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RADIATION TECHNOLOGY IN CONJUNCTION
WITH POSTHARVEST PROCEDURES AS A
MEANS OF EXTENDING THE SHELF LIFE OF
FRUITS AND VEGETABLES

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SUMMARY OF PROGRESS

During 1964, our results clearly indicated that the physiological state of fruits at the time of irradiation may determine whether the treatment is beneficial or harmful. Two examples of this phenomenon are the tomato and the banana. At the end of the 1963 season, we felt that tomatoes were doubtful subjects for an irradiation technology because of anomalous ripening in fruits treated in the mature-green state. During 1964, fruits of more advanced stages of ripeness were used, and it was found that shelf life could be extended by 4-12 days without a major adverse effect on quality. These results are described in more detail below.

In 1962-63 we irradiated bananas at very low doses (0-50 Krad) in an attempt to repeat the work of Teas et al.¹ in which they showed marked inhibition of ripening at doses of 25 Krad. We were unable to show a consistent inhibitory effect on ripening. Based on our results with other fruits, in which we showed irradiation to be ineffective once ripening was initiated, we reevaluated the Gros Michel banana during 1964 using individual fruits which were demonstrated to be preclimacteric with respect to ripening. We had immediate, and reproducible success.

Two review articles were completed during 1964 by project personnel. These are "Food Irradiation--Physiology of Fruits as Related to Feasibility of the Technology" by E. C. Maxie and Adel Abdel-Kader, and "Ionizing Radiation for Control of Postharvest Diseases of Fruits and Vegetables" by N. F. Sommer and R. J. Fortlage. These articles have been submitted to Advances in Food Research.

From the materials covered in these review articles and from our own work, certain tentative conclusions can be drawn about irradiation as a technology with fresh fruits: 1) There is nothing

¹ Teas, H. J., D. C. Quintana, and J. O. Campos. 1962. Inhibition of banana fruit ripening by gamma radiation. Second Intern. Congr. Radiation Research, Harrogate, England, Abstracts, 179.

in the literature to indicate that wholesomeness will be a problem. Doses required to significantly affect vitamins in fruits are in excess of what the fruits can tolerate. 2) Sensitivity of fruits, not fungi, to radiation injury is the limiting factor in attempts to control rots. 3) Physiological status, particularly degree of ripeness, has a marked effect on the response of some fruits. 4) Some fruits hold little promise for a practical technology; but this is true of most fungicidal agents and should have been predicted for irradiation.

Tests with Eureka lemons held at 77°F for 15 days following irradiation to 100 and 200 Krad showed that ascorbic acid content was nutritionally significant in both lots after 15 days. Fruits subjected to 200 Krad were less attractive than control fruits but were judged as acceptable. These results may have significance in some emerging nations without refrigerated storage and transportation facilities.

Dried figs and prunes were irradiated to 0, 400, 800, and 1200 Krad and stored for 6 weeks at room temperature. An expert taste panel found both products completely acceptable. The tests were terminated after 6 weeks because of worm infestation in the unirradiated samples. Fresh prunes were irradiated to 0, 100, 200, 300, 400, and 600 Krad; dried, stored at 36°F for 5 months and evaluated by the taste panel. Irradiated samples seemed to gain more weight during cooking, and were more tender than unirradiated samples. Flavor of the irradiated samples was acceptable at all levels of irradiation.

Fresh figs irradiated to 200 Krad gave good results as judged by the taste panel after a simulated marketing sequence. Fruits subjected to 300 Krad were graded down by the panel on the basis of internal appearance and softer texture.

Shasta strawberries were irradiated to 200 Krad at pulp temperatures of 32, 50, 68, and 83°F, and evaluated for taste and texture by the taste panel. No significant differences were found between the various lots. Shear press measurements showed slightly

increasing firmness with increasing temperatures, probably because of greater water loss. It was concluded that berries could be irradiated at field temperatures without adverse effects provided that they are cooled to 32-41°F immediately after treatment.

An extensive series of experiments showed that irradiation did not increase the susceptibility of Shasta strawberries to postirradiation infections with Botrytis. This means that irradiated strawberries will not require special overwraps provided they are kept cool (32-41°F). Comparisons of 200 Krad with modified atmospheres (high CO₂ - low O₂) indicated irradiation gave better quality fruit and was superior in controlling rot.

Peaches irradiated green, ripened, and evaluated by the taste panel were judged acceptable, despite difficulties in obtaining comparable stages of ripeness in irradiated and unirradiated samples. The irradiated samples ripened more rapidly than did unirradiated ones.

An extensive investigation employing electron spin resonance (EPR) showed no long-lived organic free radicals in irradiated strawberries. The Mn⁺⁺ radical was found, but is believed to have no significance in the wholesomeness of the irradiated berries.

Studies of gas exchange in Bartlett pears indicated that internal oxygen could be removed from the fruits by flushing with nitrogen in 18-40 minutes. Reentry of oxygen from air occurred at a similar rate. Irradiation did not significantly affect the rate of gas exchange.

Attempts to ripen Bartlett pears subjected to 300 Krad with 2,4-Dichlorophenoxyacetic acid and with ethylene gas were unsuccessful.

A detailed study of ozone production in a static system of air in the Mark II Irradiator showed 7.4 ppm at 50 Krad, increasing to 58.4 ppm at 1000 Krad, with G values of 6.2 at 50 Krad and 2.5 at 1000 Krad. In a system with 6,500 ml/min of air passing through the chamber the G values were relatively constant at 11.0-11.3 and the ozone concentration varied from 1.68 to 1.74 over the 50 to 1000 Krad dose range.

Most of the results with tomatoes (mature-green and breakers), reported last year, were negative with respect to using gamma irradiation treatments for extending the shelf life of the fruits. It was concluded that only under conditions where decay losses would otherwise be excessive would irradiation treatment be valuable.

Fewer adverse effects of irradiation are found with tomato fruits irradiated at the pink or table-ripe stage. An extension of shelf life for a period of 4-12 days can be obtained by doses between 200 and 350 Krad. The magnitude of shelf-life extension depends upon the condition of fruits at harvest, handling procedures, postirradiation attack by decay organisms, and storage temperature. The following two factors may limit the use of such a procedure:

1. Increased softening of fruits may affect their susceptibility to transit injury, and perhaps make them more easily infected. More work is planned to clarify this point during 1965.
2. Effect on flavor. Taste-panel evaluation of irradiated, table-ripe tomatoes (up to 300 Krad) showed no significant effect on flavor for the first week after treatment. In the second week, fruits irradiated at doses between 200 and 400 Krad were scored lower in flavor than control fruits. The effect of radiation was not consistent from fruit to fruit. Some samples were considered to be as good as the controls, while others had a distinct off flavor. It is possible that if this evaluation were done on some basis other than paired comparisons, no important differences in flavor would be detected for ripe fruits irradiated at doses up to 300 Krad. Further taste evaluations will be done to check this possibility.

In general, there is some promise for the use of gamma irradiation to extend the shelf life of pink and table-ripe tomatoes.

It is hoped that a clear answer to this question will be achieved by the end of 1965.

Gamma irradiation causes an increase in respiratory activity of tomatoes. The stimulation can be detected immediately after treatment and is dose related. The magnitude and duration of the stimulation are least in the ripest fruits. Radiation doses up to 500 Krad stimulate ethylene production; at higher doses, ethylene production is reduced. Ripening behavior can be correlated with the effect on ethylene production.

Retardation of ripening of mature-green fruits by doses of 300 Krad or more can be reversed by using ethylene treatment. However, the required dose of ethylene and duration of application are greater than those needed to hasten ripening of control fruits.

Although gamma irradiation causes a reduction in ascorbic acid content of tomatoes, the losses are not nutritionally significant. The amount of ascorbic acid in irradiated table-ripe fruits is greater than in control fruits harvested as mature-green and subsequently ripened in storage.

Combined treatments with radiation are of some importance. Chilling injury is additive to radiation damage, and thus temperature control is a very important factor in tomato transit and storage. There are some promising results for the use of some growth regulators, especially indoleacetic acid in combination with radiation for better extension of shelf life.

When unripe "Gros Michel" bananas are exposed to gamma radiation at doses of 35 Krad the ripening process is inhibited by 10-13 days. This effect is observed when experiments are done with single bananas or with whole hands.

This inhibition effect can also be observed when only one-half of an individual fruit is subjected to gamma radiation. The visual signs of senescence are first observed in the unirradiated half, although the whole fruit turns yellow upon ripening. Texture measurements confirmed this observation, since more pounds of force are required to crush the irradiated half. Further studies with bananas cut in half confirmed the inhibition effect of gamma

radiation; when bananas are cut in half the flower end ripens first; gamma radiation at doses of 35 Krad can reverse the ripening sequence in fruit halves and retard the ripening process of either half by 13-18 days.

Taste-panel evaluations on irradiated bananas showed that fruits irradiated to 35 and 50 Krad could be ripened to as good quality as unirradiated fruits under commercial ethylene treatments.

RADIATION IN THE POST-HARVEST PATHOLOGY OF FRUITS AND VEGETABLES*

The radiation sensitivity of Botrytis cinerea Pers. ex Fr., cause of the gray mold disease of strawberries and certain other fruits and vegetables, has now been explored in depth. Data on the inactivation of conidia, young mycelium, old mycelium, and sclerotia have been obtained. When comparing the sensitivity of these structures, equal fungus population sizes were used. Dry weight, as well as protein and DNA contents, provided a basis for equating fungus samples. Where possible, sensitivity was also established on a per cell basis. By use of an end-point technique, the doses required to inactivate the populations were determined.

Considerable time was expended in studies on this one very important species, because of the need to develop or adapt suitable techniques. It can be confidently predicted that similar detailed studies on other important pathogens can be pursued at an accelerated rate using the methods here found satisfactory.

It is believed that studies of the type just completed for B. cinerea are of extreme importance because they provide the needed basis for predicting the effectiveness of radiation under field

* Studies of the use of radiation for post-harvest disease control on this project are closely coordinated with the physiological and cytological studies of pathogens supported by the Division of Biology and Medicine, Contract AT(11-1)-34, Project 73. Consequently, it is sometimes necessary, for completeness, to discuss the results obtained in the reports for both projects.

conditions. They also explain why different investigators have reported widely divergent "lethal" doses for the same species of fungus (i.e. 0.95 Krad to 1.0 Mrad for *B. cinerea*). Such wide differences in sensitivity can evidently readily be obtained only if fungus population sizes differ widely. Moreover, use of the term "lethal dose" in fruit or vegetable irradiation is an unfortunate selection. Within the range of doses fruits can withstand, the probability of inactivating a fungus colony is determined as much by colony size as by the dose. Thus, a different "lethal dose" would be required for every lesion size.

Commercially harvested berries from the same farm and picked by the same people were irradiated in a series of tests during the 1964 harvest season. Estimates of the sizes of the mycelial lesions present in the harvested berries were determined on the basis of "spore equivalents," with the latter term referring to the number of spores inactivated by the same dose when irradiated in vivo. The proportion of berries containing lesions inactivatable by 175 Krad and 250 Krad and the proportion not inactivated was determined. In addition, the extent of the delay in lesion development among lesions not inactivated was noted. Under the conditions studied, about 5 to 7% of the berries included in the harvest by skilled pickers may contain lesions not inactivatable by the 175 Krad and 250 Krad doses. Development of these lesions is delayed, however, so that contact infection to adjacent berries does not occur in ten days if berries are refrigerated. At the end of this period, only berries which contained lesions at harvest have become noticeably decayed.

Considerable attention has been devoted to physiological or environmental conditions or factors which might affect the irradiation dose required for lesion inactivation. Among these has been metabolic repair of otherwise lethal irradiation injuries. A striking repair can be demonstrated with Rhizopus stolonifer, R. oryzae, and R. arrhizus under certain postirradiation environmental conditions. Tests with Botrytis cinerea,

Monilinia fructicola, Cladosporium herbarum, Penicillium expansum (both haploid and diploid strains), and Penicillium digitatum demonstrated only a very modest recovery under the conditions used for Rhizopus sp. At this time, it is not believed that metabolic repair will prove a problem in connection with the commercial irradiation of strawberries.

Under present transit conditions, carbon dioxide-enriched atmospheres are frequently provided in refrigerated rail cars, particularly when loaded with strawberries or sweet cherries. The carbon dioxide serves to suppress fungus growth during transit. Tests were made to determine if a carbon dioxide atmosphere following irradiation might provide a stress which would enhance lesion inactivation. However, results have thus far been negative.

Tests were made to study the effect on lesion inactivation of cold vs. warm temperatures following irradiation. Cold immediately following irradiation evidently did provide a stress which enhanced fungus inactivation. However, differences were relatively small.

THE INTERACTION OF GAMMA IRRADIATION, SEALED PACKAGES, AND
CONTROLLED ATMOSPHERE ON INCIDENCE OF BOTRYTIS ROT IN STRAWBERRIES

Edo Chalutz, E. C. Maxie, and N. F. Sommer

INTRODUCTION

Strawberries offer more promise for application of gamma irradiation as a protective technology than does any other fruit. Maxie et al. (5) have presented evidence that the number of rotten fruits can be stabilized at approximately the level of field infection during a normal marketing sequence. They specified quick cooling, continuous refrigeration at 32-41°F., prompt irradiation, and a dose of 200 kilorads (Krad) as requisites for success. Johnson et al. (3) studied the effects of doses of gamma rays as high as 400 Krad on the quality of 'Shasta' and 'Lassen' varieties of strawberries. They concluded that doses of 200 Krad could be used without serious adverse effects on the acceptability of the fruit.

The principal rot of strawberries grown in California is Botrytis cinerea Pers. Powelson (7) made a detailed study of the pathogen and reported evidence indicating active mycelia inside the tissues of berries at harvest. Sommer et al. (8) and Beraha et al. (1) have reported that gamma rays can penetrate the tissues of the strawberry and inhibit further development of lesions for several days. The fungal cells are not inactivated permanently, particularly in large lesions.

One problem that has not been investigated is the susceptibility of irradiated strawberries to postirradiation infection. If such infection is shown to be important, it may be necessary to use microbe-proof packages to protect the irradiated fruit. However, such a procedure could be economically infeasible because of increased packaging and handling costs.

If air-tight film overwraps or packages are used, modification of the atmosphere inside the package might affect fruit quality

adversely. Recently, the effects of artificially generated atmospheres, high in carbon dioxide and low in oxygen, have been studied on a number of fruit species. If such a modification proves adequate as a means of preventing post-harvest decay in strawberries, it might preclude the application of irradiation as a feasible technology with this species.

The objectives of this investigation were to evaluate:

1) postirradiation susceptibility of 'Shasta' strawberries to Botrytis; 2) the effects of modified atmospheres on quality of both irradiated and unirradiated strawberries kept in microbe-proof, air-tight packages; and 3) the comparative effectiveness of irradiation and artificially generated atmospheres as protective measures against post-harvest decay.

MATERIALS AND METHODS

Freshly harvested 'Shasta' strawberries were transported from Salinas to Davis, California, in a refrigerated box utilizing the "forced-air" principle of cooling. The fruit was cooled to 37°F in about 1 1/2 hours. After reaching Davis, the fruit was kept 12-24 hours at 32°F, treated, and then stored at 41°F at a relative humidity of about 93%.

For studies on susceptibility to infection following irradiation, individual berries were selected for uniformity of size and for degree of ripeness. Duplicate lots of 20 fruits each were used in each treatment. Treatments were 0, 100, 200, 300, or 400 Krad just prior to inoculation. For inoculation a uniform hole was made in the flesh of each fruit with a No. 1 cork borer. Into this wound 0.1 ml of a suspension containing 20 spores of Botrytis was pipetted. Control fruits were treated similarly except no spores were introduced. Following inoculation, each fruit was placed in a paper cup and immediately covered tightly with cellulose acetate film to avoid contamination by foreign spores. When cups of fruits were placed in jars they were left uncovered. The fruits were examined every day, and those that had clearly defined decay lesions were discarded. The samples were kept at 41°F until all fruits had

rotted or until 19 days had passed. The results are expressed as cumulative fruit lost.

Polyethylene bags (10" x 8" x 30") 1.5 mil (0.00015") in thickness were used in packaging studies. Half-crates of strawberries first were irradiated, then heat sealed in individual bags. A piece of 8 mm glass tubing was sealed into the wall of each bag. Samples of the internal atmosphere were taken with a syringe through a rubber serum cup fitted over the external end of the glass tubing. The samples were analyzed for CO₂, O₂, and N₂, with an Aerograph A-90-P (dual-detector thermal conductivity) gas chromatograph according to the method of Luh and Chaudry (4).

In another experiment holes 1/8" in diameter were made in the polyethylene bags after they were sealed. The holes comprised 40-50% of the upper surface of a bag.

Controlled atmospheres were produced by mixing O₂, CO₂, and N₂ using flowmeters developed by Claypool and Keefer (2). The precise percentage of each of the three gases was determined with the gas chromatograph. Strawberries in pint baskets were placed into 2-gallon jars, 1 kg per jar.

When evaluating results, the berries were divided into three classes: sound, soft, and decayed. Those in each class were weighed and the results calculated as per cent of the total.

Gamma irradiation was done using the Mark II Food Irradiator at a dose rate of approximately 200 Krad per hour. The fruit was placed in the center chamber of the irradiator at an air temperature of about 41°F. Aeration was maintained continuously at a rate of 6,500 ml per minute to avoid modification of the atmosphere during irradiation.

RESULTS

Table 1 compares the condition of irradiated and unirradiated 'Shasta' strawberries in three types of packages. The percentage of sound berries increased with increased dose of gamma rays in each type of package. With the exception of the unirradiated berries and berries subjected to 200 Krad in the vented polyethylene

Table 1. Effect of packaging in combination with irradiation on the condition of 'Shasta' strawberries in simulated transit and storage at 41°F.*

Treatment**	Condition of fruit (% of total)		
	Sound	Soft	Decayed
<u>Open crate</u>			
0 Krad	11.3	25.9	62.9
100 Krad	28.5	37.1	34.5
200 Krad	71.6	19.7	8.6
<u>Vented bag</u>			
0 Krad	7.3	21.8	70.8
100 Krad	32.9	25.4	41.7
200 Krad	65.6	23.4	11.0
<u>Sealed bag</u>			
0 Krad	61.3	32.2	6.5
100 Krad	71.4	28.6	0.0
200 Krad	83.3	16.7	0.0

* Fruit subjected to 0 Krad stored 16 days; irradiated fruit stored 17 days.

** 3 half-crates treatment.

bags, the order of percentage of sound berries was: sealed polyethylene > vented polyethylene > open crates. In the open crates, berries treated with 100 Krad had a higher percentage of soft fruits than did unirradiated berries. However, a dose of 200 Krad resulted in a slightly lower percentage of soft berries than was present in the unirradiated fruits. In the vented polyethylene bags, the order of percentage of soft berries was 100 Krad > 200 Krad. In each type of package the percentage of decayed fruits decreased with increasing doses of gamma rays. The percentage of decayed fruits in the vented polyethylene bags was somewhat higher at each level of irradiation than it was in the open crates. In the sealed bags, decayed fruits were present only in the unirradiated treatments.

