		At Harvest			After 7 weeks' storage		
		Firmness	CO_2	O_2	Firmness	CO_2	O_2
Pre-Climacteric	None	251	76	38	163	96	41
	0.02	272	71	37	164	99	35
	0.07	249	68	40	139	99	48
	0.26	200	75	53	163	104	61
Climacteric	None	204	96	44	166	96	44
	0.02	205	97	48	160	102	37
	0.07	182	99	55	157	105	46
	0.26	149	106	63	118	106	72
Post-climacteric	None	176	84	39	130	83	43
	0.02	169	82	41	130	86	30
	0.07	150	85	44	113	89	43
	0.26	156	91	51	112	94	65

Table 13. Loss of moisture by toluene distillation from cylinders of apple tissue irradiated at various periods of time, expressed as % fresh weight.

Duration (hours) of irradiation	Unirradiated control	0.1 Mrad	1.0 Mrad
2.4	52.3	60.2	77.2
24.0	53.6	-	74.1

Table 14. Effect of moisture content on changes caused by 0.56 Mrad gamma radiation on the pectins in the alcohol-insoluble solids (AIS) of apples.

	Total mg. anhydro-galacturonic acid per g. AIS	Percent of total galacturonic acid in fractions*			Viscosity of extracted pectins*		
		W	C	H	W	C	H
Unirradiated (Control)	360.0	26.6	13.3	60.1	17.87	15.52	10.60
Irradiated							
0% Moisture (Dry)	435.0	43.3	15.5	41.2	11.36	9.73	6.36
50% Moisture	450.4	51.6	17.1	31.3	8.69	6.55	6.13
95% Moisture	481.4	63.0	9.3	27.7	2.71	2.67	3.70

* Fractions: W = Cold water-soluble; C = Soluble in cold solution containing 0.2% Calgon and 0.8% NaCl; and H = Hot (80°C) 0.05 N HCl - soluble pectin.

** Specific viscosity/percent anhydrogalacturonic acid x 100.

Table 15. Effect of added sugars on the 0.27 Mrad gamma radiation-induced degradation of pectins in apple alcohol-insoluble solids (AIS) irradiated at various moisture contents.

	No sugar added							With sugars added*					
	Total mg. anhydro-galac-turonic acid per g. AIS	Percent of total galacturonic acid in fractions**			Viscosity of extracted pectins**			Total mg. anhydro-galac-turonic acid per g. AIS	Percent of total galacturonic acid in fractions			Viscosity of extracted pectins		
		W	C	H	W	C	H		W	C	H	W	C	H
Unirradiated (Control)	411.2	23.4	15.8	60.8	17.10	9.53	7.72	-	-	-	-	-	-	
Irradiated														
0% Moisture*** (Dry)	432.5	34.6	17.9	47.5	13.01	7.35	6.51	426.1	38.5	18.3	43.2	11.63	7.40	7.69
50% Moisture	438.6	41.7	13.1	45.2	9.77	5.00	4.27	458.5	44.1	17.9	38.0	9.68	4.67	5.58
80% Moisture	481.0	53.5	12.7	33.8	4.87	2.84	4.73	448.1	45.4	18.5	36.1	10.39	4.27	3.97
85% Moisture	449.6	49.2	26.6	24.2	4.89	2.66	4.85	502.1	41.5	28.4	30.1	7.96	3.16	3.01
90% Moisture	459.4	48.5	21.7	29.8	3.94	1.98	4.51	531.9	37.5	30.4	32.1	8.72	3.41	3.58

* Sugars added: 0.25 g. glucose, 0.25 g. sucrose, and 0.50 g. fructose with each 0.25 g. AIS sample.

** See Table 1.

*** Moisture percent calculated on weight basis including sugars.

Figure 1. Respiration rate (70°F) of McIntosh apples immediately following harvest and irradiation.