The marked reduction in the percentage of decayed fruits among unirradiated berries in sealed polyethylene bags indicated a modification of the atmosphere inside the packages. Figure 1 shows the concentrations of CO_2 and O_2 in the packages. The percentage of CO_2 increased in all packages during the 96 hours at 41°F. Packages containing fruits subjected to 200 Krad contained slightly more CO_2 than did packages containing unirradiated fruits. When the packages were transferred from 41° to 68°F, the carbon dioxide concentrations increased markedly.

The percentage of O_2 in the atmosphere in all packages decreased during the 96 hours at 41°F. Packages containing irradiated fruits contained somewhat less oxygen (1.5 to 2%) than did packages containing unirradiated fruits (about 7%). When the packages were transferred from 41° to 68°F, the oxygen concentrations decreased further. However, a sharp decrease occurred only in the packages containing unirradiated fruits; these had only 1.3% oxygen after 24 hours.

Table 2 compares condition of berries in open polyethylene bags with that of berries in sealed bags after 96 hours at 41°F followed by 24 hours at 68°F. The percentage of soft berries in unirradiated fruit was higher in open bags than in sealed bags. Irradiation decreased the percentage of soft fruit in open bags,

Figure 1. Changes in composition of atmosphere inside sealed, polyethylene packages of strawberries.

Table 2. Condition of irradiated 'Shasta' strawberries in open crates and in crates in sealed polyethylene bags after storage.*

Treatment**	Condition of fruit (% of total)		
	Sound	Soft	Decayed
<u>Open bags</u>			
0 Krad	71.8	24.8	3.3
100 Krad	79.3	19.1	1.6
200 Krad	79.1	20.4	0.5
<u>Sealed bags</u>			
0 Krad	90.4	8.4	1.2
100 Krad	84.4	15.4	0.2
200 Krad	83.6	16.4	0.1

* Berries stored at 41°F for 96 hours, then transferred to 68°F for 24 hours and evaluated.

** Half-crates treatment.

with 100 Krad being slightly better than the other treatments. In sealed bags the percentage of soft berries was higher in the irradiated fruits than in the unirradiated ones, with 100 Krad causing slightly less softening than 200 Krad. The percentage of decayed fruit was low in all treatments. This probably was due to unusually low field infection in the berries used in these experiments. Slightly less rot was noted in the irradiated samples in both open and sealed packages, with 100 Krad being slightly less effective than 200 Krad. The percentage of sound fruits in sealed bags was higher than that in open bags at all levels of irradiation. The percentage of sound fruits was higher when the berries in open bags were subjected to gamma rays. In the sealed bags irradiation did not increase the percentage of sound berries.

Increasing the concentration of CO_2 from about 0.03% (lot I) to about 10% (lot V), and decreasing the concentration of O_2 from about 21% (lot I) to about 5% (lot V), increased the percentage of sound fruits in the unirradiated treatments from 0.82 to 60.5 (Table 3). The percentage of decayed fruits decreased accordingly. When the berries were subjected to 200 Krad of gamma irradiation, the percentage of sound fruits in all combinations of CO_2 and O_2 increased, reaching a maximum of about 81% in lot V. The percentage of decayed fruits among the irradiated berries was 11% in air (lot I); it decreased to zero in the lots having about 5% O_2 and 5 or 10% CO_2 in their atmospheres (lots IV and V).

Figure 2 shows the postirradiation susceptibility of 'Shasta' strawberries to infection following inoculation with B. cinerea Pers. These results may be compared with data obtained from irradiated, noninoculated control fruits (Figure 3). All 20 inoculated unirradiated berries had rotted by the 16th day of storage. The rate of rot development in the irradiated fruits was slightly slower. In the control, the 20 unirradiated berries and the 20 berries subjected to 100 Krad had rotted by the 18th day of storage. Larger doses of gamma rays delayed rot development; after 19 days in storage only 16, 9, and 4 berries had rotted after treatment with 200, 300, and 400 Krad, respectively.

Table 3. Effect of controlled atmospheres alone, or in combination with gamma rays, on condition of 'Shasta' strawberries.*

Lot No.	Atmospheric Composition	Dose in Krad	Condition of fruit (% of total)		
			% of fruit in each class		
			Sound	Soft	Decayed
I	CO ₂ : 0.035	0	0.8	21.8	77.3
	O ₂ : 21.53	200	40.5	48.5	11.0
II	CO ₂ : 2.80	0	8.4	34.5	57.1
	O ₂ : 3.26	200	74.2	25.1	0.7
III	CO ₂ : 2.84	0	5.2	59.6	35.2
	O ₂ : 5.43	200	69.6	25.5	4.9
IV	CO ₂ : 5.46	0	22.7	52.0	25.3
	O ₂ : 5.29	200	83.3	16.7	0.0
V	CO ₂ : 10.46	0	60.5	33.4	6.1
	O ₂ : 4.73	200	80.9	19.1	0.0

* Fruit stored for 16 days at 41°F.

Figure 2. Postirradiation susceptibility of inoculated 'Shasta' strawberries to infection by Botrytis cinerea. Each fruit was inoculated with 20 spores following irradiation at doses indicated.

Figure 3. Rot development in noninoculated, irradiated 'Shasta' strawberries. Compare to Figure 2.

Figure 4 depicts the susceptibility of 'Shasta' strawberries to infection by B. cinerea Pers. after each fruit had been inoculated with 1020 spores following irradiation at the doses indicated. All 20 berries in each treatment had rotted after 12-13 days in storage. Only small differences in rate of decay of fruits were noted among the various treatments. In the control (Figure 5) all 20 unirradiated berries had rotted after 17 days storage. After 19 days storage, only 16, 9, and 8 had rotted following doses of 200, 300, and 400 Krad, respectively.

The effect of various levels of CO₂ and O₂ on development of lesions by B. cinerea in irradiated strawberries was pronounced. Inoculated berries developed more rot than noninoculated berries in every (CA) treatment. This was expected, since both control and inoculated fruits were irradiated with 200 Krad prior to use in this experiment (Table 4). The degree of rot development decreased when CO₂ levels were increased and O₂ levels decreased. An atmosphere containing about 3% CO₂ and 3.25% O₂ was effective in delaying rot development in the control fruit, but only slightly decreased the development of rot in inoculated berries.

DISCUSSION

The data presented here are in agreement with those of Beraha et al. (1), Maxie et al. (5), and Nelson et al. (6). Gamma irradiation of 'Shasta' strawberries markedly delayed development of lesions following field infections with Botrytis cinerea Pers. The percentage of sound berries in fruit stored in open crates or in vented polyethylene bags and subjected to 200 Krad was 7-8 times greater than in unirradiated fruit. In sealed polyethylene bags the magnitude of the difference in favor of irradiated fruits was much less. This can be attributed to the modified atmosphere inside the bags. High concentrations of CO₂ and low concentrations of O₂ are known to inhibit rot development. In general, gamma irradiation at 200 Krad is more effective in maintaining berry condition than is a modified atmosphere (Table 3). The relatively low percentage of sound fruit and the high percentage of soft

Figure 4. Postirradiation susceptibility of inoculated 'Shasta' strawberries to infection by *Botrytis cinerea*. Each fruit was inoculated with 1020 spores following irradiation at doses indicated.

Figure 5. Rot development in noninoculated, irradiated 'Shasta' strawberries. Compare to Figure 4.

Table 4. Effect of controlled atmospheres on development of B. cinerea lesions in 'Shasta' strawberries after 16 days storage at 41°F.

Lot No.	Atmospheric Composition	Treatment	Number of fruit in each grade*					
			0	1	2	3	4	5
I	CO ₂ : 0.035	Control**	14	4	2	-	-	-
	O ₂ : 21.53	Inoculated***	-	-	1	2	5	12
II	CO ₂ : 2.80	Control	18	2	-	-	-	-
	O ₂ : 3.26	Inoculated	-	-	8	9	3	-
III	CO ₂ : 2.84	Control	18	2	-	-	-	-
	O ₂ : 5.43	Inoculated	-	4	12	4	-	-
IV	CO ₂ : 5.46	Control	20	-	-	-	-	-
	O ₂ : 5.29	Inoculated	2	16	2	-	-	-
V	CO ₂ : 10.46	Control	20	-	-	-	-	-
	O ₂ : 4.79	Inoculated	17	3	-	-	-	-

* Berries were graded 0-5 according to amount of decay.

** Field infection only.

*** Each fruit inoculated with 1020 spores of B. cinerea.

berries in the lot subjected to 200 Krad and held under normal atmospheric conditions (lot II) might reflect a high level of field infection. Normally the effectiveness of 200 Krad of gamma irradiation is more pronounced (Tables 1, 2). Inhibition of rot development was always greater when irradiation and a modified atmosphere were used jointly. However, the irradiation treatment seems to contribute the major protective action. Fruit kept best when irradiated berries were stored in about 5% CO₂ and about 5% O₂ (Table 3, lot IV). Berries subjected to 200 Krad, then stored in sealed polyethylene bags also kept well. In this instance, after 16 days storage the percentage of CO₂ inside the bags exceeded 5% and the concentration of O₂ was less than 5% (Figure 1). Even though the amount of field infection might have been different in the berries in these two experiments, these findings strengthen the view that irradiation was more effective than modified atmosphere in controlling development of lesions.

The apparent delay in development of decay in strawberries subjected to irradiation, then inoculated with B. cinerea spores, (Figure 2) probably reflects delayed lesion development from field infections. The unirradiated berries contained active spores which, when supplemented by inoculation, caused earlier decay of these berries. This concept is supported by the data in Figure 3, which indicated that development of rot in noninoculated fruit was progressively delayed with increasing dose. When 1020 spores were used per inoculation, the development of field infections was masked in the unirradiated berries (Figure 4). That these berries contained active field infections at the time of irradiation is shown in Figure 5. Here again, as the dose increased there was a progressive delay in development of lesions.

The postirradiation susceptibility of 'Shasta' strawberries to infection by B. cinerea was not affected by the doses used in this experiment, even though the highest dose (400 Krad) was well above the threshold dose for severe injury to the fruit (5). This is an important consideration in the application of radiation technology to strawberries and other fruits. If irradiation

increased the susceptibility of the fruits to infection, it might be necessary to use spore-proof packages to protect the fruit. Our studies indicated no need for such packages (Figures 2, 4). Air-tight packaging does contribute a relatively small degree to improving the condition of irradiated fruit. However, this contribution may be attributable to the modification of the atmosphere inside the package.

SUMMARY

The comparative effectiveness of gamma irradiation and controlled atmospheres as protective measures against post-harvest decay in 'Shasta' strawberries was studied. The irradiation treatments were more effective than were modified atmospheres in controlling development of lesions of B. cinerea. Inhibition of rot development was greater when berries were subjected both to irradiation and to a modified atmosphere.

Modified atmospheres inside sealed polyethylene bags had a marked effect on delaying rot development in unirradiated strawberries, but contributed little additional protection to fruit subjected to 200 Krad of gamma irradiation.

Our studies also indicated that irradiation treatments do not increase the susceptibility of strawberries to infection by B. cinerea. Therefore, there is no need for microbe-proof, air-tight packages to protect irradiated strawberries from reinfection during commercial storage, provided that berry temperatures are maintained at 32 to 41°F.

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GAS EXCHANGE IN 'BARTLETT' PEARS IN RELATION TO GAMMA IRRADIATION

Joseph Bussel and E. C. Maxie

INTRODUCTION

The internal atmosphere of fruit is of considerable physiological significance. Analysis of its composition may reveal responses to external stimuli. Fruits contain sizeable volumes of gases in their inter- and intracellular spaces (8, 17). In mature but unripe fruits, oxygen makes up about 20% of the gas (17, 22). Gamma radiation produces ozone from oxygen in the air (14). Ozone is highly reactive, particularly with the classes of compounds found in fruits and thus may cause much of the injury attributable to irradiation (1). Fruits offer a unique system for comparing the primary effects of radiation and the secondary effects (ozone) on a living organism.

Some fruits are injured severely by gamma radiation at doses required to inhibit rot significantly. The most serious effect of irradiation is on fruit texture (16). Severity of the injury, however, can be reduced by irradiating the fruit under anaerobic conditions (17). Unfortunately, this technique increases resistance of the pathogens, thus necessitating a higher dose for their control. This, in turn, offsets the benefits to the fruit of anoxic irradiation. Irradiation can be limited to the surface of fruit by the use of an electron beam. However, this procedure is not practical at present because of the additional handling necessary, and because of the mechanical injury received by the individual fruits as they are rotated under the beam to insure uniform coverage. Another possible means of reducing radiation injury to the fruit without protecting the pathogens is to remove the oxygen from the internal atmosphere of the fruit, then move the fruit into air at the moment of irradiation. Hopefully, the fungi being near the surface of the fruit, would be in oxygen

approaching the concentration in air while the internal atmosphere of the fruit would still be at a low level. Information is needed on the rate of gas exchange in fruits to evaluate the potential of this approach.

MATERIALS AND METHODS

Freshly harvested 'Bartlett' pears were sorted for uniformity of color, size, weight, and quality. The fruits were stored at 32°F and a relative humidity of 93% until used.

When needed, samples containing 15 fruits each were transferred to 68°F or 86°F; 18 hours were allowed for the fruit to reach temperature equilibrium before treatments were made. Small, wide-mouthed pint jars containing individual fruits were connected to flowmeters (6) and flushed with air saturated with water vapor, at a rate of 200 ml per minute. To deplete the oxygen from the internal atmosphere of the fruits, nitrogen was flushed through the jars at a rate of 250 ml per minute for varying periods of time. The procedure was reversed when measuring the reentry of oxygen into the tissues.

The internal atmosphere of fruits was extracted by a modification of the method of Freebairn (9). The jar, with the flushing gas passing through it, was immersed in a saturated solution of sodium chloride (NaCl) contained in a pressure cooker. The jar then was opened, and the solution displaced the gas from around the fruit. This procedure precluded contamination of the fruit with air.

The fruit was kept submerged, and a large funnel completely filled with the saturated salt solution inverted and placed over it. The spout of the funnel was fitted with a short piece of rubber tubing sealed with a solid glass plug. This tube provided access for a hypodermic needle with which to withdraw samples of gas for analysis. The pressure cooker was sealed and a vacuum of 22.5 inches of mercury applied to the system for 1 minute. The extracted gases from the fruit displaced the solution in the spout

of the funnel. Each pear yielded approximately 11 ml of gas. A sample of the gas in the funnel was taken with a gas-tight syringe fitted with a 1-inch needle.

The gas was analyzed by the methods of Burg and Burg (4), using an Aerograph A-90-P dual-detector, thermal-conductivity gas chromatograph for CO₂, O₂, and N₂, and an Aerograph A-600 Hi-fy flame ionization gas chromatograph for ethylene.

To verify that the samples of gas withdrawn from whole fruits were representative of the entire internal atmosphere, the above procedure was repeated using fruits that were sliced after submersion under the funnel. Samples taken in this manner showed the same concentration of O₂ as did whole fruits.

Irradiation was done in the Mark II Experimental Food Irradiator (18). The dose rate in the source was approximately 250 Krad per hour.

To define the status of fruits relative to the climacteric, respiratory rates were measured by the method of Claypool and Keefer (6) using three replicates of ten fruits each.

RESULTS

The times required to remove oxygen from the internal atmosphere of unirradiated 'Bartlett' pears at 68° and at 86°F, and from irradiated pears (300 Krad) at 68°F, are given in Figure 1. During the first 18 minutes there was a marked reduction in the percentage of internal oxygen in all treatments. There was always a minute peak at the oxygen position on the chromatographic chart even after correction for argon. Analyses of the nitrogen used to flush the jars containing the fruit showed enough oxygen as a contaminant to account for the discrepancy.

Ethylene was barely detectable in unirradiated fruit at 68°F (Figure 2). In the other two groups, the concentration increased considerably during the first 6 minutes of nitrogen flushing. Thereafter it declined rapidly in both lots, and after 42 minutes was the same as in unirradiated fruits at 68°F.

Figure 1. Time required to remove the oxygen from the internal atmosphere of 'Bartlett' pears held in nitrogen flowing at a rate of about 250 ml/minute

Figure 2. Concentration of ethylene in the internal atmosphere of 'Bartlett' pears held in nitrogen flowing at a rate of about 250 ml/minute.

The rate of reentry of oxygen into fruits after they were placed in air following 2 hours in nitrogen was very rapid in all three lots during the first 18 minutes (Figure 3). The movement of oxygen into the irradiated fruits was slightly slower than that into unirradiated fruits. However, the amount of internal oxygen in all three lots seemed to stabilize between 18 and 42 minutes.

The concentration of ethylene in the internal atmosphere of fruits in all lots was low at conclusion of the nitrogen flushing (Figure 4). It remained low in the unirradiated lots throughout the experiment. However, production of ethylene in pears subjected to 300 Krad of gamma rays was stimulated immediately after exposure of the fruit to air. The concentration of ethylene in the irradiated pears increased gradually during the first 30 minutes in air and then declined.

All the above experiments were done with preclimacteric pears. In other experiments, the velocity of gas exchange was determined as the fruit went through the respiratory climacteric, since softening of the tissues occurs in association with this phenomenon.

The climacteric pattern of 'Bartlett' pears is shown in Figure 5. Rates of gas exchange were determined for pears in the following stages of ripeness: preclimacteric, 1 day before the climacteric peak, and 1 and 6 days after the climacteric peak. The pears in the final sample (no. 4) were in bad condition, full yellow in color and very soft.

The times required to remove oxygen from the internal atmosphere of the fruits at different stages of the climacteric are given in Figure 6. During the first 18 minutes there was a marked reduction in the percentage of internal oxygen at all stages, even though there were differences in the initial percentages of oxygen. The rate of oxygen removal from the senescent fruit 6 days past the climacteric peak was slightly slower than from fruit in the other samples, despite a low initial concentration.

Initial concentrations of ethylene in the internal atmosphere of the pears were: preclimacteric, 0.5 ppm; 1 day after peak, 179 ppm; and 6 days after peak, 93 ppm.

Figure 3. Time required for reentry of oxygen into the internal atmosphere of 'Bartlett' pears. Fruit was held in nitrogen 2 hours, then transferred to air flowing at about 200 ml/min.

Figure 4. Concentration of ethylene in the internal atmosphere of 'Bartlett' pears. Fruit was held in nitrogen 2 hours, then transferred to air flowing at about 200 ml/min.

Figure 5. Respiratory rate of ripening 'Bartlett' pears at 68°F.

Figure 6. Time required to remove the oxygen from the internal atmosphere of ripening and senescent 'Bartlett' pears held at 68°F in nitrogen flowing at about 250 ml/min.

Figure 7 shows the ethylene concentrations in the internal atmosphere of the pears during the nitrogen flush. Data for the preclimacteric sample are not shown because the change from the low initial concentration was negligible. After the first 6 minutes in nitrogen the concentration of ethylene was higher in the pears sampled 1 day before, and 1 day after the climacteric peak. Then declined in both these lots to about 15 ppm during 42 minutes. The concentration of ethylene in the pears sampled 6 days after their climacteric peak increased from 93 to 199 ppm during the first 18 minutes in nitrogen, and then declined to 9 ppm after 42 minutes.

The reentry of O_2 into the pears after they were placed in air was very rapid during the first 18 minutes (Figure 8). There was little difference between the rate of movement of oxygen into pears sampled 1 day before and those sampled 1 day after the climacteric peak; both rates were slower than that into the preclimacteric pears, but faster than that into the senescent pears. After 42 minutes, the percentage of oxygen in the internal atmosphere of these pears was about the same as in the other samples.

The concentration of ethylene increased rapidly during the first 18 minutes of flushing with air, in pears sampled either 1 day before or 1 day after the climacteric peak (Figure 9). After 42 minutes the concentrations had declined to 265 and 204 ppm respectively. The concentration of ethylene in the pears sampled 6 days after the climacteric peak increased from 4.5 to 42 ppm during the first 6 minutes in air, but remained low and nearly constant during the next 36 minutes.

DISCUSSION

Gas exchange in 'Bartlett' pears is very rapid. During the first 18 minutes of flushing with nitrogen, 98% of the oxygen in the internal atmosphere was removed. The same rate was found for reentry of oxygen from the air into the fruit.

Figure 7. Concentration of ethylene in the internal atmosphere of ripening and senescent 'Bartlett' pears held at 68°F in nitrogen flowing at about 250 ml/min.

Figure 8. Time required for reentry of oxygen into the internal atmosphere of ripening and senescent 'Bartlett' pears. Fruit was held in nitrogen 2 hours, then transferred to air flowing at about 200 ml/min at 68°F.

Figure 9. Concentration of ethylene in the internal atmosphere of ripening and senescent 'Bartlett' pears held at 68°F in air flowing at about 200 ml/min.

The initial concentration of oxygen in irradiated pears and in those held at 86°F was lower than in unirradiated pears held at 68°F. This probably reflects the increased respiratory rate induced by higher temperature (11) or by gamma irradiation (15). That the lower initial values for oxygen did not reflect a reduced capacity for gas exchange is shown in Figure 1. Irradiated fruits lost their oxygen even more rapidly than did the unirradiated ones. However, the differences were slight and may reflect only a greater utilization of oxygen.

The rate of reentry of oxygen from air into pears (Figure 3) following 2 hours of anoxia was different in the irradiated pears and those held at 86°F. At 86°F, the rate of oxygen accumulation in the internal atmosphere was about the same for the first 6 minutes as in fruits held at 68°F. Thereafter, the rate was lower, as was the final concentration after 42 minutes. Irradiated pears accumulated oxygen more slowly, and reached a final concentration of almost 3% less than in unirradiated pears at 68°F. It is likely that the differences in both irradiated fruits and those held at 86°F reflect greater utilization of oxygen in respiration rather than an altered permeability of the fruits to the gas.

Hansen (11) reported that an anaerobic condition causes a depression in production of ethylene in 'Bartlett' pears. As shown in Figure 2, ethylene concentration in the internal atmosphere of irradiated pears at 68°F and of unirradiated pears at 86°F increased during the first 6 minutes of anoxia. Thereafter, the concentration of ethylene in both lots declined. It seems that the ethylene production was unaffected in unirradiated pears at 68°F during a 42 minute flush with nitrogen. These data confirm the presence of detectable amounts of ethylene in the internal atmosphere of preclimacteric pears (12).

In the present work oxygen stimulated production of ethylene in pears, following 2 hours anoxia, only in the irradiated samples (Figure 4). The ability of these irradiated pears to produce ethylene when oxygen was supplied was immediate. Probably the

unirradiated pears needed more than 42 minutes flushing with air in order to respond with an increase in production of ethylene. The results of this experiment also could be interpreted to indicate that irradiation stimulates the formation of a precursor for ethylene that does not require free oxygen. For conversion to ethylene, such a precursor, if one exists, apparently requires the presence of oxygen (3).

Williams and Patterson (22) reported a high concentration of oxygen in the core area when the respiratory rate of 'Bartlett' pears was low. With increased respiration during the climacteric rise, the intercellular oxygen decreased. During senescence, when the respiratory rate declined, the oxygen in the internal atmosphere increased again. The initial concentrations of oxygen in Figure 6 are in agreement with the data of Williams and Patterson.

The declining concentration of oxygen in the internal atmosphere of pears during the ascending portion of the climacteric may reflect a decrease in permeability of the tissues to oxygen, as well as the higher utilization of oxygen. Permeability might decline because of increased wax deposition in the cuticle (10, 19) or, more likely, because of clogging of internal spaces by cell solution as the tissues soften (2). The higher oxygen concentration in pears sampled 6 days after the climacteric peak seems in conflict with both these concepts. A possible explanation for this anomaly may be found in the relative rates of respiration at the various stages of the climacteric (Figure 5). Between the preclimacteric minimum and the climacteric peak there was an increase in respiratory rate of approximately 300%. During the 6 days between the climacteric peak and the final sampling, the respiratory rate declined to about half that noted during the climacteric rise. Thus, it may be that the rate of oxygen utilization by the tissues declined more than did the rate of movement of O_2 into the tissues. There is some evidence that aerobic fermentation may occur in senescent fruit tissues (13, 21). This would be reflected in reduced oxygen consumption and a higher respiratory quotient.

That oxygen exchange is not severely restricted as pears ripen can be seen by the similarity between Figures 1 and 6 (oxygen removal) and between Figures 3 and 8 (reentry of oxygen).

The onset of the rise in ethylene production closely follows the respiratory rise (Figure 7). This is in agreement with the work of Hansen (11). However, the peak in ethylene production lagged 2 days behind the peak rate of respiration.

Flushing with nitrogen stimulates ethylene production for the first few minutes, then depresses it (Figures 2, 7). Apparently, at least for short period of time, 'Bartlett' pears can produce ethylene while having an internal oxygen concentration considerably below that normally present in air.

It was desirable to determine the internal concentration of CO_2 in 'Bartlett' pears simultaneously with measurements of the internal oxygen concentration. However, the vacuum method of extracting gases from within fruits could yield misleading data (5, 7), since the CO_2 of the air in the intercellular spaces is in a state of equilibrium with the dissolved CO_2 (20). Any change in the CO_2 content of the internal atmosphere would lead to the passage of CO_2 from solution into the gaseous state. For this reason data on internal carbon dioxide concentration are not included in this report.

SUMMARY

A study was made of the rate of gas exchange in 'Bartlett' pears. Movement of oxygen into or from the internal atmosphere was very rapid, and was not restricted severely as the fruits ripened.

Irradiation with 300 Krad of gamma rays, or a temperature of 86°F , stimulated production of ethylene. Flushing the fruit with nitrogen inhibited production of ethylene. Upon transfer of anoxic fruits to air, production was renewed, during the 42 minutes of the experiment, only in the irradiated pears.

Production of ethylene by pears at different stages of the climacteric was inhibited when the fruits were transferred to nitrogen, and was renewed upon their return to air.

Because of the rapid movement of gases into or from the internal atmosphere of 'Bartlett' pears, irradiation of the fruits in anaerobic conditions seems impractical.

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CHERRIES

Carol F. Johnson, Camilla Boyd, and E. C. Maxie

A second study on the sensory evaluation of Bing cherries was conducted this year.

The cherries, obtained as field run from Stockton, were sorted for maturity and only the riper cherries used. They were irradiated to 0, 200, 300, and 400 Krad. Storage and irradiation temperature was 32°F throughout. The cherries were sampled three consecutive days the first, second, and third week following irradiation. Ten judges were selected after three days preliminary testing. The judges were asked to evaluate flavor, texture, and appearance in comparison to a reference which was an unirradiated control. Scores were assigned as follows: much better than reference = 7, neither better nor poorer than reference = 4, much poorer than reference = 1. Flavor and texture evaluations were made on whole cherries with stems removed. To evaluate appearance, fruits were displayed under grading lights and the judges instructed to evaluate both fruits and stems.

Shear press readings were made each week on 100 gram samples of pitted cherries warmed to room temperature. The universal cell was used with a 60-second stroke time. Four replications were made each week on each treatment.

An analyses of variance was applied to the data and the results are reported in Tables 1 and 2.

Since there was no significant difference from week to week, the overall average flavor scores are shown in Table 1. Although significant differences in treatments occurred, the scores for the irradiated cherries are only slightly lower than controls. Some judges described the treated samples as "sweeter" while others described them as "lacking in flavor." A few comments indicated fruits subjected to 300 and 400 Krad were off flavor. The scores for appearance are also shown in Table 1. The irradiated fruit was scored lower than the controls for drier

Table 1. Average panel scores for flavor and appearance of Bing cherries irradiated and held three weeks.

	0	200	300	400
	Krad			
Flavor	4.11	<u>3.72</u>	<u>3.52</u>	3.42
Appearance	3.91	<u>3.41</u>	<u>3.30</u>	3.09

Any figures connected by the same line are not significantly different from each other at $P = 0.05$.

Table 2. Average taste panel scores and shear press readings of texture for irradiated cherries held three weeks.

	Taste Panel			Shear Press ^a		
	Weeks after irradiation			Weeks after irradiation		
	1	2	3	1	2	3
0 Krad	3.97	4.07	4.07	223.8	250.0	248.0
200 Krad	3.03	3.50	3.50	121.5	138.5	177.0
300 Krad	2.87	3.27	3.17	107.8	134.5	172.2
400 Krad	2.83	2.63	3.10	99.0	115.2	158.0

^a Average of four readings expressed in pounds force.

stems the first week and for shriveled fruit as well as drier stems the last 2 weeks.

The texture scores from taste panel and shear press readings are given in Table 2. A significant difference was distinguished in all treatments by the panel. The scores the last two weeks were significantly higher than the scores the first week. The panel as a group felt the softening effect due to irradiation yielded a poorer cherry. However, some judges found the slight softening effect of the lower doses preferable to the firmer controls.

Analyses of the shear readings showed a significant difference for all treatments except between 200 and 300 Krad. There was a significant increase in the firmness of the cherries each week. This could probably be attributed to water loss.

A correlation coefficient was calculated between weekly average taste panel scores for texture and the average shear readings. This figure was 0.93, which is very highly significant, and indicates that the panel's preference was closely related to the shear readings.

Some panelists commented that ripe cherries seemed less affected by the softening action of gamma rays than partially ripe fruit. An experiment was conducted to test this possibility.

Bing cherries from San Jose, California, were sorted into fully ripe and partially ripe groups. Each class was bagged and irradiated at 0, 100, 200, 300, and 400 Krad. Storage and irradiation were both done at 32°F.

The cherries were brought up to 68°F quickly by forcing warm air through the fruit mass in a specially designed box. Four replications were made per treatment on 100-gram samples of pitted cherries. The Universal shear cell was used with a 60-second stroke as noted above.

As was expected (Table 1), the immature controls were firmer than the ripe controls. At each dose used the loss in firmness was greater when expressed as percent of the unirradiated fruits in the less ripe lots. The degree of difference was too small to have any practical significance.

Table 3. Shear press readings on Bing cherries of two stages of ripeness irradiated and sheared on the same day as treated.

	0	100	200 Krad	300	400
Ripe	228.0 ^a	175.7	137.5	105.0	93.5
Immature	248.0	181.2	131.2	102.0	87.0

^a Average of four readings, expressed in pounds force.

THIAMINE AND NIACIN ANALYSIS OF IRRADIATED STRAWBERRIES

Henry Rae, Camilla Boyd, and E. C. Maxie

The thiochrome method of thiamine analysis and the cyanogen bromide method of niacin analysis were used to measure the effect of gamma rays on the content of these vitamins on strawberries analyzed immediately and 10 days after treatment to 200 Krad.

Frozen, whole, unsweetened berries (Bel-Air brand, Safeway Stores) were used throughout the study. Berries were divided into four lots of about 60 g each for the thiamine assay (40 g; niacin assay). Of these, two were control samples and two were irradiated. One control lot and one irradiated lot was analyzed the same day irradiated, while the other lots were held for 10 days at 41°F. Duplicate samples (20 g, thiamine assay; 10 g, niacin assay) of blended strawberry material were run for each treatment. Two days were required to complete each analysis and each experiment was completed twice. The results are shown in Tables 1 and 2.

These data show clearly that irradiation did not cause a nutritionally significant reduction in thiamine. In the case of niacin, irradiated fruits contained somewhat higher amounts of the vitamin. The reason for this has not been determined, but may reflect greater extractibility of the vitamins from the irradiated tissues.

Table 1. Effect of gamma rays on thiamine content of strawberries.
Results expressed as μg thiamine/100 g fruit.

Experiment	Days Storage	0 Krad	200 Krad
I	0	10.18	9.11
		9.64	9.28
	10	11.17	9.76
		---	10.08
II*	0	13.69	12.37
		---	11.65
	10	11.50	11.36
		11.21	12.88

* Values obtain in Experiment II may be higher because method of absorption and elution of the thiamine assay solution was changed and believed to be more efficient.

Table 2. Effect of gamma rays on niacin content of strawberries.
Results expressed as $\mu\text{g}/100 \text{ g}$ fruit.

Experiment	Days Storage	0 Krad	200 Krad
I	0	799	1238
		688	1078
	10	554	589
		620	577
II	0	754	789
		620	719
	10	723	841
		603	830

Mn²⁺ IN Co⁶⁰ GAMMA-IRRADIATED STRAWBERRIES¹

J. Shah,² W. C. Landgraf,³ and E. C. Maxie

The formation of free radicals in biological systems exposed to ionizing radiation is well established (1-7). Some of the induced radicals, as well as some of those occurring naturally (8), have long lives (9-11). The surprising stability of the induced radicals may have far reaching biological implications. As an example of stability, cystine, on irradiation in vacuo at room temperature, gave EPR signals even after 35 days (12). Numerous studies in connection with irradiation damage of proteins and amino acids confirm the stability of these radicals (13-18). This stability must be considered carefully in assessing the wholesomeness of foods preserved by ionizing radiation. Such assessment involves the concepts of stored chemical energy and consequent radiomimetic hazard (19-20). Chromosomal aberrations have been shown to occur in germinating barley and onion seeds following treatment with orange on apple juice, which had been irradiated with 200 Krad of gamma rays (21). Although the radiomimetic precursors generated by irradiation of fruit juices have not been ascertained, preliminary studies indicate that peroxides may be implicated.

From research in our laboratory, strawberries appear to be one of the most promising fruits for application of irradiation to extend shelf life (22). This communication reports on an investigation to determine whether long-lived organic radicals were present in irradiated fruits. Greenhouse-grown 'Shasta' strawberries were irradiated at 200 Krad at 0°C and maintained at

¹ This work was not formally a part of contract activities. It is reported here because of its significance to project objectives.

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this temperature for 64 hours. Samples taken at regular intervals, starting immediately after irradiation, were held in evacuated quartz tubes submersed in liquid nitrogen until EPR measurements. The spectra were determined with a Varian EPR spectrometer having a 9-inch magnet system, over the temperature range of 77°K to 0°C. The scan was varied from 500 to 1000 gauss at roughly g 2. The samples were evacuated continuously during the measurements.

We were able to detect only Mn^{2+} in the samples. Similar results were recorded from commercially available fruit irradiated over a dose range of 200 to 600 Krad. Atomic absorption spectroscopy showed 19 ppm Mn^{2+} (dry weight basis) in the greenhouse strawberries.

Preservation of foods by radiation, as a novel concept, led to extensive investigations concerning the wholesomeness of irradiated foods. Among the various general principles used to evaluate the biological safety of such foods, sub-threshold toxicity and delayed response are particularly significant when considering wholesomeness in relation to long-lived free radicals.

Due to the absence of any long-lived organic free radicals, the above concept is of no significance in irradiated strawberries. Comprehensive feeding trials of the irradiated foods have shown no adverse effects in test animals. Likewise, in trials conducted by the U. S. Army Quartermaster Corps, troops fed sterilized rations showed no ill effects. Manganese resonance also has been detected in various unirradiated plant materials, such as fallen oak leaves and pine needles and naturally dried ivy stems.

On the basis of the evidence available, it is logical to assume that wholesomeness of irradiated strawberries is not affected by the presence of Mn^{2+} free radicals. We have been unable to locate any references in the literature as to the fate of ingested transition metal free radicals. More definitive evidence regarding the biological safety of Mn^{2+} free radicals will come from such an investigation.

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GAMMA RAY RADIOSYNTHESIS OF OZONE FROM AIR

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Ozone is the principal product obtained from irradiation of air. Other products include the oxides of nitrogen--NO, NO₂, NO₃, N₂O, N₂O₃, N₂O₄, and N₂O₅. The presence of water vapor will lead to the formation of nitric acid.

The radiochemical synthesis of ozone from pure oxygen first was investigated by Lind (1). He measured the yield of ozone from a slow stream of oxygen exposed to alpha radiation and found ion-pair yields to be erratic, with a maximum value of about 0.5. Minimal yields of ozone were reported by D'Olieslager (2) in a static radon-oxygen mixture. In a flowing system, yields varied directly with flow rate and inversely with dose. By varying these parameters, he obtained yields from oxygen of 0.34 to 1.25 molecules of ozone per ion-pair. Lind and Bardwell (3) confirmed these results, showing that higher flow rates yielded up to 2 molecules/ion-pair. Mund and D'Olieslager (4) showed that inconsistent yields resulted from a difference in rate between two opposing reactions, ozonization and deozonization. The former reaction is dependent on oxygen pressure; the latter is proportional to concentration of ozone. As the dose approaches zero, maximum yields of ozone are obtained. At higher doses or longer dwell time of the ozone in the irradiation chamber, the yields are less. From a theoretical analysis, Mund (5) concluded that the net yield is proportional to the square root of radiation intensity, which indicates the efficiency of deozonization. Lewis (6) investigated the irradiation of pure ozone with alpha rays and confirmed that deozonization was dependent of oxygen pressure. Capron and Cloetens (7), using alpha radiation, showed that decomposition of ozone was proportional to its concentration in O₂ - O₃ mixtures having a large excess of O₂.