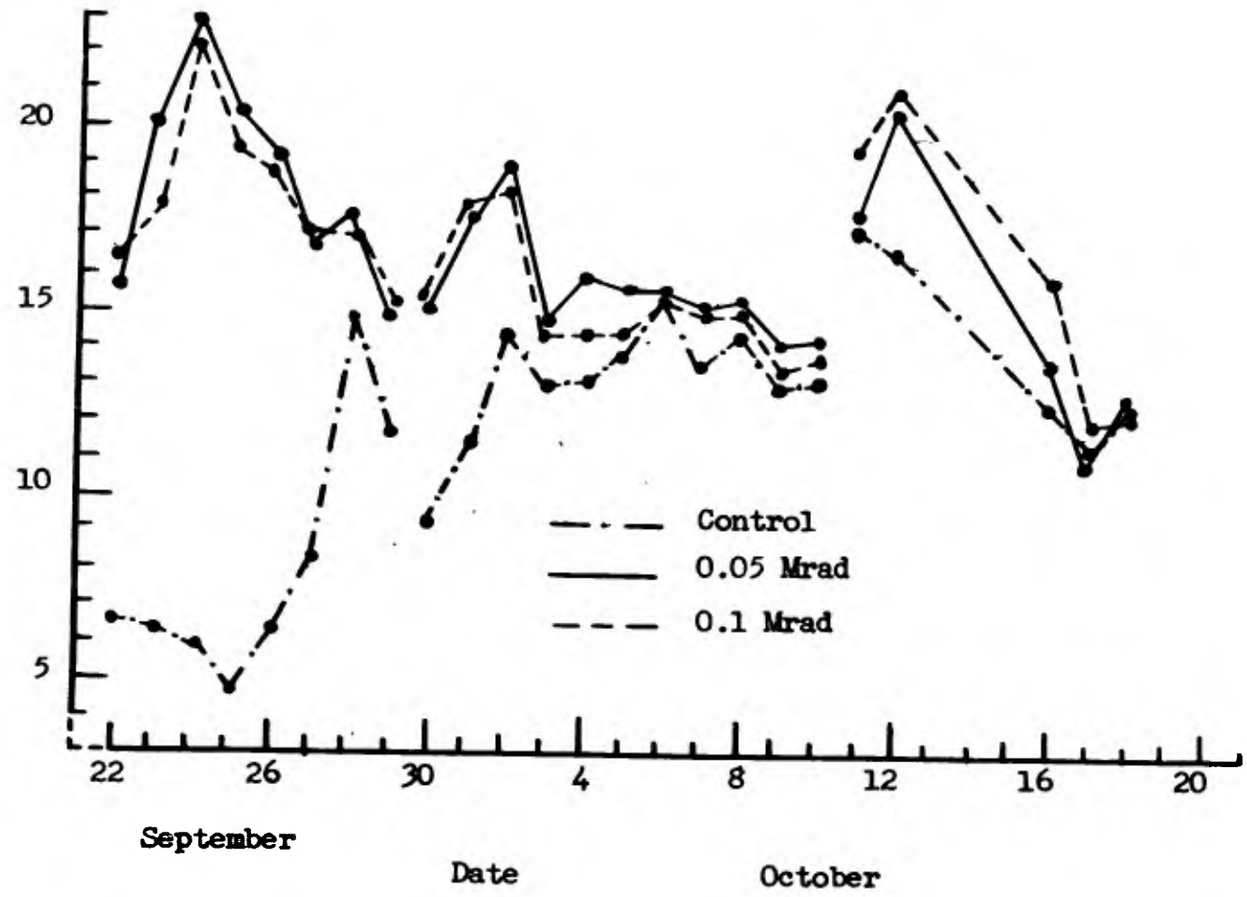


Figure 2. Respiration rate (70°F) of Rome Beauty apples immediately following harvest and irradiation.