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The same authors (8) found ionic yields from the decomposition of ozone increased with increasing dose. The theoretical maximum ion yields as calculated by Hirschfelder and Taylor (9) are in the range of 2.6 to 4 O_3 molecules, with the lower experimental yields being due to deozonization. Based on Lind's data (loc. cit.), and assuming that, in air, alpha and gamma rays have the same ion yield products, Newton (10) estimated that a 10^4 r/hr dose in air would produce 2.57 ppm O_3 , 1.17 ppm NO_2 , and 0.118 ppm N_2O .

Magee and Burton (11) have suggested a mechanism for the formation of ozone in irradiated oxygen. Mechanisms leading to the formation of oxides of nitrogen in oxygen-nitrogen mixtures have been discussed by Harteck and Dondes (12) and by Pshenzhetsky and Dmitriev (13).

As carbon dioxide is relatively unaffected by radiation (14), for practical purposes air can be considered a two-component system composed of oxygen and nitrogen. From such a system one would expect lower yields of ozone than from pure oxygen.

Kertesz and Parsons (15) measured formation of ozone from air exposed to a 4,000 curies cobalt source, both in static and flowing systems. In air flowing through a glass structure placed near the source, concentrations as high as 0.1 ppm were recorded. In a static system the concentration of ozone was as high as 18 ppm. The investigators suggested that formation of ozone may be a factor in the germicidal activity of ionizing radiation.

Toward the end of our investigation, Less and Swallow (16) published measurements, made to estimate the hazard of radiolytic products of air, on formation of ozone with gamma irradiation.

In preparation for studies on the effects of radiation, per se, on plant tissue, as opposed to the effects of radiation-ozone in combination, it was essential to obtain data on formation of ozone in our radiation facility--a Brookhaven Mark II Research Irradiator (17) having three radiation chambers. The center chamber was used in the present investigation. At the time of these studies the source contained about 22,360 curies and had a dose rate of about 223 Krad/hr.

Most of our studies on irradiation of fruit are done with an air flow of 6,500 ml/minute; in some instances, however, no air is circulated through the radiation chamber. This study was limited to the estimation of ozone concentration under such conditions. Due to the design features of the chamber, formation of ozone in a static system had to be studied in a glass vessel, which consisted of three interconnected cylinders, provided with suitable inlet and outlet and had a volume of 11.7 liters.

EXPERIMENTAL METHODS

Formation of ozone in a flowing system was studied by passing 6,500 ml air/min through the chamber. The outgoing gases were conducted through stainless steel tubing into an impinger containing 2% neutral-buffered KI solution (18). After the required dose, the inflow of air was continued for 5 minutes to ensure complete removal of radiolytic products from the chamber. Color intensity of the iodide solution was read on a Bausch and Lomb spectronic 20 at 380 m μ , with suitable dilution where necessary.

In the static system, the glass vessel was filled with air from a cylinder and brought to atmospheric pressure. After the required dose, the vessel was removed from the chamber and its contents passed, by a slow current of air, through neutral-buffered KI solution. Absorbance was measured as described earlier.

OZONE PRODUCTION

Ozone concentration in a static system varied from 7.4 ppm at 50 Krad to 58.1 ppm at 1000 Krad (Table 1, Figure 1). Values of G (Figure 2) attained a maximum of 6.2 at the lowest dose, and then gradually fell to 2.5 at 1000 Krad. It is evident that the relationship between formation of ozone and dose is nonlinear.

In the flowing system, the G values were relatively constant (11.0 to 11.3) over the entire dose range; the concentration of ozone varied from 1.68 to 1.74 ppm (Table 2). Our results confirm that maximum formation of ozone occurs in a flowing system.

Table 1. Ozone concentration and G values for ozone production by gamma rays in a static system of air.

Dose (Krad)	O ₃ (ppm)	G value
50	7.36	6.20
100	12.40	5.20
150	17.49	4.96
200	23.24	4.94
250	23.76	4.04
300	28.39	4.02
350	31.66	3.85
400	32.64	3.47
500	36.68	3.12
550	38.19	2.95
600	42.43	3.00
700	45.04	2.74
800	46.36	2.46
900	53.52	2.53
1000	58.09	2.04

Figure 1. Formation of ozone on gamma irradiation of air in a static system.

Figure 2. Ozone G values on gamma irradiation of air in a static system.

Table 2. Ozone concentration and G values for ozone production by gamma rays in a flowing system of air.

Dose (Krad)	O ₃ (ppm)	G value
50	1.74	11.3
100	1.72	11.2
150	1.71	11.1
200	1.72	11.2
250	1.68	11.0
300	1.69	11.0
350	1.70	11.0
400	1.73	11.2
450	1.71	11.1
500	1.70	11.0
550	1.69	11.0
600	1.74	11.3
700	1.72	11.1
800	1.68	11.0
900	1.70	11.0
1000	1.69	11.0

The greater the dwell time of ozone in the irradiation chamber, as in the static system, the greater is its decomposition, and thus the lower the G values at higher doses.

Fruits are bulky and contain sizeable volumes of gas in their intercellular spaces. The concentration of oxygen in this gas is about 20% in freshly harvested fruits at temperatures of 25°C or less. Thus, at a given dose, the production of ozone per unit volume of gas inside the fruit probably would approximate the values given above for the static system. The ozone produced would react immediately with tissue components.

While gas exchange in fruits held in a flowing system may be relatively rapid (19), it is doubtful that such a system would reduce significantly the amount of ozone produced within the tissues. However, it would reduce markedly the concentration of ozone in the ambient atmosphere around the fruits.

Ozone in low concentration is strongly germicidal (20); also, it may cause injury to plant tissues (21, 22, 23). For these reasons, studies need to be made of irradiation in static and flowing systems as it may affect the response of pathogens and fruit tissues. Similarly, studies are needed of the protective action of anaerobic conditions, using inert gases to displace the O₂ from the internal atmosphere of fruits.

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EFFECT OF GAMMA IRRADIATION ON FRESH PRUNES

E. C. Maxie, Carol F. Johnson, and Camilla Boyd

One possible application of gamma irradiation to fruits is the treatment of fresh prunes with the objectives of: 1) increasing the drying rate, thus reducing the time the fruit is in the dehydrater with perhaps a reduction in the loss of volatile flavor components; 2) decreasing the rehydration time (and thus cooking time of the dried product); and 3) obtaining a more tender product.

Before making a detailed study of this use of gamma irradiation, we wanted to know its effect on the quality of the dried product. The information below describes the results of an experiment to ascertain this effect. Fresh, French prunes were irradiated to 0, 100, 200, 300, 400, and 600 Krad, then dried to a moisture content of 20%. The dehydrated fruits were stored at 36°F for about 5 months, then evaluated.

SENSORY PANEL

Preparation of samples. Evaluations were made on the aroma of prune syrup, texture of prune halves, and flavor of pureed samples. The prunes were cooked covered at a slow boil in batches of 30-40 (number required for irradiated samples in any one day). The prunes were cooked for 30 minutes. The ratio of distilled water to prunes was 2 parts to 1. Following cooking, the prunes were cooled approximately 30 minutes, drained, and reweighed. The volume of the remaining syrup was measured.

Prunes to be used for texture evaluation were halved, pitted, and served in coded paper cups. For flavor evaluations the fruits were pitted and pureed in a Waring blender. Some syrup was always added to aid blending (approximately 16% of the weight of the prunes). An effort was made to equalize the consistency of the puree before serving in coded darkened beakers. For aroma evaluation, 50 ml of syrup was placed in dark 8-oz water glasses and capped with aluminum foil.

Taste panel. Three preliminary sessions were held to retrain 11 judges from a previous prune panel. Two brands of commercially packaged prunes were prepared and presented as they would be for the experiment.

During each of three tasting sessions the panel judged aroma of the prune syrup, flavor of pureed samples (both duo-trio tests), and texture of prune halves (paired test) for the following comparisons:

Control (0 Krad) vs. 100 Krad

Control vs. 300 Krad

Control vs. 600 Krad

The reference samples in the duo-trio tests were unirradiated, control prunes. The judges also evaluated the flavor of the odd sample in comparison to the reference. Numerical scores of 7 to 1 were assigned to the following descriptive scale: much better than reference (7), neither better nor poorer than reference (4), much poorer than reference (1). For the paired test the judges indicated which sample had the softer texture.

PHYSICAL MEASUREMENTS

In addition to finding the per cent gain in weight of the prunes during cooking, soluble solids on the remaining syrup and on syrup reconstituted to its original volume was taken.

Four shear readings on 50 gm of dried prunes and 75 gm of cooked prunes were made at each level of irradiation. The standard shear compression cell and 30-second stroke were used.

RESULTS AND DISCUSSION

The results of the difference test are summarized in Table 1. Limited replications were made because of the quantity of prunes available. Differences were significant, however, in flavor and aroma. Average flavor scores were only slightly lower than 4.0 (same as reference) for 2 treatments. The only comments made by the judges with any consistency were less flavor for 100 Krad prunes,

Table 1. Results of difference tests between control and irradiated prunes for aroma, flavor, and texture.

	100 Krad	300 Krad	600 Krad
Aroma (duo-trio test)	19/32	24/32**	25/32** judgments
Flavor (duo-trio test)	23/32*	24/32**	22/32* judgments
average flavor scores	3.8	3.9	4.0
Texture (paired test-softer)	13/32	19/32	20/32 judgments

* Significant at P = 0.05.

** Significant at P = 0.01.

and tart or tangy for 300 and 600 Krad prunes. In a difference test such as this, variations in consistency of the puree may have contributed to detection of the odd sample.

In most cases, the irradiated prunes appeared to have a higher gain in weight (Table 2). Soluble solids of the syrup was always greater in the irradiated samples and may account for differences the judges found in aroma.

Average shear press readings are given in Table 3; a treatment of 400 Krad or greater is necessary before a difference in texture could be measured in dried prunes. Following stewing, all irradiated samples were significantly softer than controls.

Table 2. Results of physical measurements taken from two replications of prunes stewed 30 minutes.

		% gain in weight	Soluble solids of syrup	Soluble solids of reconstituted syrup
Rep I	0 Krad	32.0	2.2	1.4
	100 Krad	31.9	2.9	1.7
	300 Krad	35.1	3.6	1.8
	600 Krad	30.5	3.3	1.8
Rep II	0 Krad	31.1	2.3	1.0
	100 Krad	31.9	3.0	1.5
	300 Krad	30.2	2.8	1.6
	600 Krad	33.1	3.9	2.1

Table 3. Results of shear readings¹ taken on dried and stewed prunes treated at different levels of irradiation.*

	200	0	300	100	400	600 Krad
Dried	<u>648.8</u>	<u>607.5</u>	<u>567.5</u>	545.0	<u>552.5</u>	396.2
	0	100	200	300	400	600 Krad
Stewed for 25 min.	155.5	128.5	<u>102.0</u>	93.5	<u>89.8</u>	64.8

¹ Average of four shear readings expressed in pounds.

* Any scores not connected by the same line are significantly different at $P = 0.05$.

EFFECT OF GAMMA IRRADIATION ON LEMON FRUITS
HELD IN NON-REFRIGERATED STORAGE

E. C. Maxie, Henry L. Rae, and Camilla Boyd

We have shown with reasonable conclusiveness that lemons destined for long-term cool storage cannot be irradiated at levels that might be effective against the common citrus pathogens. However, there is the possibility that gamma irradiation could still be used in countries without refrigerated cold storages, trucks, and railway cars as a means of inhibiting rot development during a transit period of a few days at warm temperatures.

To investigate this possibility, we obtained Eureka lemons from a commercial packing house near Riverside, California. Fruits were irradiated at 0, 100, 200, and 300 Krad. After treatment, the fruits were placed at 77°F in an atmosphere containing 93% relative humidity. Triplicate samples of 10 fruits each were withdrawn for analysis after 8 and 15 days.

Table 1 shows the effect of gamma rays on the ascorbic-acid content of lemons held at a warm temperature, and Table 2 shows some other chemical and physical attributes of the fruit. From these data, it appears that lemons would still contain nutritionally significant levels of ascorbic acid, even after 15 days, when subjected to 200 Krad. There was a decided loss in attractiveness of the fruit subjected to 200 Krad after 15 days, but it seems possible that the fruit might be acceptable to people who might otherwise be deprived of this fruit.

The appearance and the chemical composition of fruits subjected to 300 Krad were so adversely affected that we doubt that this level of irradiation could be used even for short a period as 8 days at 77°F.

Table 1. Effect of gamma rays on ascorbic acid content of Eureka lemons stored at 77°F. Results expressed as mg ascorbic acid per 100 ml juice. Triplicate samples.

Time in Days	CONTROL			100 KRAD			200 KRAD			300 KRAD		
	Total	Reduced	Dehydro	Total	Reduced	Dehydro	Total	Reduced	Dehydro	Total	Reduced	Dehydro
0	51.32	47.69	3.63									
8	51.44	50.69	0.75	44.57	44.58	---	39.98	34.20	5.78	22.86	12.58	10.28
15	47.26	48.63	---	43.42	42.42	1.00	24.92	22.57	2.35	4.47	1.28	3.82

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^a Total acidity as % anhydrous citric acid.

^c % juice reamed based on storage wt.

* Two samples only.

EFFECT OF PULP TEMPERATURE DURING IRRADIATION ON QUALITY ATTRIBUTES OF SHASTA STRAWBERRIES

Juanita Manalo, Camilla Boyd,
Carol F. Johnson, and E. C. Maxie

If gamma irradiation becomes a practical technology with fruits, product temperature during treatment may be an important consideration. The nature and cost of a commercial irradiator may be determined in part by the refrigeration requirements of the products. Fruit temperature during irradiation may affect the quality of the product. Therefore, this study was designed to evaluate the effect of fruit temperature during irradiation on the quality attributes of strawberries.

It is becoming more and more evident that adverse effects of gamma radiation on the texture of fruits is more often limiting than any other attribute. Therefore, texture of strawberries was measured by sensory (taste panel) and physical (Allo-Shear) means. An attempt was made to correlate the findings from these two methods.

Shasta berries were obtained directly from the field, cooled immediately, trucked to Davis under refrigeration, stored at 32°F for 5 days, sorted for maturity, and the fully ripe fruits divided into the various experimental lots. The effect of high temperatures on the quality of strawberries is so profound that temperatures must be raised and lowered very rapidly if the data obtained are to be meaningful. Thus, a miniature "forced-air" box was constructed. The box consists of a screen-wire floor mounted half-way between the closed bottom and the open top of the box. Into one end of box and below the screen floor is mounted a high-speed "squirrel cage" fan which rapidly exhausts air from the box. The strawberry baskets were placed on the screen floor of the box. Strips of paper were placed underneath the edges of the bottom of the baskets to close off the open areas in the screen. This created a plenum chamber underneath the screen. When the fan was turned on, air

was rapidly pulled downward through the baskets of fruit, thus affording maximum contact between the individual fruits and the air. When the box was moved into the various warm rooms, it was possible to raise the fruit temperature from 32°F to 50, 68, and 83°F in 20, 45, and 90 minutes, respectively. To expedite the rate of temperature change, each lot of fruit was warmed with air of a temperature 5°C higher than the desired final temperature. Pulp temperatures were measured with a fruit thermometer and the fruits removed from the warming box as soon as they reached the desired temperature. The berries were transported to and from the irradiator in an insulated box and irradiated at air temperatures the same as the berry temperature. Following irradiation, the berries were stored at 32°F.

Two crates were used at each of the following temperatures; 32, 50, 68, and 83°F. One crate was held as a control and the other irradiated at 200 Krad with air passing through the irradiator at a rate of 6,500 ml per minute.

Preliminary tests on taste were conducted on two days just prior to the regular session to give the judges a warm-up. Taste panel and shear press measurements were then conducted on the second and third days after irradiation.

A paired comparison test was used to evaluate the flavor of the fruit. The paired samples were: control at 32°F vs. 200 KR at 32°F; control at 50°F vs. 200 KR at 50°F; control at 68°F vs. 200 KR at 68°F; and control at 83°F vs. 200 KR at 83°F. The paired samples were served at random to the trained judges who were also asked to score the samples. For flavor evaluation the fruits were stemmed, mashed, and served in amber-colored beakers. Three sets of results were obtained in the two-day period of testing. Two sets were served on the second day.

The same judges evaluated the fruits for texture. For this the fruits were stemmed, halved, and served in coded paper cups. Two sets of texture samples were served the first day and a single set served the second day. Three sets for flavor and texture were employed, making a total of 30 readings. Multiple comparison with

a reference was used, using the 68° control samples as a reference. Numerical scores of 1-7 were assigned to the judge's ratings in sequence of 1 "much softer than reference" to 7 "much firmer than reference." A score of 4 was neither softer nor firmer than the reference.

On the day that the judges evaluated flavor and texture, an evaluation of texture by physical measurement was also conducted with the shear press. Two baskets for each treatment were warmed to room temperature 3-5 hours before shearing. Samples of 100 grams were weighed and stemmed. A 200-pound setting with 1-minute stroke was used. The samples were placed in the universal shear cell and the results of compression and shear automatically recorded. Five replicates were done on each treatment each day.

RESULTS

I. TASTE PANEL

A. Flavor

Comments on flavor varied from off, sour, bland, "canny" to metallic. Most of the judges, however, admitted they could not tell any difference between the paired samples.

As shown in Table 1, statistical analysis showed that only the panel's preference for irradiated berries at 32°F over controls at 32°F was significant. One other preference which was apparent but not statistically significant was the preference for the irradiated berries at 68°F over the controls at 68°F. All others were not significant.

B. Texture

As shown in Table 2, there was no significant difference between the irradiated samples subjected to different temperatures. Only the controls at 68°F and 50°F were significantly firmer than any of the other samples. It is interesting to note that the judges scored the texture of the 83°F control the same as the 32°F control, while the 50°F and 68°F were scored the firmest.

Table 1. Summary of flavor evaluation of strawberries.

	Comparisons ^a							
	0 Krad--200 Krad 32°F 32°F		0 Krad--200 Krad 50°F 50°F		0 Krad--200 Krad 68°F 68°F		0 Krad--200 Krad 83°F 83°F	
Sum of preference	8	21*	15	15	11	19	16	14
Average of scores	2.86	3.43	3.24	3.17	3.17	3.37	3.11	2.97

^a Possible total of 30 evaluations for each comparison.

* Significant P = 0.05.

Table 2. Average readings^a for panel texture and shear press measurements.

PANEL								
	200 Krad 32°F	200 Krad 83°F	200 Krad 50°F	0 Krad 83°F	200 Krad 68°F	0 Krad 32°F	0 Krad 68°F	0 Krad 50°F
Ave. panel readings	<u>3.03</u>	3.23	3.37	3.47	<u>3.50</u>	<u>3.50</u>	4.30	<u>4.37</u>

SHEAR PRESS								
	200 Krad 32°F	200 Krad 68°F	200 Krad 50°F	200 Krad 83°F	0 Krad 32°F	0 Krad 50°F	0 Krad 68°F	0 Krad 83°F
Ave. shear press readings	<u>59.4</u>	61.4	<u>62.2</u>	69.9	<u>85.6</u>	<u>88.1</u>	<u>88.4</u>	<u>95.0</u>

^a Any scores not connected by the same line are significantly different at P = 0.05.

This same pattern was observed with irradiated samples and might possibly be a reflection of variation in maturity of berries. Only further testing will indicate if this trend is real.

II. SHEAR PRESS

As shown in Table 2, fruits tended to be more firm with increased temperatures. As expected, the controls were firmer than the irradiated samples. Temperature treatments did not show any marked effects on texture except in the 83°F samples, where irradiated fruits were significantly different from comparable samples subjected to the three other temperatures. Control fruits at 83°F were significantly firmer than those at 32°F. This probably reflects higher loss of water, resulting from the larger exposure to high air velocity at the warmest temperature, rendering the fruits tougher. This could possibly explain why readings on the second day were generally higher than on the first day.

In contrast to these results were the panel's observations which considered controls at 68°F and 50°F significantly firmer than the rest. They did not consider controls at 83°F and 32°F different.

TASTE AND SHEAR CORRELATION

A correlation coefficient was calculated between the average panel scores and the average shear readings for each of the two days. This value was found to be 0.57, which was significant at $P=0.05$. Even though significant, this figure indicates that only 32% ($0.57^2 \times 100$) of the total variation in the panel scores is due to the linear relationship between the shear press readings and panel texture scores; the rest of the variation is due to unexplained factors.

EFFECT OF GAMMA IRRADIATION ON RIPENING OF GROS MICHEL BANANAS

Rafael Amezcuita, E. C. Maxie, and Carol F. Johnson

Bananas, like many other tropical fruits, are subject to chilling injury. This phenomenon is not caused by freezing of the tissues, but occurs at temperatures between 32°F and 54-58°F (the maximum temperature at which chilling occurs varies with variety). Symptoms of chilling in bananas consist initially of a cinnamon-colored speckling of the subepidermal tissues of the peel. With increasing severity of chilling, the affected tissues become more deeply browned, and finally the entire peel becomes darkened. Severe chilling has a strongly adverse effect on subsequent ripening of bananas. The peels may turn very dark, the pulp remain starchy and firm, and the characteristic aroma and flavor of ripe bananas may not develop.

Since bananas must be transported and stored at temperatures above the 54-58°F range, they often ripen to a considerable extent in the ship's holds. Ripe and turning bananas cannot be shipped for any appreciable distance via truck or rail car because of their great sensitivity to impact and vibration injury. Premature ripening is particularly troublesome on bananas that grow from flower stalks thrown in late winter when growing temperatures are somewhat lower than for the rest of the year. These fruits may take 30-40 days longer on the plant to reach an acceptable maturity grade, and are often referred to as "tree stale" bananas.

Teas *et al** reported that gamma irradiation to 25 Krad markedly inhibited the ripening of banana fruits. When irradiated fruits subsequently were ripened they were of good quality. In 1963 we attempted to repeat the work of Teas and his co-workers, but were

* Teas, H. J., D. C. Quintana, and J. O. Compos. 1962. Inhibition of banana fruit ripening by gamma radiation. Second Intern. Congr. Rad. Res., Harrogate, England, Abstracts, 179.

unable to do so. We, therefore, suspended our work with this fruit. When we found that the stage of several fruits in the climacteric (ripening sequence) had such a profound effect on the response of the fruits to radiation, we felt that our experiments should be repeated. Our observations that ripening of pears and tomatoes were much less affected by gamma irradiation after the onset of the climacteric (ripening) led us to use individual bananas upon which we made daily measurements of respiratory activity. In this way we were able to choose fruits that were at precise stages of the climacteric sequence for irradiation. We had immediate and reproducible success in inhibiting ripening.

Figure 1 shows the days delay in onset of the respiratory climacteric in bananas subjected to 10 and 20 Krad in comparison with unirradiated fruits. Doses of 10 Krad caused delays of 10-12 days and 20 Krad caused delays of 16-20 days.

Figure 2 shows the rate of ethylene production by the bananas as they ripened. Here again, the radiation-induced delay in ripening is evident. In later experiments we found doses of 25-35 Krad to be more effective. These experiments will be reported in 1966.

Considering these results, we felt that we should evaluate the effects of the inhibitory treatments on ripened fruit quality. These experiments were as follows:

Several boxes of green Chiquita brand bananas (United Fruit Company) were obtained directly from the boat in San Francisco. On the following day the fruit was sorted for uniformity of green color and grade, then irradiated at ambient temperatures to 0, 35, and 50 Krad. The fruit was subsequently ripened as follows:

Lot 1. One-half of the fruit was taken immediately after irradiation to a commercial banana ripening plant and treated with ethylene to initiate ripening. On the 4th day after irradiation when the fruit color was more yellow than green, the samples were returned to the laboratory and held at 68°F until tasted on the 6th, 7th, and 8th days after irradiation. Bananas ranged in ripeness from green tipped to all yellow for this tasting session.

Figure 1. Effect of gamma irradiation on respiratory rate of Gros Michel bananas.

Figure 2. Effect of gamma irradiation on rate of ethylene production by Gros Michel bananas.

Lot 2. The second half of the fruit was placed at 68°F to ripen slowly. When it became evident that the ripening process was proceeding too slowly these fruit were placed in a "gassing box" and given an ethylene treatment on the 8th, 9th, and 10th day after irradiation treatment. The fruit was again placed at 68°F until it reached an all yellow color at which time it was removed to 59°F. Control fruit was placed at 59°F on the 13th day and irradiated fruit the following day. Unfortunately, these bananas, especially the irradiated samples, dried out considerably while being treated with ethylene. This condition improved when the fruit was placed in the humidified 59°F room. Tasting was done 13, 14, 15, 18, 19, and 20 days after irradiation treatment. Bananas ranged in ripeness from full yellow color to slightly overripe as indicated by degree of "speckling" on the skin of the fruit.

SENSORY EVALUATION

Preparation and presentation of samples. Bananas, at room temperature, were skinned and cut-up shortly before serving. Using only the center portion, each banana was cut into six servings. The servings were placed in paper cups coded with random two-digit numbers.

A triangle test was used to determine differences. In this test, sets of three samples were presented to each judge. Two samples contained the same treatment and one a different treatment. In addition to indicating which was the odd sample the judge also stated whether he preferred the odd or duplicates and the reason for his preference. The following comparisons were made:

Control vs. 35 Krad

Control vs. 50 Krad

35 Krad vs. 50 Krad

Four comparisons or triangle tests were made by each judge on each of three days for a possible total of 44 judgments per comparison.

JUDGES

Preliminary training and panel selection was made on similar bananas which had been permitted to ripen without the use of ethylene. A total of 12 triangle comparisons were tasted by each of 14 volunteers. The 11 judges with the highest number of correct answers were selected for the study.

DATA ANALYSES

The triangle tests were analyzed by chi-square using $P=1/3$ to establish significant differentiation and $P=1/2$ to establish preference from those making correct differentiations.

RESULTS AND DISCUSSION

The results of the study are summarized in Table 1. The panel's ability to detect sensory differences is probably related to the apparent maturity of the fruit with regard to flavor and/or texture. Irradiated samples (especially those treated to 35 Krad) were slower to ripen and though an attempt was made to select samples of equal maturity, some differences remained. It was also noted that skin color is not always an accurate measure of maturity in the irradiated samples.

During the first tasting session, the fruit was barely eating ripe and the judges especially noted that the control fruit was riper and better flavored than the 35 Krad treated samples. Comments on maturity were fewer during the second three days of tasting. By the end of the study, the fruit was getting very ripe and judges noted that the 50 Krad treated fruit was slightly overripe and soft in texture. Throughout the three tasting sessions the judges consistently commented that the 35 Krad treated bananas were less mature than other samples.

It is believed that differences in ripeness were too great to allow detection of differences between fast and slow ripened fruit. Evidently irradiation at these dose levels does not have an adverse effect on bananas, as no preference for any treatment occurred

Table 1. Results of sensory evaluation of banana.

Comparison	Correct Identification of Odd Sample	Preference
<u>Judgments</u>		
I. Fast Ripening		
Control vs. 35 Krad	26 of 44***	Control=16; 35 Krad=10
Control vs. 50 Krad	17 of 44	Control=11; 50 Krad=6
35 Krad vs. 50 Krad	14 of 44	50 Krad=8; 35 Krad=6
II. Slow Ripening-1		
Control vs. 35 Krad	22 of 42**	35 Krad=12; Control=9
Control vs. 50 Krad	21 of 40*	50 Krad=12; Control=9
35 Krad vs. 50 Krad	14 of 42	35 Krad=7; 50 Krad=7
III. Slow Ripening-2		
Control vs. 35 Krad	21 of 41*	35 Krad=14; Control=7
Control vs. 50 Krad	24 of 41***	35 Krad=14; 50 Krad=10
35 Krad vs. 50 Krad	34 of 41***	35 Krad=20; 50 Krad=14

* Significant at $P = 0.05$.

** Significant at $P = 0.01$.

*** Significant at $P = 0.001$.

during the three weeks of tasting. From judges' comments it is clear that preferences vary widely between fruits of different stages of ripeness; some liking fruits just barely ripe and others preferring overripe fruits.

EFFECT OF GAMMA IRRADIATION ON PEACHES

E. C. Maxie, Carol F. Johnson, Camilla Boyd,
Henry L. Rae, and R. F. Stallman

In our reports for 1962 and 1963, we presented evidence indicating that gamma irradiation stimulated ripening of freestone peaches and nectarines. This phenomenon was investigated further during 1964 with several varieties. The methods used were as described previously.

Figure 1 shows the respiratory rate, at 68°F, of Bonita peaches subjected to doses of 0, 100, 200, and 300 Krad. Immediately after treatment there was a marked increase in the rate of CO₂ evolution, giving a curve resembling the autogenous climacteric which is a characteristic of ripening peaches.

Figure 2 shows the rate of ethylene evolved by the fruit. Immediately after treatment the ethylene production of irradiated peach fruits increased. The pattern of ethylene evolution closely paralleled the respiratory activity (Figure 1), and all irradiated lots were ripe 4-6 days before the unirradiated fruits.

It seems clear that irradiation does not alter the sensitivity of peach fruits to the ripening action of ethylene. Thus, ethylene production induced by gamma irradiation triggers the ripening process in this species.

The degree of ripeness of fruits at the time they are irradiated may determine whether beneficial or adverse effects occur. In most cases, fruits that are ripe or nearly so at the time of treatment will respond more favorably to gamma irradiation. We were interested in determining whether peaches irradiated green, then ripened, would be as acceptable to our taste panel as fruits irradiated ripe. The peach is one of the few fruits where gamma irradiation stimulates ripening.

Two varieties of peaches were irradiated and subjected to sensory analysis. The fruit was harvested from the Wolfskill

Figure 1. Effect of gamma irradiation on the respiratory rate of Bonita peaches.

Figure 2. Effect of gamma irradiation on the rate of ethylene production by Bonita peaches.

Experimental Orchards at an advanced, commercial stage of maturity. They were stored at 32°F until irradiated. At the beginning of each sub-study a third of the peaches were irradiated green and all were placed at 68°F to ripen. After ripening, another third of the fruit was irradiated to the same level one or two days prior to sensory evaluation. The remaining fruit served as control. An attempt was made to ripen the fruit to the same stage of ripeness; however, the peaches irradiated green tended to ripen faster.

Sensory evaluations were made by 10-12 judges on Roberta freestone peaches irradiated at 0 and 100 Krad, and on Merrill Fiesta freestones irradiated at 0 and 100 Krad and 0 and 200 Krad. The peaches were peeled, pitted, sliced, and dipped into citric-acid solution (1/4 tsp/100 ml water) for 5 minutes to retard browning. For texture evaluations the peaches were served in slices in a paired comparison test. The panelists were asked to indicate the sample with the softer texture and score on a like (5) to dislike (1) scale. Flavor was evaluated on crushed peaches to obscure texture differences. The judges indicated which sample in a pair had the better peach flavor and scored both samples using the same scale as described for texture. Samples were tested four consecutive days following ripening and/or irradiation. The first day was always used as a warm-up session.

Results. There was some difficulty in ripening the irradiated fruit and unirradiated fruit to the same degree. There was a definite tendency for the peaches irradiated green to ripen more rapidly. The greatest difference in ripeness occurred in Merrill Fiesta peaches treated at 100 Krad. The fruit was generally lacking in quality and the control lot did not soften adequately. This test was the only one in which the panel could detect major differences. The panel chose the fruit irradiated green for its sweeter and better peach flavor. The controls and ripe fruit irradiated at 100 Krad were much more sour and tart. A few people described a bland or off flavor in both irradiated lots.

Although there were no significantly better flavor choices in Merrill Fiesta peaches irradiated at 200 Krad, the same

description of the fruit as for fruits subjected to 100 Krad was given by the panel. The lack of agreement among the panel as to the better flavor can be attributed to the fact that some judges considered the difference due to irradiation as resulting in sweeter, less tart fruit, and others considered the flavor as "bland" or "off".

For the Roberta variety the only significant difference in flavor was between the control and green fruit irradiated to 100 Krad. Some panelists described a bitter after taste or off flavor in both the irradiated samples.

The panel showed a tendency to rate control fruit as softer than fruits irradiated when ripe. The reason for this is not known, but it may be a reflection of mealiness induced in ripe fruit by irradiation. Unfortunately, inadequate sample size precluded shear-press determinations on these samples.

Table 1. Paired test on Roberta peaches irradiated at different maturities.

	Comparisons					
	Con-100 Krad Green		Con-100 Krad Ripe		100 Krad-100 Krad Green Ripe	
Better flavor	25*	11	21	15	16	19
Softer texture	15	21	27***	8	24*	12
Average Scores	Control		100 Krad Green		100 Krad Ripe	
Flavor	3.36		3.07		3.35	
Texture	2.93		2.96		3.55	

* Significant at 0.05 level of probability.

*** Significant at 0.001 level of probability.

Table 2. Paired test on Merrill Fiesta peaches irradiated at different maturities.

	Comparisons					
	Con-100 Krad Green		Con-100 Krad Ripe		100 Krad-100 Krad Green	100 Krad Ripe
Better flavor	9	26**	21	14	28***	7
Softer texture	1	34***	26*	8	35***	0

Average Scores	Control	100 Krad Green	100 Krad Ripe
Flavor	2.62	3.24	2.48
Texture	2.97	3.54	2.70

	Con-200 Krad Green		Con-200 Krad Ripe		200 Krad-200 Krad Green	200 Krad Ripe
Better flavor	16	18	22	12	22	12
Softer Texture	12	22	22	12	25**	8

Average Scores	Control	200 Krad Green	200 Krad Ripe
Flavor	3.19	3.03	2.82
Texture	3.32	3.34	3.02

* Significant at 0.05 level of probability.

** Significant at 0.01 level of probability.

*** Significant at 0.001 level of probability.

THE EFFECT OF GAMMA IRRADIATION ON THE POST-HARVEST BEHAVIOR OF TOMATO FRUITS

Adel S. Abdel-Kader, L. L. Morris, and E. C. Maxie

INTRODUCTION

The economic importance of tomatoes and the increased interest in the possible use of gamma irradiation to reduce spoilage stimulated the present study. The factors investigated should help in the evaluation of gamma irradiation for extending shelf life of tomatoes. The results of work done in 1962 and 1963 were reported in 1964.¹ This report presents results of experiments conducted during 1964 and gives an evaluation of results obtained until now.

A limited number of physiological studies of tomato irradiation had been done prior to this work. However, the conclusions were contradictory because the various workers did not take into consideration the extreme importance of the effect of stage of ripeness at which tomato fruits were irradiated. Thus, much of the literature--especially on changes in composition--is inconsistent, and even meaningless in some cases.

In this study careful attention was given to the selection of fruits of comparable maturity, size, and ripeness. Most of the work in the first two seasons was carried out on mature-green and breaker fruits. In the work reported here, emphasis was given to fruits of advanced ripeness.

GENERAL MATERIALS AND METHODS

Fruits. The variety Earlypak No. 7 was used in most experiments. Fruits at the indicated stage of ripeness were

¹ Maxie, E. C., N. F. Sommer, and D. S. Brown. 1964. Radiation technology in conjunction with postharvest procedures as a means of extending the shelf life of fruits and vegetables. Annual Report, Contract No. AT(11-1)-34, Project Agreement No. 80, U. S. Atomic Energy Commission, pp. 7-53.

obtained from the Merced, Tracy, or Vegetable Crops farm at the University of California, Davis. Fruits were transported to Davis, sorted for class of ripeness and visual quality, then stored until treated.

Samples. To minimize variability within and among the samples, extensive sorting and matching were done. Nine to ten fruits in each of 3 replications were used for respiration and ethylene production measurements; 25 or more fruits were used in samples for ripening or shelf-life observations, and individual fruits within the sample were observed. All experiments were repeated at least once.

Irradiation. Gamma irradiation treatments were made, using the Mark II Experimental Food Irradiator, and variability of doses was $\pm 5\%$. Fruits were irradiated at the same temperature as that used in subsequent storage.

Storage. Fruits were held in controlled temperature rooms where temperature fluctuations were within very narrow limits and the relative humidity was about 90%.

Respiration measurements. The colorimetric method of Claypool and Keefer² was used to measure CO₂ evolution. Determinations were made at short intervals during the first 2 or 3 days after irradiation, then every day until 50% or more of the sample was discarded. Samples were sorted every 2 days and decayed fruits discarded.

Ethylene determinations. Measurements at the same intervals as for respiration measurements were made by the method of Burg and Burg,³ using an Aerograph Hi-fy flame ionization gas chromatograph.

Ripeness score. The following table shows the classes of ripeness used and the numerical score assigned to each class:

² Claypool, L. L., and R. M. Keefer. 1942. A colorimetric method for CO₂ determination in respiration studies. Proc. Amer. Soc. Hort. Sci. 40: 177-186.

³ Burg, Stanley P., and Ellen A. Burg. 1962. Role of ethylene in fruit ripening. Plant Physiol. 37: 179-189.

<u>Ripeness score</u>	<u>Class</u>	<u>Description</u>
0	Mature-green	Entirely green but mature
1	Breaker	First appearance of coloring
2	Light pink	Approximately equal amount of green and pink
3	Dark pink	No green; entirely pink
4	Table-ripe	Fully red color; minimum eating ripeness
5	Canning-ripe	Intense red coloring

To obtain a ripeness score for each treatment at each sorting, the scores of individual fruits were averaged. Sorting continued until more than 50% of the sample was discarded.