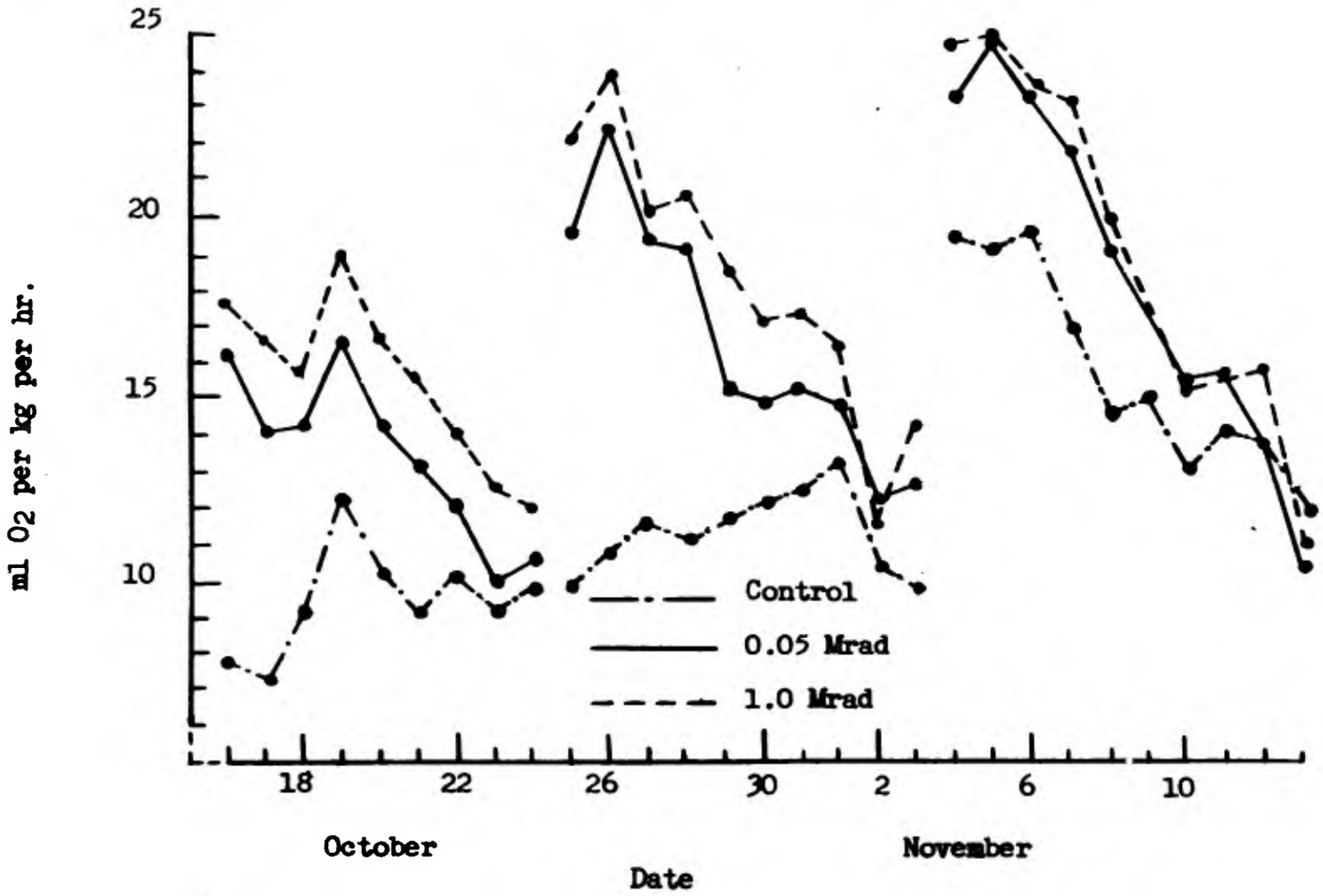


Figure 3. Evolution of carbon dioxide by lettuce leaves as brought about by a one hour irradiation period compared to that brought about by a subsequent 16.5 hour continuous irradiation, at two dose rates, expressed as percent of unirradiated.