Quality score. A visual evaluation of quality was made at 2-day intervals until more than 50% of the sample was discarded. At each sorting, individual fruits in each sample were given a score according to the following system:

<u>Score</u>	<u>Description</u>
9	Excellent - Field-fresh; no defects
7	Good - Minor defects
5	Fair - Defects present but not serious
3	Poor - Major defects; unsalable but still usable
1	Unusable - Not usable under ordinary conditions

These scores were averaged for each sample. Shelf life was expressed as the number of days required for the sample to deteriorate to an average score of 5.

RESULTS AND DISCUSSION

EFFECTS ON RATE OF RESPIRATION, ETHYLENE PRODUCTION, RIPENING, AND SHELF LIFE

Fruits irradiated at the mature-green stage. Figure 1 shows the rates of respiration, ethylene production, and the ripening behavior of tomatoes irradiated at the mature-green stage. Five replications of one fruit each were used to avoid variability in

Figure 1. Effect of gamma irradiation (400 Krad) on respiration rate, ethylene production, and ripening of tomatoes irradiated at the mature-green stage and stored at 68°F.

physiological maturity within the sample. Results obtained with individual fruits agreed in general with those obtained in previous experiments with samples of 10 fruits each.

Irradiation at 400 Krad stimulated ethylene production and respiration for the first 6 or 7 days after treatment. Thereafter the rate of ethylene and CO_2 production became lower in the irradiated fruits than in the controls, but ripening was retarded. Stimulation of ethylene production by irradiation did not seem to trigger endogenous production of C_2H_4 by the fruit, as observed when ethylene treatment was applied artificially to the fruits.

The climacteric was very clear in the control fruits, but no true climacteric or at best a low one was observed in the irradiated fruits. The slow rate of CO_2 and C_2H_4 production following the first 7 days in irradiated fruits can be correlated with the slower ripening of these fruits as measured by color development. It can also be argued that retardation of ripening caused a slower climacteric rise and a lower enzymatic ethylene production in the irradiated fruits.

Fruits irradiated at the pink stage. Tomato fruits irradiated at the pink stage showed an immediate increase in respiratory rate, as shown in Figure 2. The magnitude of the response increased with dosage between 300 and 700 Krad. Two days after treatment, the respiration rate of fruits in the 100-Krad treatment returned to a pattern similar to that of the control. Fruits in the 300- and 500-Krad treatments required 4 days to resume the respiratory pattern of untreated fruits. Fruits irradiated at 700 Krad showed a higher respiration rate than that of any other treatment throughout the storage period.

Figure 3 shows the ethylene production of fruits irradiated at the pink stage. The 700-Krad treatment caused a reduction in ethylene production throughout the storage period. Dosages of 100, 300, and 500 Krad caused an increase in ethylene production relative to that of the control for the first 6 days after irradiation. Thereafter the differences became relatively small.

Figure 2. Effect of gamma irradiation (dosages between 100 and 700 Krad) on respiration rate of tomatoes irradiated at the pink stage and stored at 68°F.

Figure 3. Effect of gamma irradiation on ethylene production of tomato fruits irradiated at the pink stage and stored at 68°F.

As shown in Figure 4, dosages of 300, 400, and 500 Krad retarded ripening--as expressed by color development--by 1, 2, and 5 days, respectively, but all fruits reached table ripeness. Fruits irradiated to 700 Krad failed to reach the table-ripe stage. This can be correlated with the reduction in rate of C_2H_4 production.

Shelf life of fruits was extended by 2-4 days by dosages between 300 and 500 Krad (Figure 4). Dosages lower or higher than 300 Krad had little or no effect. Dosages above 500 Krad shortened shelf life. The extent of physical damage to the fruit tissue at 700 Krad was less than that observed with fruits irradiated at the mature-green or breaker stages.

Fruits irradiated at the table-ripe stage. Results similar to those reported for fruits irradiated at the pink stage were obtained for fruits irradiated at the table-ripe stage. Respiration rate was stimulated slightly by dosages between 50 and 500 Krad for the first 3 days after irradiation; then the respiration rate of all treatments followed a pattern similar to that of the unirradiated fruits (Figure 5). Irradiation stimulated ethylene production except in the case of the 500 Krad treatment, when a reduction in rate was observed throughout the storage period (Figure 6). The magnitude of respiration rate and C_2H_4 production in fruits harvested at the table-ripe stage was much lower than in fruits harvested at the pink stage.

The effects upon color changes were small. Fruits irradiated at 500 Krad took 2-3 days longer than other treatments to reach the canning-ripe stage, then deteriorated faster than unirradiated fruits. A dose of 300 Krad extended shelf life of the fruit by 4 days.

Fruits ripened in storage and irradiated at various stages of ripeness. Fruits harvested at the mature-green stage were stored at 68°F to ripen. Samples of mature-green fruits (at the beginning of the experiment), and breaker, light-pink, and dark-pink fruits which became available 11 days after harvest were used in this experiment.

Figure 4. Effect of gamma irradiation on ripening and shelf life of tomatoes irradiated at the pink stage and stored at 68°F.

Figure 5. Effect of gamma irradiation on respiration rate of tomato fruits irradiated at the table-ripe stage and stored at 68°F.

Figure 6. Effect of gamma irradiation on ethylene production by tomato fruits irradiated at the table-ripe stage and stored at 68°F.

Doses of 400 Krad caused an increase in the respiration rate of fruits in all ripeness classes (Figure 7). The higher respiratory rate in irradiated samples persisted for most of the storage period, except in the case of the dark-pink fruits. Irradiated dark-pink fruits had a respiratory rate comparable to that of control fruits after 4 days following irradiation.

Irradiation at various stages of ripeness caused a stimulation of ethylene production (Figure 8). The magnitude of the stimulation was lower in the advanced ripeness classes than in the less ripe ones.

As shown in Figure 9, irradiation retarded ripening 1-2 days in the case of breaker, light-pink, and dark-pink fruits and 4-5 days in mature-green fruits, but all samples reached the table-ripe stage. Samples of breaker, light-pink, and dark-pink fruits that were supposedly of comparable maturity and ripeness at harvest reached the table-ripe stage before the original samples did. This can be attributed mainly to variability within the sample of mature-green fruits, even if sorted and matched to the best ability of the human eye.

Irradiation did not increase the shelf life of any sample. There was a noticeable loss in quality of irradiated fruits, especially those treated in the mature-green stage. This was a reflection of loss in texture of irradiated fruits and consequent increased susceptibility to mechanical injury. This study has particular significance in that it demonstrates that maturation and ripening on the plant has a profound and beneficial effect on the response of fruits to irradiation, which is lacking in fruits harvested in an unripe stage and subsequently ripened.

Effect of irradiation in combination with C_2H_4 treatment. The purpose of this experiment was to see if C_2H_4 treatment can offset the retarding effect of gamma irradiation on ripening of mature-green tomatoes. Fruits of the variety A-1 were obtained from the Salinas area in California and irradiated to 400 and 600 Krad. The ethylene treatment was 1000 ppm for 3 days at 68°F and 93% relative

Figure 7. Effect of gamma irradiation on respiration rate of tomato fruits ripened in storage at 68°F and irradiated at various stages of ripeness.

Figure 8. Effect of gamma irradiation on ethylene production by tomato fruits ripened in storage at 68°F and irradiated at various stages of ripeness.

Figure 9. Effect of gamma irradiation on ripening behavior of mature-green tomato fruits ripened in storage at 68°F and irradiated at various stages of ripeness.

humidity. Combined treatments were included, and each treatment had 3 replicates of 10 fruits each.

The effect of combination treatment upon respiration rate is shown in Figure 10 (A and B). Ethylene alone stimulated respiration during the treatment, but thereafter the rate was lower than that of control fruits. Irradiation only (400 or 600 Krad) stimulated respiration somewhat more than did C_2H_4 alone. About 7 or 8 days after irradiation, the respiratory pattern of irradiated fruits became similar to that of the control fruits. The effect of combination treatments was comparable to that of C_2H_4 alone when irradiation preceded the ethylene treatment. On the other hand, when ethylene treatment preceded irradiation, a rise in respiration rate was observed during the first 4 days after harvest, followed by a still higher rise after irradiation. The respiratory rate remained higher than in any other treatment throughout most of the storage period.

Ethylene treatment stimulated ethylene production by mature-green tomatoes (Figure 11), which remained higher than that of the control fruits for all the storage period. Irradiation to 400 Krad stimulated ethylene production for 9-10 days after treatment. Thereafter the rate of ethylene production was lower in fruits treated with 400 Krad than in the control fruits. Fruits treated to 600 Krad showed a lower ethylene production rate than that of control fruits during the entire storage period. Treatment with C_2H_4 after irradiation caused higher ethylene production than was observed in control fruits or fruits subjected to C_2H_4 alone until 10 days after treatment. Thereafter fruits treated with C_2H_4 alone had a higher C_2H_4 production rate than those subjected to 400 Krad plus C_2H_4 . Fruits treated with 600 Krad plus C_2H_4 had a higher rate of ethylene production than did fruits subjected to 600 Krad alone, but their rate was lower than either the controls or those subjected to C_2H_4 alone during the whole storage period. Irradiation applied after C_2H_4 treatment induced an immediate increase in ethylene production, and in the case of the 400-Krad treatment the rate remained higher than for other treatments. In fruits subjected to

Figure 10. Effect of gamma irradiation and ethylene treatment, alone and in combination, on respiration rate of mature-green tomato fruits stored at 68°F.

Figure 11. Effect of gamma irradiation and ethylene treatment, alone and in combination, on ethylene production of mature-green tomato fruits stored at 68°F.

600 Krad the rate of ethylene production dropped to a lower value after 6 days than either control or fruits treated with ethylene alone but higher than fruits treated with 600 Krad alone or those given 600 Krad plus C_2H_4 .

Ethylene treatment hastened ripening (Figure 12), while irradiation to 400 and 600 Krad retarded ripening. Ethylene treatment was effective to some extent in offsetting the retarding effect of irradiation on ripening. Ethylene applied after irradiation was more effective than when applied before irradiation. The majority of the fruits treated with 600 Krad never reached table ripeness, whether treated with C_2H_4 or not.

No great differences in shelf life were observed among fruits subjected to 0 Krad, 400 Krad, and ethylene. Treatment with 600 Krad caused a reduction in shelf life of 4-5 days. The principal adverse effects were softening, loss of characteristic attractive appearance, and irregular and retarded color development.

GENERAL DISCUSSION AND CONCLUSIONS

1. Gamma irradiation causes an increase in the respiratory activity of tomato fruits immediately after irradiation which is proportional to dosage. The magnitude of stimulation depends upon the stage of ripeness of the fruits at the time of irradiation. The more advanced in ripening at time of irradiation, the smaller is the stimulation of respiration in comparison to unirradiated fruits. The duration of the stimulation also varies with ripeness and dose. In general, it is shorter with advanced ripeness and becomes longer at higher doses. The temperature effect on respiration rate due to irradiation is relatively comparable at all temperatures.

2. Irradiation of tomato fruits at dosages up to 500 Krad stimulates ethylene production for a period after treatment. Dosages of 500 Krad in the case of table-ripe fruits or more than 500 Krad for other ripeness classes cause a decrease in ethylene production. The results indicate that these higher dosages not

Figure 12. Effect of gamma irradiation and ethylene treatment, alone and in combination, on ripening behavior of mature-green tomatoes stored at 68°F.

only cause a reduction in the rate of ethylene production by the fruit, but also cause the fruit to be less responsive to ethylene treatment. The reduction of ethylene production may result from destruction of some enzymatic system that controls its production, or it may be due to oxidation by the free radicals produced at the high doses. Stimulation of C_2H_4 production by lower dosages did not seem to enhance subsequent ethylene production by the fruits as was observed if fruits were subjected to ethylene treatment.

3. The retarding effect on ripening becomes lower in magnitude with advanced ripeness of the fruit when radiation is applied (Figure 13). At dosages above 500 Krad for mature-green tomatoes and above 600 Krad for more advanced ripeness classes, irradiation prevented fruits from attaining table ripeness.

4. Irradiation has an adverse effect on color development. It causes unevenness in color formation, with yellow spots that make the fruits less attractive. This is more pronounced at dosages above 400 Krad, especially for the mature-green fruits. Relatively low dosages of gamma irradiation (0-100 Krad) have little or no effect on ripening. Dosages between 100 and 400 Krad have a retarding effect that increases with dosage.

5. The effect of gamma irradiation on ripening behavior can be related to some extent with its effect on ethylene production, especially at dosages above 500 Krad. Reduction of ethylene production at these dosages is accompanied by inhibition of color development. Dosages below 500 Krad stimulate ethylene production for a period immediately after irradiation which varies with dosage and stage of ripeness. From all the tests made to date no clear correlations can be found between ethylene production, ripening behavior, dosage effect, and respiration in samples containing 10 or more fruits. The use of individual fruits in two tests with mature-green fruits gave better correlations. These results were reported at the beginning of this section.

6. No effect has been observed on maturity or ripening of fruits harvested and treated at a relatively immature stage

Figure 13. Effect of dosage of gamma irradiation on ripening of tomatoes irradiated at various stages of ripeness and held at 68°F. Mature-green fruits irradiated at 600 Krad and all stages irradiated at 700 Krad failed to reach table ripeness.

(5-10 days from the mature-green stage) by using low dosages of radiation (0-25 Krad).

7. Figure 14 compares the effects of irradiation at various levels on the shelf life of tomato fruits irradiated at five stages of ripeness. The highest practical dose for possible benefit in shelf-life extension seems to be 400-500 Krad, based upon visual quality; and 200-300 Krad, based upon flavor, chemical changes, and other quality factors, as will be shown later in the report. A dose of 300 Krad had little or no effect on fruits irradiated at the mature-green or breaker stage. On the other hand, an extension in shelf life of 4-8 days was obtained in fruits irradiated at the more advanced stages of ripeness.

EFFECT ON DETERIORATION, VISUAL QUALITY, AND TASTE

The economic importance of methods that would reduce losses of tomatoes from decay cannot be overemphasized. Spoilage losses of fruits destined for the fresh market amounted to \$97.3 million at 1959-63 U. S. retail prices (U.S.D.A. Agr. Statistics, 1963).

In this portion of the study, the fruits were sorted at intervals of 2 days, and unsalable fruits (usually due to decay) were discarded. Deterioration is expressed as per cent of discarded fruits. It should be noted, however, that preserving the soundness of tomatoes by preventing decay does not necessarily mean preserving quality (flavor, texture, taste, and nutritive value).

Fruits irradiated at the mature-green stage. In general, tests with commercial packages of mature-green tomatoes gave results similar to those obtained last year. Figure 15 shows that irradiated mature-green fruits had a larger percentage of decay than unirradiated fruits. This was probably a reflection of increased softening of the fruits, and thus increased susceptibility to postirradiation infections. These results were the reason for emphasizing use of fruits of more advanced stages of ripeness during the 1964 season.

Fruits irradiated at the pink stage. Two tests were conducted with sorted samples of 25 fruits each, and two tests with commercial

Figure 14. Effect of gamma irradiation on shelf life of tomatoes irradiated at various stages of ripeness and held at 68°F.

Figure 15. Effect of gamma irradiation on deterioration of tomatoes in commercial packages (30 lb L.A. Lug) irradiated at the mature-green stage and held at 68°F.

packages of so-called "vine-ripened" fruits. Doses were 0, 200, 250, 300, 350, and 400 Krad. Following treatment, the fruits were stored at 68°F and sorted every other day for evaluation of ripening and quality changes. Figure 16 shows that doses below 250 Krad were ineffective in reducing decay. Doses above 250 Krad reduced the amount of rot, 300 Krad being most effective, the fruits showing only about 40% decay after 20 days in storage. Similar results were obtained in the case of sorted samples, but better control of decay and longer shelf life were obtained (Figure 17). This emphasizes the importance of fruit condition at the time of treatment in determining the effectiveness of radiation pasteurization.

Fruits irradiated at the table-ripe stage. Six tests were done to determine the effect of various doses of radiation on extending shelf life of tomatoes irradiated at the table-ripe stage. In each test, radiation treatments included most or all of the following irradiation doses: 0, 50, 200, 300, and 400 Krad. Twenty-five fruits were used per treatment and were stored at 68°F. Another two tests included a series of storage temperatures--41°F for one week then at 68°F; 50°F; 59°F, and 68°F. Doses were 0, 200, 300, and 400 Krad in these tests.

Figure 18 shows the results of one experiment with fruits held continuously at 68°F. While all treatments gave some extension of shelf life, 300 Krad was most effective, reducing decay by 50% after 18 days in storage. This amounts to extending the shelf life by 10 days. The range of shelf-life extension varied among the 6 tests. Table 1 summarizes the results of these various tests.

Factors that may have contributed to the differences among tests are: fruit condition at harvest, level of field infection, handling procedures during transit, sorting, and time of harvest during the season.

Figure 19 shows the effect of storage temperature and irradiation on shelf life. All treatments had some beneficial effect, with 300 Krad giving the best results. Temperatures of 50°F (borderline chilling temperature) and 41°F (chilling temperature) caused a higher percentage of decay than did higher temperatures, especially in the

Figure 16. Effect of gamma irradiation on deterioration of tomatoes in commercial packages (2-layer flat) irradiated at the pink stage and held at 68°F.

Figure 17. Effect of gamma irradiation on deterioration of tomatoes irradiated at the pink stage and held at 68°F.

Figure 18. Effect of gamma irradiation on deterioration of tomatoes irradiated at the table-ripe stage and held at 68°F.

Table 1. Effect of radiation dosage on shelf life of tomatoes irradiated at the table-ripe stage.

Test No.	Shelf Life--Days Required For Indicated Cullage									
	25% of sample					50% of sample				
	0	50	200	300	400 Krad	0	50	200	300	400 Krad
1	8	10	12	18	14	13	15	16	21	20
2	5		8	9		8		12	12	
3	16	16	18	20	20	21	21	22	26	26
4	8	10	14	17	12	12	14	16	20	18
5	10	11	16	19	18	14	16	20	24	21
6	9		14	18		13		19	22

Figure 19. Effect of gamma irradiation on the deterioration after 2 and 3 weeks of tomatoes irradiated at the table-ripe stage and held at various temperatures.

irradiated lots. This probably reflects a combination of chilling injury and radiation damage. After 2 weeks in storage, fruits stored at 59°F had less decay in all treatments than those stored at 68°F.

Comparison of varieties. Table-ripe fruits of 12 varieties were obtained from the Vegetable Crops farm at Davis. These included both commercial and non-commercial varieties. A control and a 300-Krad treatment were included for each variety (25 fruits per treatment). Fruits were stored at 68°F and sorted every other day. Table 2 shows the effect on quality and shelf life of the varieties used.

These preliminary results indicate that varieties may differ in their response to radiation pasteurization. The shelf-life extension due to irradiation varied from 0-8 days. It can also be seen that extension of shelf life does not necessarily mean better quality.

Sensory evaluation of tomatoes irradiated at the table-ripe stage.⁴ A taste evaluation was made on Earlypak 7 tomatoes harvested and irradiated at the table-ripe stage. The treatments included 0 (control), 200, 300, and 400 Krad. Ten experienced judges were trained for 3 days and data were taken 5, 6, 7, 8, 11, 12, 13, and 14 days following irradiation.

A paired comparison test was used, with fruits from each irradiation treatment paired with the control tomatoes. The judges sampled all three pairs each day, indicated which sample in each pair had the better flavor, and scored both samples on a 5 (like) to 1 (dislike) scale.

Eight to ten tomatoes per treatment were sampled each day after cutting a portion of each tomato into small pieces. The samples were served in random order in cups coded with random, 2-digit numbers.

The results of the paired comparison test are summarized in Figure 20 and in Table 3. Although in the first week the panel consistently indicated that the controls had a better flavor, only the comparison of controls versus 400-Krad treatment was significant.

⁴ The assistance of Miss Carol F. Johnson is greatly appreciated.

Table 2. Effect of gamma irradiation on quality and shelf life of tomato varieties irradiated at the table-ripe stage.

Variety	Treatment (Krad)	Days To Reach		Days Until	
		Fair quality	Poor quality	25% discard	50% discard
1. Earlypak No. 7	0	10	20	12	18
	300	12	24	18	24
2. 428	0	12	18	10	16
	300	10	18	11	18
3. Ace	0	9	20	11	16
	300	11	22	14	20
4. VF-36	0	8	20	10	15
	300	10	20	15	23
5. Fireball	0	7	12	6	8
	300	7	14	9	14
6. Burpee Globe	0	8	20	15	20
	300	8	22	21	25
7. Yellow Jubilee	0	12	23	15	20
	300	10	23	15	22
8. 1402	0	8	20	11	16
	300	10	22	17	22
9. Roma	0	8	22	16	23
	300	8	22	16	23
10. VF-13L	0	8	20	12	16
	300	8	20	14	19
11. Red Cherry	0	10	20	18	--
	300	8	20	22	--
12. Yellow Pear	0	10	24	20	--
	300	11	24	25	--

Figure 20. Average taste panel scores after 1 and 2 weeks of storage of tomatoes irradiated at the table-ripe stage and held at 59°F.

Table 3. Flavor preferences between untreated and treated tomatoes irradiated at the table-ripe stage and held at 59°F.

Tasting period	Indicated Preference For Control vs. Irradiated Sample					
	0 Krad	vs. 200 Krad	0 Krad	vs. 300 Krad	0 Krad	vs. 400 Krad
First week	20	15	21	14	28***	7
Second week	20**	9	29**	9	32***	6

** Significant at $P = 0.01$, using chi-square analyses.

*** Significant at $P = 0.001$.

The second week all three pairs were significantly different, with the controls again having the better flavor. The average scores are illustrated in Figure 20, and indicated that all samples deteriorated somewhat in quality the second week. All three irradiated treatments were described as lacking in flavor--flat, off-flavor, and softer than the controls. The effect of irradiation was not consistent from fruit to fruit, some samples being as good as the controls, others having a distinct off-flavor.

Effect on susceptibility to transit injury. Tomatoes are bruised by pressure, impact, and/or vibrations during handling and transit. The extent of bruising injury depends largely on the degree of ripeness of the fruit, with susceptibility increasing directly with advancing ripeness.

Mature-green and pink tomatoes were used for these experiments. One L.A. Lug (50-60 fruits, size 5 x 6) was used per treatment. Treatments were: control, vibration only, irradiation only (300 Krad), vibration followed by irradiation, and irradiation followed by vibration. The vibration treatment consisted of 40 minutes at 1.1 x gravity, simulating transit to eastern markets. Fruits were stored at 68°F after treatment, and ripening and quality changes were observed. Table 4 shows results for mature-green fruits.

Vibration in combination with irradiation was more injurious than vibration alone, but differences among treatments were small. The vibration treatment was more injurious when applied after irradiation than before. Similar results were obtained with the pink fruits. While shelf life was extended 5 days by 300 Krad without vibration, an extension of only 2 days was obtained when vibration treatment was applied to irradiated fruits. Vibration alone decreased shelf life by 2 days. These results show the desirability of conducting simulated transit tests and actual shipment tests to answer the question of how much radiation-induced softening will affect susceptibility of fruits to transit injury.

Effect of application methods. The following factors were investigated to determine their effect on ripening and shelf life of tomatoes:

Table 4. Effect of gamma irradiation and vibration treatments on ripening and shelf life of tomatoes treated at the mature-green stage.

Treatment	No. Of Days To Reach	
	Table-ripe stage	50% discarded
1. Control	19	22
2. Vibration only*	18	22
3. Irradiation only**	22	21
4. Vibration + irradiation	22	19
5. Irradiation + vibration	22	20

* To simulate vibrations in transit.

** 300 Krad.

1. Preirradiation conditioning period (time between harvest and irradiation). Fruits were held at 68°F after harvest then were irradiated (400 Krad) at the mature-green stage immediately, 24, 48, or 72 hours after harvest. The fruits were held at 68°F after irradiation. The results are shown in Table 5. Fruits irradiated after 48 or 72 hours ripened normally without retardation. Fruits in these treatments were breakers at the time of irradiation. The length of the conditioning period had no appreciable effect on quality or shelf life of fruits irradiated at the table-ripe stage.

2. Applied dose rate. Fruits were irradiated (400 Krad) at the mature-green stage, either in one dose or in a number of subdoses. The results are shown in Table 6. When 400 Krad was applied in 4 subdoses, no retardation of ripening was obtained, but shelf life was decreased relative to the other treatments, because of increased handling. From this experiment and a comparable one with table-ripe tomatoes, it seems best to give the recommended dose in the shortest possible time and with minimum handling.

3. Temperature during irradiation. Mature-green tomatoes were irradiated with 400 Krad at various temperatures. The results are shown in Table 7. No appreciable effect on ripening or quality was observed during subsequent storage except in fruits irradiated at chilling or near chilling temperatures. It is advisable to use a temperature close to the storage temperature, to avoid chilling, and it should not exceed 77°F, because of possible effects on color development.

4. Possible effects of season. There are two main seasons for tomato production in California: 1) early summer (June to August), and 2) early fall (September to December). Toward the end of the fall season, tomatoes tend to be weaker and more subject to mechanical injury and decay organisms, especially Alternaria rot. Thus the effectiveness of radiation pasteurization may depend in part upon harvesting time in the season.

Gamma irradiation at the mature-green stage gives better results with fall tomatoes--if they have not been subjected to chilling injury in the field--than with summer tomatoes. Early in the summer

Table 5. Influence of conditioning periods on the effect of gamma irradiation on quality and ripening of tomatoes harvested in the mature-green stage.

Treatment	Number Of Days To Reach	
	Fair quality	Table-ripe stage
0 Krad	15	20
400 Krad immediately after harvest	14	23
400 Krad 24 hours after harvest	14	23
400 Krad 48 hours after harvest	15	20
400 Krad 72 hours after harvest	15	19

Table 6. Effect of gamma irradiation, applied as single and split treatments, on quality and ripening of tomatoes harvested at the mature-green stage and held at 68°F.

	Treatment				Number Of Days To Reach	
	Total dosage (Krad)	Sub-dosage (Krad)	Number of sub-dosages	Interval between subdosages (days)	Fair quality	Table-ripe stage
1	0	-	-	-	15	20
2	400	-	1	-	13	28
3	400	200	2	1	13	25
4	400	200	2	3	14	23
5	400	100+	1+	3	15	24
		300	1			
6	400	100	4	1	12	21

Table 7. Effect of temperature during irradiation on quality and ripening of tomatoes harvested at the mature-green stage and held at 68°F.

Temperature during irradiation*	Number Of Days To Reach	
	Fair quality	Table-ripe stage
°F		
32 ± 2	11	17
41	12	18
50	13	17
59	15	18
68	14	16
77	12	19
86	11	20

* 400 Krad.

(June), a large percentage of relatively immature-green fruits is harvested and shipped as mature-greens. These fruits are subject to physiological stresses, and radiation does more harm than good to them.

GENERAL DISCUSSION AND CONCLUSIONS

1. For Mature-Green And Breaker Tomatoes

a. Most of the results obtained with mature-green and breaker tomatoes were negative with respect to using gamma irradiation as a treatment for extending the shelf life of tomatoes. In this work it was concluded that irradiation would be valuable only under conditions where decay losses would be excessive. No single treatment controlled the pathological organisms completely. Although dosages of 400 Krad caused an extension of 1-3 days in shelf life, dosages of 100-300 Krad did not extend shelf life, and dosages above 500 Krad caused a reduction in shelf life, due to tissue damage. The shelf-life extension with 400 Krad varied among tests and was more evident in fall-grown than in summer-grown tomatoes.

b. Irradiated tomatoes seemed to be more susceptible to postirradiation attack by organisms than unirradiated fruits. Alternaria tenuis seemed to be the tomato post-harvest pathogen most resistant to medium dosages of irradiation (300-400 Krad). Studies of quantitative dose response will have to be made to verify this assumption. Other causes of losses were: Rhizopus, Fusarium, and Penicillium rots, physiological softening, and shriveling.

c. According to the taste panel, doses as low as 200 Krad caused off-flavor.

d. Storage and transit temperatures are very important for irradiated tomatoes. Irradiation cannot substitute or modify the temperature requirements of this fruit.

e. An additional handling operation during irradiation may contribute to the severity of bruising injury. It seems that gamma irradiation intensifies the symptoms of internal bruises rather than increasing their severity.

2. For Pink And Table-Ripe Tomatoes

a. The limitations of irradiation with respect to application for shelf-life extension are less in fruits irradiated at the pink or table-ripe stage. An extension of shelf life of 4-12 days can be obtained by doses of 200-350 Krad.

b. The length of shelf-life extension resulting from irradiation is affected by condition at harvest, level of field infection, handling procedures, and storage temperature.

c. Loss of texture and development of off-flavor may limit the use of irradiation with fruits at pink and table-ripe stages despite the control of rot. However, the irradiated fruits were still acceptable to the panel and might have had better rating if some technique other than paired comparisons had been used. Indeed, irradiated table-ripe fruits may taste better than fruits picked when mature-green and ripened in storage to the same stage.

d. The procedures for handling tomatoes destined for irradiation seem to be as follows:

1. Harvest at the dark-pink or table-ripe stage.
2. Wash.
3. Irradiate to 250-300 Krad.
4. Wax.
5. Pack in consumer packages covered with perforated cellophane paper.
6. Transport at temperatures between 55° and 65°F.

EFFECT ON SOME CHEMICAL, PHYSICAL, AND
PHYSIOLOGICAL PHENOMENA

Effect on ascorbic acid content. Ascorbic acid is one of the major nutritive constituents of tomatoes. Its concentration depends upon inheritance factors, climate, position of fruit on the plant, size of fruit, stage of maturity and ripeness at harvest, and storage conditions. Thus, variable composition of morphologically comparable fruits is not uncommon.

The effect of gamma irradiation on the ascorbic acid content of tomatoes at the mature-green, breaker, pink, and table-ripe stages was determined immediately after treatment and at 5-day intervals thereafter. Special attention was given to selecting samples for analysis. Each sample consisted of approximately equal opposite segments from 10-15 fruits at each determination. The photometric method⁵ was used for analysis.

Figure 21 shows the effect on mature-green tomatoes. An irradiation dose of 600 Krad caused an immediate loss of about 25% of the ascorbic acid content; thereafter the pattern of loss was similar to that of the control fruits. An immediate reduction after treatment was observed at 200 and 400 Krad, but soon thereafter the ascorbic acid content returned to normal. In general, doses between 5 and 400 Krad had no significant effect on the ascorbic acid content. In the case of breakers (Figure 22), 200 Krad had no effect, while 300 and 400 Krad caused a reduction in ascorbic acid content of 8% and 15%, respectively, after 15 days in storage. Similar results were obtained with fruits irradiated at the pink stage (Figure 23). A small loss in ascorbic acid content was observed with all doses used.

Figure 24 shows results with fruits irradiated at the table-ripe stage. Doses of 50, 200, 300, and 400 Krad caused a reduction in ascorbic acid content of 6, 21, 12, and 24%, respectively, after 15 days in storage. Most of the loss in ascorbic acid content

⁵ Loeffler and Ponting. 1942. Photometric method for the determination of ascorbic acid. Ind. Eng. Chem. Anal. Ed. 14: 846.

Figure 21. Effect of gamma irradiation on ascorbic acid content of tomatoes irradiated at the mature-green stage and held at 68°F.

Figure 22. Effect of gamma irradiation on ascorbic acid content of tomatoes irradiated at the breaker stage and held at 68°F.

Figure 23. Effect of gamma irradiation on ascorbic acid content of tomatoes irradiated at the pink stage and held at 68°F.

Figure 24. Effect of gamma irradiation on ascorbic acid content of tomatoes irradiated at the table-ripe stage and held at 68°F.

seemed to occur immediately after irradiation, and in the 400-Krad treatment some recovery was observed. It should be noted that, during the first 10 days in storage (the important marketing period), for table-ripe fruits the ascorbic acid content was always higher than 15 mg/100 g in fruits of all treatments. On the other hand, fruits harvested as mature-greens and ripened in storage had only 7.5 mg ascorbic acid/100 g at the table-ripe stage. Although gamma irradiation causes some reduction in ascorbic acid content, table-ripe tomatoes still contain more ascorbic acid than do unirradiated mature-green fruits when they ripen.

Effect on texture. Textural changes--as measured by the Magness-Taylor pressure tester--in tomatoes irradiated at the mature-green stage were reported in the 1963-64 report. There was an immediate and progressive decrease in firmness with increasing dosage. Because of the importance of fruit softening in susceptibility to decay and transit injury, a more detailed study of textural changes was included in the present work.

Textural changes of tomatoes irradiated at the mature-green, breaker, pink, and table-ripe stages were measured, using the L.E.E.-Kramer shear press,⁶ before and after irradiation and at 5-day intervals thereafter. Measurement of the required force in this method was provided by the compression of a proving ring dynamometer (maximum capacity, 250 lbs). A recorder was used to obtain a time-force curve for the entire stroke, which was approximately 30 seconds in all cases. Ten fruits were used for each treatment at each determination time. Each fruit was cut from the stem scar end, to obtain a piece of 100 grams (uniform sample size), and this piece was placed in a small chamber across the slots. A plunger made of parallel stainless steel plates was forced through the sample. The amount of force required was an index of flesh firmness. In every fruit the area under the curve, as well as the peak height, was measured. Although there was good correlation

⁶ Kramer, A. 1961. The shear-press, a basic tool for the food technologist. Food Science 5: 7-16.

between the two methods of measurement, only the data on the area under the curves were used. Each point represents the average of 10 fruits.

Figure 25 shows the relative and immediate effect of gamma irradiation on textural changes in fruits irradiated at the mature-green, breaker, pink, and table-ripe stages. It is clear that an immediate loss of firmness takes place after irradiation, which increases with dosage. At 400 Krad the per cent of softening relative to the control fruits was 74, 59, 55, and 44 for fruits irradiated at the mature-green, breaker, pink, and table-ripe stages, respectively. The more advanced the ripening stage of the fruit at the time of irradiation, the less the radiation-induced loss of texture. If we compare fruits at the table-ripe stage, we see that the differences between fruits irradiated at various stages of ripeness are much smaller than those differences observed immediately after irradiation (Figure 26). However, the differences in texture between fruits irradiated at various stages of ripeness were much smaller when table-ripeness was reached than those observed immediately after irradiation (Figure 26). At this stage (table-ripe) there was still a relatively large difference between the control and the irradiated fruits. For fruits irradiated at the table-ripe stage and stored for 15 days at 68°F, the percentages of textural loss relative to the control were 10, 27.5, 29, and 33 at 50, 200, 300, and 400 Krad, respectively. The question now is how much this induced softening of fruits affects their susceptibility to transit damage and decay organisms. More work is needed to answer this question.

Effect on weight loss and permeability of fruit skin. The tomato fruit is unique in that it has a nearly impermeable skin. Gas exchange occurs primarily through the stem scar. In 1963 it was concluded that fruits irradiated at the mature-green stage showed a marked weight loss during and immediately after irradiation. These studies were continued as a part of the 1964 work. Fruits were irradiated at the mature-green and table-ripe stages at various doses and stored at 68°F and ~90% relative humidity. Twenty fruits

Figure 25. Comparative textural values of tomatoes immediately after irradiation at various stages of ripeness.

Figure 26. Comparative textural values at the table-ripe stage of tomatoes irradiated at various stages of ripeness and held at 68°F.

were used per treatment and were stored 5 to a flat to avoid variability within the storage room. The weight of the individual fruits was recorded before and after irradiation, every 4 hours for the first 3 days after treatment, then every few days thereafter until more than 25% of the total sample had been discarded.

Figure 27 shows the effect on fruits irradiated at the mature-green stage. These results are generally in agreement with those reported previously. The most rapid weight loss occurred during irradiation and in the 24-hour period thereafter. The per cent weight loss after 14 days in storage was 5.9, 6.4, 7.1, 7.3, and 7.6% for 0, 200, 400, 500, and 600 Krad, respectively. Thus the amount of weight loss due to irradiation pasteurization was much less than was found in 1963. Similar results were obtained with fruits irradiated at the table-ripe stage (Figure 28). Fruits subjected to 300 and 400 Krad lost only about 1% more in weight than the control fruits after 15 days in storage. This difference is not significant.

Treatments with paraffin wax were used to determine if water is lost from the stem scar, fruit skin, or both. Mature-green fruits were used, and the treatments were: no waxing, waxing stem scar only, waxing skin only, and waxing the entire fruit for irradiated (600 Krad) and control fruits. Weight of individual fruits was recorded periodically. The results are shown in Figure 29. It was clear that the increase in weight loss induced by irradiation was due to changes in both the skin and the stem scar. Gamma irradiation increases the permeability of the skin and thus increases water loss. This observation was also supported by our preliminary work on measuring permeability changes by a conductivity meter of small disks from the outer wall tissue of fruits.

The increased weight loss of irradiated fruits when the skin was waxed may be due in part to permeability changes of the skin, effect on the wax material, and in part to increased respiratory activity following irradiation. Waxing the stem scar of tomatoes delayed color formation of mature-green fruits. Waxing the entire

Figure 27. Effect of gamma irradiation on weight loss of tomatoes irradiated at the mature-green stage and held at 68°F.

Figure 28. Effect of gamma irradiation on weight loss of tomatoes irradiated at the table-ripe stage and held at 68°F.

Figure 29. Effect of gamma irradiation and various waxing treatments on weight loss of tomatoes irradiated at the mature-green stage and held at 68°F.

surface except the stem scar had no appreciable effect on the ripening process. When the whole fruit was waxed, resulting internal anaerobic conditions caused the fruits to be discarded 5 days after treatment.