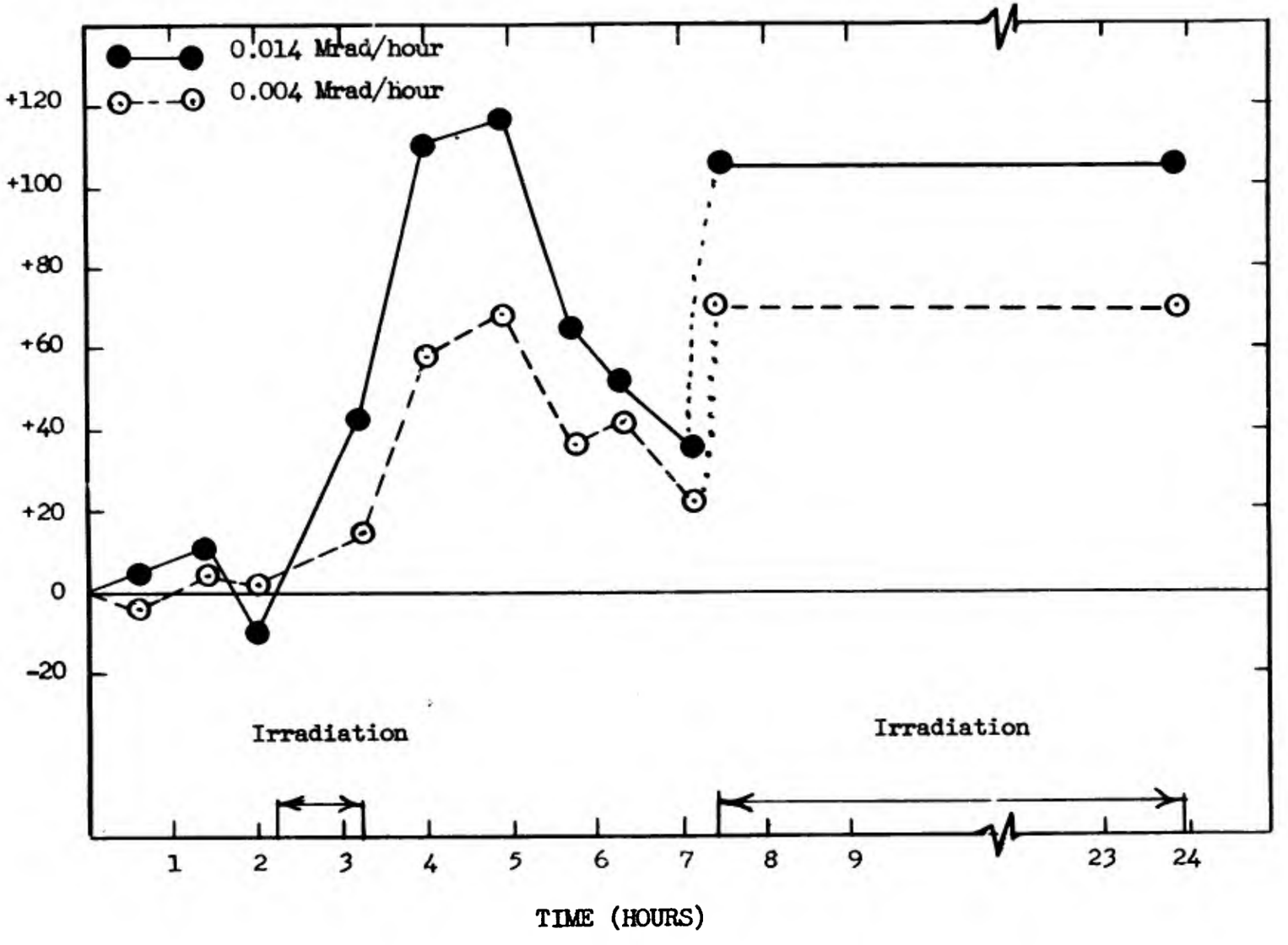


Figure 4. Evolution of carbon dioxide by lettuce leaves as brought about by successive one hour irradiation periods compared to that brought about by a subsequent 16.5 hour continuous irradiation, at two dose rates, expressed as percent of unirradiated control.

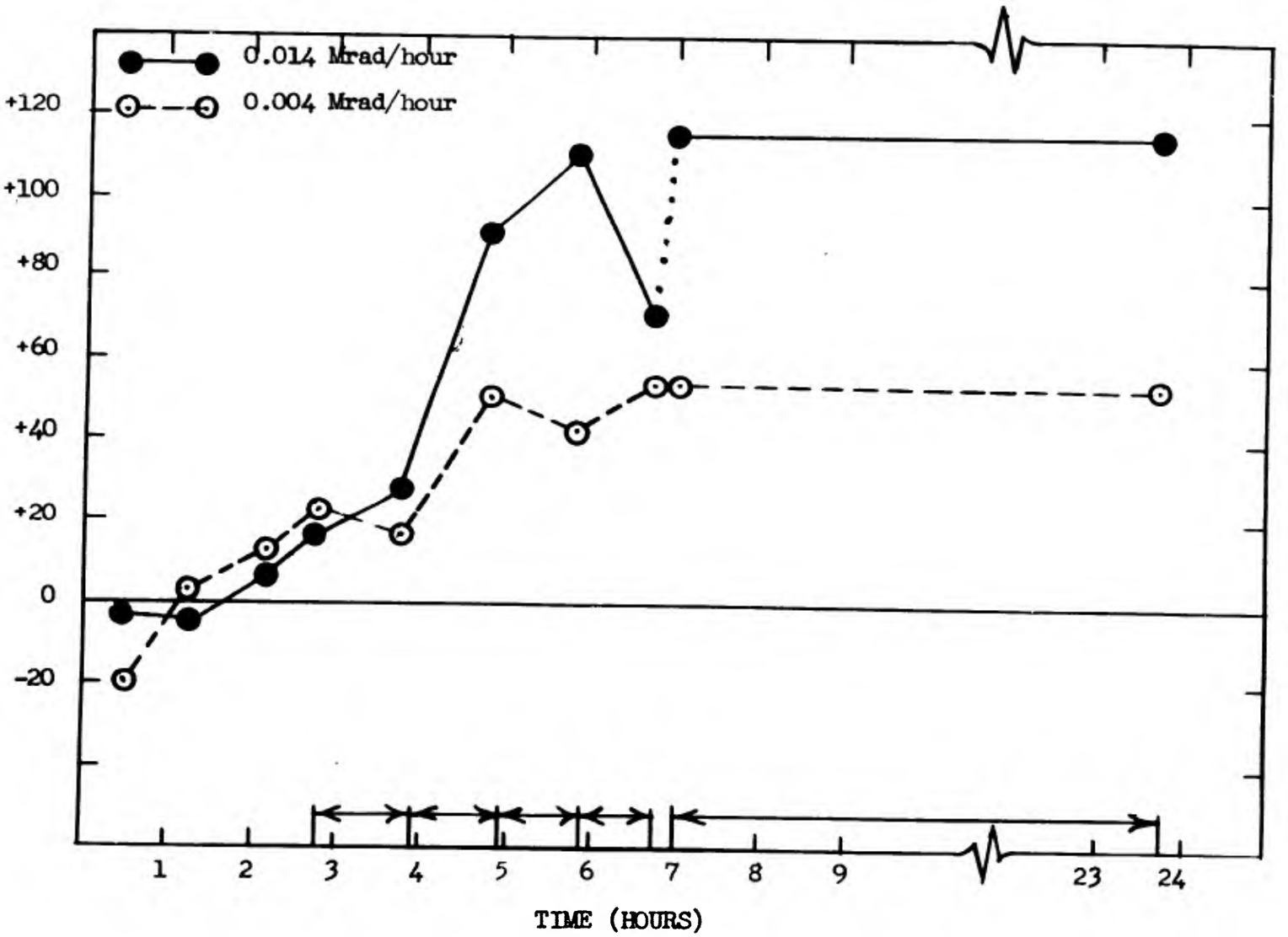


Figure 5. Loss of moisture by toluene distillation from cylinders of Rome Beauty apples which have been irradiated with various doses of gamma radiation.

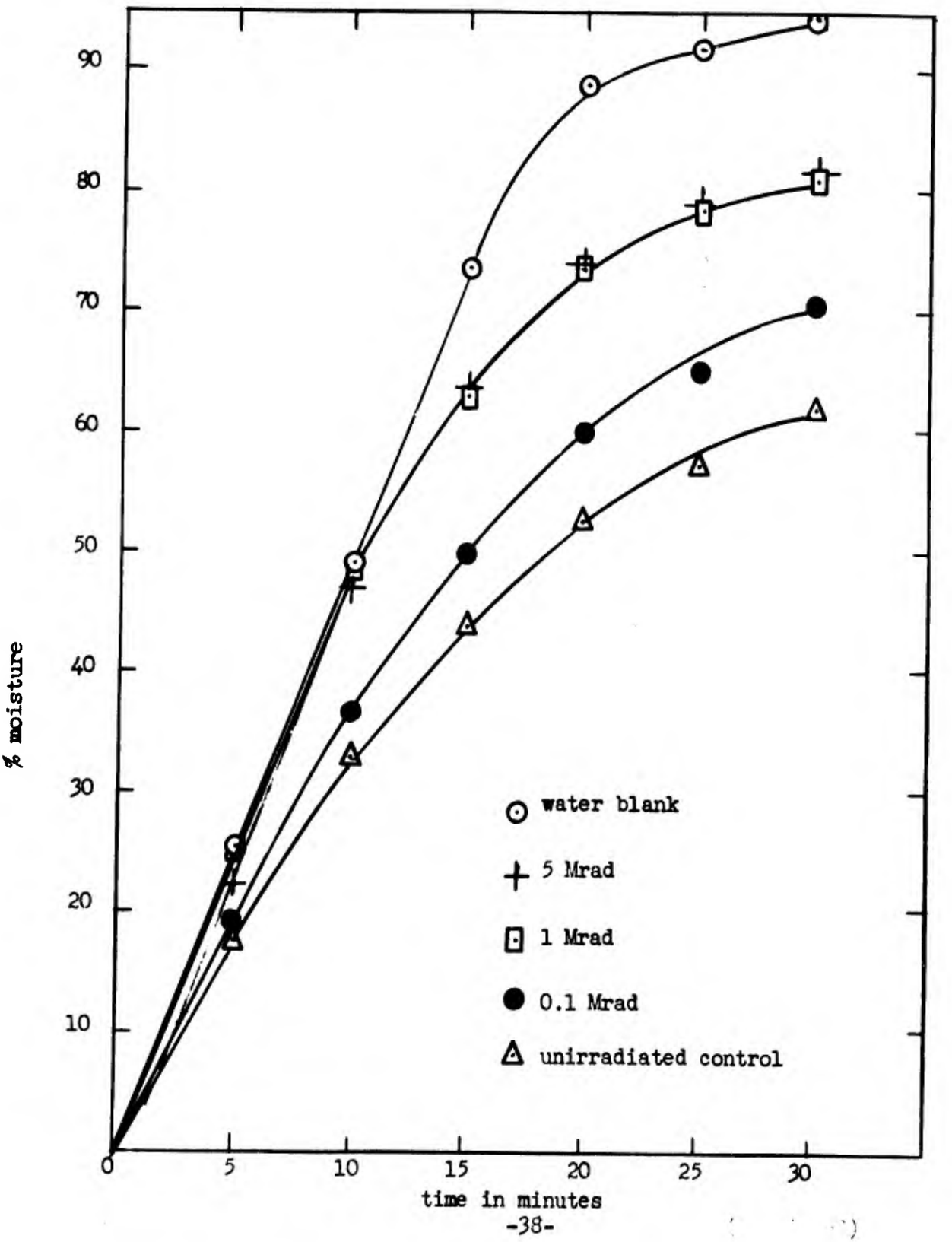


Figure 6. Dosage response expressed as percent increased rate of moisture loss from tissue cylinders at 20 minutes over that of the unirradiated control.

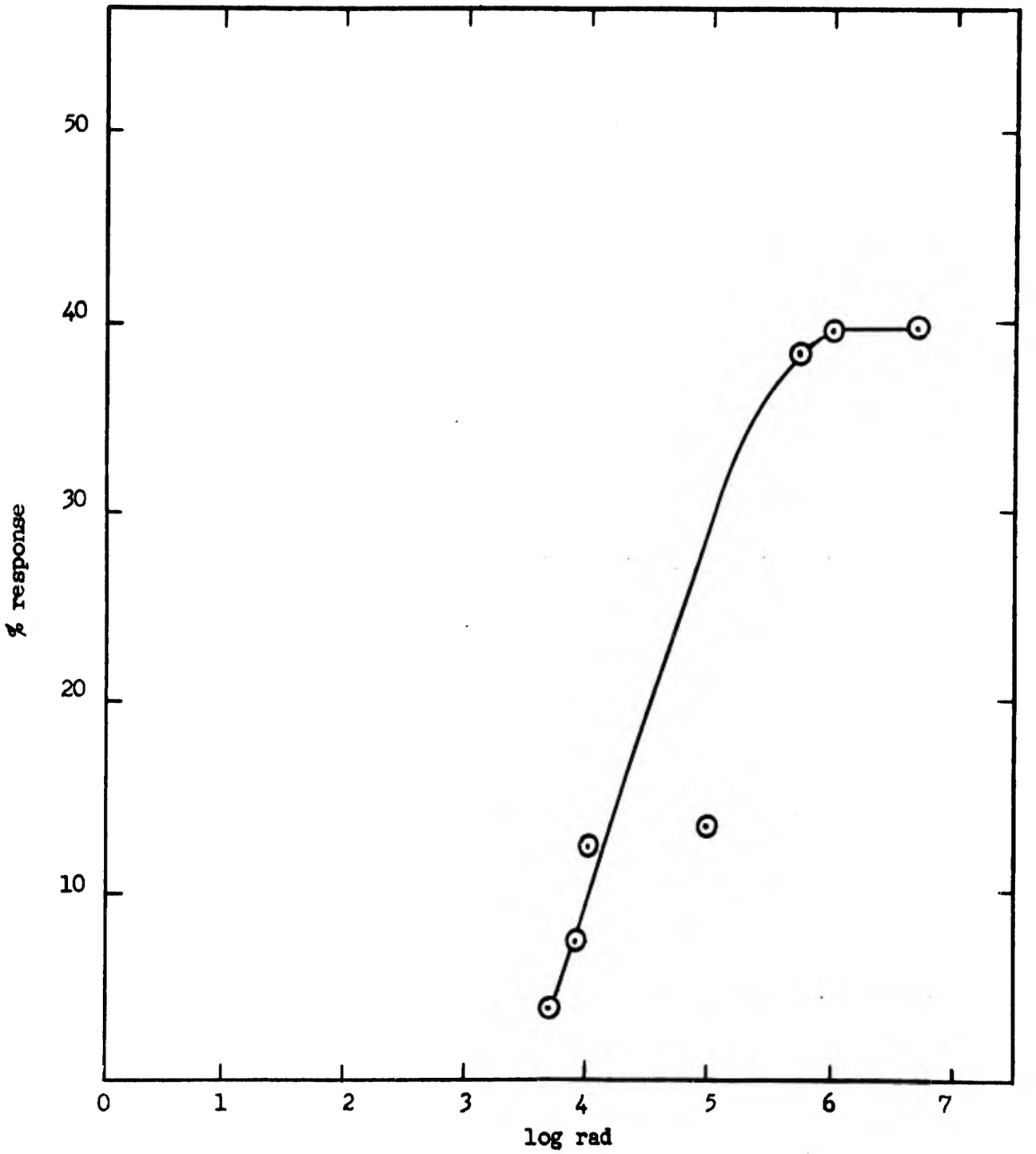
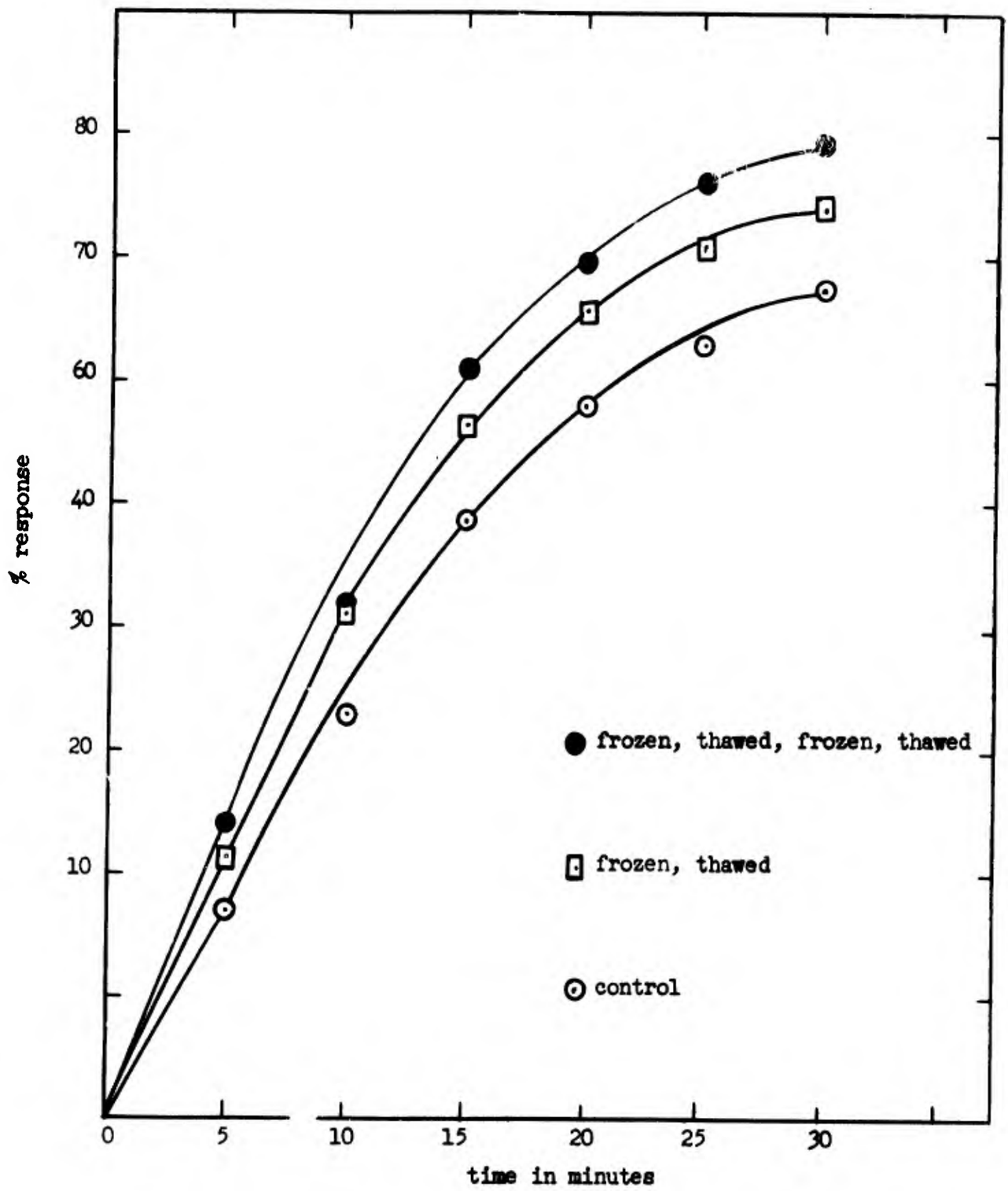


Figure 7. Loss of moisture by toluene distillation from cylinders of Rome Beauty apples which have been subjected to freezing and thawing.



Unclassified
Security Classification

DOCUMENT CONTROL DATA - R&D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1 ORIGINATING ACTIVITY (Corporate author) New York Agricultural Experiment Station, Cornell University, Geneva, New York	2a REPORT SECURITY CLASSIFICATION Unclassified 2b GROUP

3 REPORT TITLE

A STUDY OF THE RADIATION-INDUCED SOFTENING OF PLANT TISSUES

4 DESCRIPTIVE NOTES (Type of report and inclusive dates)

Final report 1 June 1960 - 31 August 1962

5 AUTHOR(S) (Last name, first name, initial)

KERTESZ, Z. I.
MASSEY, L. M., JR.

6 REPORT DATE	7a TOTAL NO. OF PAGES	7b NO. OF REFS
October 1965	40	12

8a CONTRACT OR GRANT NO DA 19-129-QM-1584 b PROJECT NO. 7-84-01-002 c d	9a ORIGINATOR'S REPORT NUMBER(S) 9b OTHER REPORT NO(S) (Any other numbers that may be assigned this report) FD-27

10 AVAILABILITY/LIMITATION NOTICES

Distribution of this document is unlimited.
Release to CFSTI is authorized

11 SUPPLEMENTARY NOTES	12 SPONSORING MILITARY ACTIVITY
	Radiation Sources Branch, Food Division, U. S. Army Natick Laboratories, Natick, Mass. 01762

13 ABSTRACT

The radiation-induced changes in a large variety of fresh fruits and vegetables were investigated, and the physiology and biochemistry of some changes, particularly softening, investigated in detail. Possible beneficial results with several commodities, particularly apples, were noted.

DD FORM 1 JAN 64 1473

Unclassified
Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Softening	8					
Plant tissues	1		9			
Fruits	1		9			
Vegetables	1		9			
Apples	1		9			
Irradiation	10					
Storage stability	4					
Physiology			8			
Biochemistry			8			
Irradiated			0			

INSTRUCTIONS

1. ORIGINATING ACTIVITY: Enter the name and address of the contractor, subcontractor, grantee, Department of Defense activity or other organization *(corporate author)* issuing the report.

2*a*. REPORT SECURITY CLASSIFICATION: Enter the overall security classification of the report. Indicate whether "Restricted Data" is included. Marking is to be in accordance with appropriate security regulations.

2*b*. GROUP: Automatic downgrading is specified in DoD Directive 5200.10 and Armed Forces Industrial Manual. Enter the group number. Also, when applicable, show that optional markings have been used for Group 3 and Group 4 as authorized.

3. REPORT TITLE: Enter the complete report title in all capital letters. Titles in all cases should be unclassified. If a meaningful title cannot be selected without classification, show title classification in all capitals in parenthesis immediately following the title.

4. DESCRIPTIVE NOTES: If appropriate, enter the type of report, e.g., interim, progress, summary, annual, or final. Give the inclusive dates when a specific reporting period is covered.

5. AUTHOR(S): Enter the name(s) of author(s) as shown on or in the report. Enter last name, first name, middle initial. If military, show rank and branch of service. The name of the principal author is an absolute minimum requirement.

6. REPORT DATE: Enter the date of the report as day, month, year; or month, year. If more than one date appears on the report, use date of publication.

7*a*. TOTAL NUMBER OF PAGES: The total page count should follow normal pagination procedures, i.e., enter the number of pages containing information.

7*b*. NUMBER OF REFERENCES: Enter the total number of references cited in the report.

8*a*. CONTRACT OR GRANT NUMBER: If appropriate, enter the applicable number of the contract or grant under which the report was written.

8*b*, 8*c*, & 8*d*. PROJECT NUMBER: Enter the appropriate military department identification, such as project number, subproject number, system numbers, task number, etc.

9*a*. ORIGINATOR'S REPORT NUMBER(S): Enter the official report number by which the document will be identified and controlled by the originating activity. This number must be unique to this report.

9*b*. OTHER REPORT NUMBER(S): If the report has been assigned any other report numbers *(either by the originator or by the sponsor)*, also enter this number(s).

10. AVAILABILITY/LIMITATION NOTICES: Enter any limitations on further dissemination of the report, other than those imposed by security classification, using standard statements such as:

(1) "Qualified requesters may obtain copies of this report from DDC."

(2) "Foreign announcement and dissemination of this report by DDC is not authorized."

(3) "U. S. Government agencies may obtain copies of this report directly from DDC. Other qualified DDC users shall request through _____."

(4) "U. S. military agencies may obtain copies of this report directly from DDC. Other qualified users shall request through _____."

(5) "All distribution of this report is controlled. Qualified DDC users shall request through _____."

If the report has been furnished to the Office of Technical Services, Department of Commerce, for sale to the public, indicate this fact and enter the price, if known.

11. SUPPLEMENTARY NOTES: Use for additional explanatory notes.

12. SPONSORING MILITARY ACTIVITY: Enter the name of the departmental project office or laboratory sponsoring *(paying for)* the research and development. Include address.

13. ABSTRACT: Enter an abstract giving a brief and factual summary of the document indicative of the report, even though it may also appear elsewhere in the body of the technical report. If additional space is required, a continuation sheet shall be attached.

It is highly desirable that the abstract of classified reports be unclassified. Each paragraph of the abstract shall end with an indication of the military security classification of the information in the paragraph, represented as *(TS)*, *(S)*, *(C)*, or *(U)*.

There is no limitation on the length of the abstract. However, the suggested length is from 150 to 225 words.

14. KEY WORDS: Key words are technically meaningful terms or short phrases that characterize a report and may be used as index entries for cataloging the report. Key words must be selected so that no security classification is required. Identifiers, such as equipment model designation, trade name, military project code name, geographic location, may be used as key words but will be followed by an indication of technical context. The assignment of links, rules, and weights is optional.