In conclusion, gamma irradiation causes a relatively small increase in weight loss of tomatoes, but this is not a problem under normal conditions. This increase in weight loss is attributed to skin permeability changes and increased metabolic activity.

Combined effects with waxing of fruits. The purpose of this study was to determine the modifying effects of waxing on the response of tomatoes to irradiation. Treatments with paraffin wax were: waxing stem scar only, waxing skin only, and waxing the entire fruit surface. Irradiation doses were 0 and 400 Krad. Three replications of 8-10 mature-green fruits per treatment were used. Rates of respiration, ethylene production, ripening behavior, and quality changes were recorded.

Figure 30 shows the respiratory pattern throughout the storage period for these treatments. Waxing the stem scar on the skin reduced the respiratory rate, but the former was much more effective. This supports the known fact that most of the gas exchange takes place through the stem scar of the tomato fruit. When the whole surface of the fruit was waxed, a large reduction in respiratory rate occurred and within a few days all fruits were discarded. This apparently reflects internal anaerobic conditions due to CO₂ accumulation and oxygen depletion. Irradiation caused relatively comparable respiratory stimulation in the various waxing treatments for 6 days after treatment. Thereafter, the rates for the irradiated lots were somewhat lower than for the controls up to the twelfth day after treatment; then all followed a similar pattern until the end of the storage period.

Irradiation (400 Krad) stimulated ethylene production in unwaxed tomato fruits, starting immediately after treatment and continuing throughout most of the storage period (Figure 31). Waxing the skin, only, caused a reduction in the rate of ethylene

Figure 30. Effect of gamma irradiation and waxing treatments on respiration rate of tomatoes irradiated at the mature-green stage and held at 68°F.

Figure 31. Effect of gamma irradiation and waxing treatments on ethylene production of tomatoes irradiated at the mature-green stage and held at 68°F.

production, but with irradiated lots the reduction was much smaller than with control lots. Waxing the stem scar only, with or without irradiation, caused a reduction in ethylene production of 70-80% from the non-waxed treatment. Waxing the entire surface of the fruit also reduced ethylene production considerably. The effect on ethylene production can be correlated with the effects on respiration rate and ripening.

Figure 32 shows the effect of waxing and irradiation treatments on the ripening behavior of tomatoes. Irradiation retarded ripening, whether applied alone or with waxing treatments. While waxing of the skin, only, retarded ripening by 3-4 days, waxing of the stem scar retarded ripening much more. Most fruits with the stem scar waxed were discarded before reaching table-ripeness.

The effect on shelf life is shown in Table 8. A rapid decay of fruits due to anaerobic conditions resulted when the entire surface was waxed. Waxing the stem scar also caused a reduction in shelf life of 20-30% from that of the control. But waxing the skin, only, had no effect on shelf life. Fruits with no wax or with only the skin waxed showed no response to irradiation as regards shelf life. When the stem scar was waxed after irradiation, shelf life compared with that of control fruits was decreased by about 20%. This could be due to increased CO_2 production, caused by irradiation, that was not exchanged through the waxed stem scar, thus forming unfavorable anaerobic conditions in the internal atmosphere and enhancing decay.

From the results reported in this section, we conclude that one of the contributing factors in stimulating respiration and ethylene production by irradiated tomatoes is change in permeability of skin and stem scar tissues. None of the waxing treatments used, alone or in combination with irradiation, gave favorable results with respect to shelf-life extension of ripening mature-green tomatoes.

Combined effects of irradiation and chilling injury. Tomato fruits are subject to chilling injury if stored at temperatures between their freezing point and 50°F. Chilling injury is a stress

Figure 32. Effect of gamma irradiation and waxing treatments on ripening behavior of tomatoes irradiated at the mature-green stage and held at 68°F.

Table 8. Effect of irradiation and various waxing treatments on quality of tomatoes harvested at the mature-green stage and ripened at 68°F.

Treatment		Days to reach fair quality
Irradiation	Waxing	
0 Krad	None	19
0 Krad	Stem scar only	15
0 Krad	Skin only	20
0 Krad	Entire fruit surface	5
400 Krad	None	18
400 Krad	Stem scar only	12
400 Krad	Skin only	20
400 Krad	Entire fruit surface	4

condition that results in abnormal ripening behavior and increased susceptibility to decay, especially by Alternaria rot. These symptoms do not become apparent until the tomatoes have been in the ripening room for 2 or 3 days. The injurious effects of low temperature are cumulative and additive to the effects of other stresses, such as gamma irradiation. Results of earlier experiments with mature-green tomatoes reported in the 1963-64 report showed that these two stresses are additive and the effect is both qualitative and quantitative. Experiments were conducted during 1964 to investigate the effects of chilling in combination with irradiation on pink and table-ripe tomatoes. The effects on respiration rate and ethylene production by fruits treated at the mature-green stage were also investigated, in the hope of obtaining evidence of the causes of injury to the fruits.

Fruits harvested at the pink and table-ripe stages were irradiated at 0, 200, 300, and 400 Krad. For each of these treatments, one lot of 25 fruits was stored continuously at 68°F and another two lots chilled at 32°F for 4 or 8 days, then transferred to 68°F for the remainder of the storage period. Table 9 shows the percentages of discarded fruits for each lot of fruits treated at the pink stage.

Similar results were obtained with fruits treated at the table-ripe stage. In all cases the chilling injury symptoms showed much more clearly when fruits were chilled for 8 days than for 4 days. Chilling increases the susceptibility of fruits to attack by decay organisms. Radiation intensifies this effect, presumably because of induced softening of the fruit. This makes chilling injury much more serious when radiation is used as a pasteurization treatment on fruits subjected to chilling temperatures.

The effect of various combined treatments of chilling and irradiation on the respiratory rate of tomatoes is shown in Figure 33. Irradiation, only, applied immediately after harvest or after 8 days in storage at 68°F, caused a rise in the respiratory rate; but the magnitude of stimulation was much lower for fruits irradiated after 8 days at 68°F than for those irradiated immediately after harvest.

Table 9. Effect of irradiation and chilling injury on per cent of discarded tomatoes irradiated at the pink stage.

Treatment		Per cent of discarded fruits after 20 days in storage
Irradiation	Temperature	
0 Krad	Held continuously at 68°F	80
0 Krad	Chilled for 8 days at 32°F	89
200 Krad	Held continuously at 68°F	70
200 Krad	Chilled for 8 days at 32°F	82
300 Krad	Held continuously at 68°F	53
300 Krad	Chilled for 8 days at 32°F	75
400 Krad	Held continuously at 68°F	62
400 Krad	Chilled for 8 days at 32°F	78

Figure 33. Combined effect of irradiation and chilling injury on respiratory rate of tomatoes treated at the mature-green stage.

Fruits irradiated immediately after harvest and stored at 32°F for 8 days showed a small increase, then followed the respiratory pattern of control fruits stored at 32°F for 8 days. In both cases, when fruits were transferred to 68°F, the respiratory rate increased 12-15 fold in response to the increased temperature. Fruits irradiated after being kept at 32°F for 8 days had even a larger increase in respiratory rate, which remained higher than for all other treatments throughout the storage period. This increase was a result of both temperature change and irradiation.

Ethylene production was stimulated by irradiation, as noted earlier, when applied after harvest, after 8 days at 68°F, or after 8 days at 32°F (Figure 34). All fruits that were stored at 32°F for 8 days produced much less ethylene when transferred to 68°F than did fruits held continuously at 68°F. The effect of irradiation and/or chilling treatments on ethylene production can be correlated with the ripening behavior of the fruits. Figure 35 shows that irradiation alone, applied immediately after harvest, delayed ripening by 4 days but had no effect when applied at 68°F 8 days after harvest. Fruits that were subjected to 32°F failed to reach the table-ripe stage before they were discarded.

From the data presented here and in the 1963-64 report, it can be concluded that:

1. The two stresses of gamma irradiation and chilling injury are similar in many of their symptoms, such as intensification of mechanical injury, delay of ripening, and increased susceptibility of decay.

2. The effects of these two stresses are qualitatively and quantitatively additive when applied to the same fruits at any of the stages of ripeness tested (mature-green, pink, and table-ripe).

3. The correlation between the respiratory patterns, ethylene production, and other symptoms of these stresses cannot be used to postulate any specific mechanism.

4. Both chilling injury (as reported by other workers) and gamma irradiation increase cell membrane permeability in tomatoes, indicating that some of the mechanisms of the two stresses are similar.

Figure 34. Combined effect of irradiation and chilling injury on ethylene production by tomatoes treated at the mature-green stage.

Figure 35. Combined effect of irradiation and chilling injury on the ripening behavior of tomatoes treated at the mature-green stage.

5. If gamma irradiation is to be used for extending the shelf life of tomatoes harvested at the pink or table-ripe stages, temperature control during transit and storage will be very important. Chilling temperatures should be avoided both before and after irradiation. Gamma irradiation treatment should not be used with fruits subjected to extensive chilling injury in the field.

Combined effect of gamma irradiation and growth regulators.

Growth regulators of various types have been used as post-harvest treatments on fruits and vegetables. Their effects vary with species and variety, depending on concentration, method of application, and time of treatment. One of the proposed mechanisms of radiation damage is that irradiation at doses as low as 10 Krad causes the destruction of endogenous auxin by the disruption of the auxin-synthesizing enzymes.⁷ The present study was initiated to investigate the effects of treating irradiated tomatoes with exogenous auxin (indoleacetic acid), gibberellic acid, and kinetin.

Fruits were treated at the mature-green, pink, and table-ripe stages of ripeness. Radiation doses of 300 and 400 Krad were used. Indoleacetic acid (1000 ppm) was applied as a dip treatment for 30 minutes before or after irradiation. Control fruits were dipped in distilled water for the same period. Fruits were held at 68°F after treatment and observations on ripening behavior and shelf life were recorded.

In the case of tomatoes treated at the mature-green stage (Figure 36), IAA applied alone had little effect on ripening. Irradiation with 400 Krad retarded ripening by 4-5 days. IAA applied after irradiation had little or no effect on ripening but, when applied before irradiation, retarded ripening by 1-2 days. The difference in the effect of IAA applied before and after irradiation may reflect differences in the actual amount of the auxin that enters the fruit because of increased permeability of

⁷ Gordon, S. A. 1957. The effects of ionizing radiation on plants: biochemical and physiological aspects. Quart. Rev. Biol. 32: 3-14.

Figure 36. Effect of gamma irradiation and indoleacetic acid (1000 ppm) on ripening of tomatoes treated at the mature-green stage and held at 68°F.

the skin upon irradiation. Figure 37 shows the effect on shelf life expressed as percentage of fruits discarded throughout the storage period. While irradiation alone or irradiation plus IAA had no effect, IAA alone and IAA plus irradiation decreased the per cent decayed by 20 and 32%, respectively.

Ripening of fruits irradiated at the pink stage was retarded for 2 or more days by all treatments used (Figure 38). The percentage of discarded fruits after 20 days in storage was decreased by 37% when fruits were treated by IAA plus 300 Krad (Figure 39). When irradiation or IAA was applied alone, decay control was much less; however, when IAA was applied after irradiation, decay amounted to 25%.

Figure 40 shows the effects on shelf life of fruits treated at the table-ripe stage. Treatment with irradiation plus IAA or IAA alone increased decay to some extent. Irradiation alone and IAA plus irradiation slightly decreased the percentage of decay. Some damage observed after IAA treatment may have been caused by the relatively high concentration of 1000 ppm. Thus, the effects of a series of concentrations should be investigated.

In conclusion, we can say that there is some promise in using IAA in combination with gamma irradiation as a post-harvest treatment to extend the shelf life of tomatoes. More experiments are under way or will be initiated during the 1965 season to investigate the effects of indoleacetic acid, gibberellic acid, and kinetin on respiration, ethylene production, ripening, and shelf life of tomatoes.

SUMMARY AND CONCLUSIONS

Most of the results with tomatoes (mature-greens and breakers) reported in 1963-64 were negative with respect to using gamma irradiation for extending shelf life. It appeared that only under conditions where decay losses would otherwise be excessive would irradiation be valuable. From experiments completed during the 1964 season, we can conclude the following:

Figure 37. Effect of gamma irradiation and indoleacetic acid (1000 ppm) on deterioration of tomatoes treated at the mature-green stage and held at 68°F.

Figure 38. Effect of gamma irradiation and indoleacetic acid (1000 ppm) on ripening of tomatoes treated at the pink stage and held at 68°F.

Figure 39. Effect of gamma irradiation and indoleacetic acid (1000 ppm) on deterioration of tomatoes treated at the pink stage and held at 68°F.

Figure 40. Effect of gamma irradiation and indoleacetic acid (1000 ppm) on deterioration of tomatoes treated at the table-ripe stage and held at 68°F.

1. The adverse effects of irradiation are less in fruits irradiated at the pink or table-ripe stage. An extension of shelf life of 4-12 days can be obtained by irradiation at doses between 200 and 350 Krad. The magnitude of shelf-life extension depends upon the condition of fruits at harvest, handling procedures, postirradiation attack by decay organisms, and storage temperature.

The two factors which may limit the use of irradiation with tomatoes are:

a. Increased softening of fruits by irradiation may affect their susceptibility to transit injury. More work is planned to clarify this point during 1965.

b. Effect on flavor. Taste panel evaluation of irradiated table-ripe tomatoes (up to 300 Krad) showed no significant effect on flavor for the first week after treatment. In the second week, fruits irradiated at doses between 200 and 400 Krad were scored lower in flavor than control fruits. The effect of radiation was not consistent from fruit to fruit. Some were comparable to control fruits, while others had a distinct off-flavor. It seems likely that, if this evaluation were done on a basis other than paired comparisons, no important differences in flavor would be detected for ripe fruits irradiated at doses up to 300 Krad. Further taste evaluations will be made to check these speculations.

2. Gamma irradiation causes an immediate increase in the respiratory activity of tomatoes, with the magnitude of the increase directly proportional to dose. The magnitude and duration of this stimulation decline with increasing ripeness. Doses up to 500 Krad stimulate ethylene production; at higher doses, ethylene production is reduced. Ripening behavior can be correlated with the effect on ethylene production.

3. Retardation of ripening of mature-green fruits by doses of 300 Krad or more can be reversed by using ethylene treatment.

However, the required concentration of ethylene and the duration of application are greater than those needed to hasten ripening of control fruits.

4. Although gamma irradiation causes a reduction in ascorbic acid content, the remaining quantity is significant nutritionally. There is more ascorbic acid in irradiated table-ripe fruits than in control fruits harvested as mature-green and ripened in storage.

5. Gamma irradiation causes an increase in weight loss, but this is not of great significance. It also increases permeability of the fruit skin.

6. Chilling injury is additive to radiation damage, and thus temperature control is a very important factor in tomato transit and storage.

7. There are some promising results from the use of indoleacetic acid in addition to radiation for better extension of shelf life for mature-green tomatoes.

COMPARATIVE INACTIVATION OF CONIDIA, MYCELIA, AND SCLEROTIA OF
BOTRYTIS CINEREA BY GAMMA RADIATION^{1,2}

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ABSTRACT

The radiation sensitivity of conidia, mycelia, and sclerotia of Botrytis cinerea was compared using an "end-point" technique. Young mycelia were more resistant than either mature mycelia or sclerotia when populations of these structures were equated by dry weight, protein, or DNA content. Sclerotia were more resistant than mature mycelia and conidia on the basis of equivalent protein and more resistant than conidia on a dry weight basis.

INTRODUCTION

The fungus Botrytis cinerea Pers. ex Fr. is the cause of important post-harvest diseases of a wide variety of fruits and vegetables. Since the fungus is able to grow and decay the host at -1°C or less, modern temperature management in cold storage and transit slows but does not prevent disease development (Harvey and Pentzer, 1960). Chemical protective fungicides are usually ineffective if infection precedes harvest, and preventing the disease in the field may be impractical or impossible. Particularly under such conditions, ionizing radiation may be of value as a therapeutic fungicidal treatment.

Despite widespread interest in the control of Botrytis rots by ionizing radiation, no detailed studies appear to have been made of the radiation sensitivity of the fungus. Published accounts of "lethal doses" have varied widely. Skou (1960) reported the "lethal

¹ Received for publication _____.

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dose" to be greater than 470 Krad. Beraha et al. (1960) reported that the approximate doses lethal to young mycelium in vitro varied from 95-203 Krad. In the host, a comparable effect required 274-456 Krad. Saravacos et al. (1962) reported a "lethal dose" of 500 Krad for the fungus in culture. Kljajić (1960) found that 600-1,000 Krad was needed for a "lethal" effect. However, Sommer et al. (1964) pointed out the importance of considering fungus population size or extent of lesion development in estimating the fungicidal effectiveness of a radiation dose. In the latter report, end-point studies provided an experimental estimation of the doses required to inactivate all fungus cells in conidial populations of various sizes.

The fungus population of disease lesions is composed of mycelia, sometimes accompanied by sclerotia and conidia. The various morphological structures presumably might vary in sensitivity. In fact, Schwinghamer (1958) estimated that mycelia of a rust fungus were ca. 20 times more sensitive than its uredospores. If so, the relative abundance of the different morphological structures might influence the effectiveness of a given dose.

The investigation reported here was made to compare the radiation sensitivities of conidia, mycelia of various ages, and sclerotia. The multicellularity of structures other than conidia prevent the ready determination of sensitivity on a per cell basis. Consequently, inactivation of populations of the various structures was studied. Dry weight, protein and deoxyribose nucleic acid (DNA) were utilized to equate sample populations.

METHODS

The culture of B. cinerea used was isolated from a fruit of Prunus persica L. (nectarine) which had developed the gray mold disease in -1°C storage. A subculture was selected for abundant sporulation in culture. Tests revealed that the isolate thus obtained was highly pathogenic to fruits of Fragaria and Prunus sp.

Conidia were produced by growing the fungus cultures in 500-ml Erlenmeyer flasks on 100 ml of V-8 juice agar (1 liter of

Campbell's V-8 vegetable juice plus 10 gm dextrose with 2% agar adjusted to pH 7.0 before autoclaving). The cultures were normally grown for at least two weeks in an air-conditioned room (ca. 22-24°C). Conidia were harvested in water with added "Tween 80" (3 drops per 200 ml water) and were dislodged by scraping with a glass rot and agitating with glass beads. The conidial suspension was strained through several layers of sterile cheesecloth to remove mycelial fragments. The spores were centrifuged down at least once, and the liquid replaced with fresh "Tween" solution. Concentrations of conidia were measured by relating the optical density of the suspension to a curve previously established with aid of a counting chamber. The concentration was verified by plating appropriate volumes of the suspension on potato-dextrose agar in Petri dishes and counting the colonies that developed.

Mycelium was obtained by growing the colony on cellophane circles of about 4.4 cm diameter (approximately 15 sq cm) over 10 ml of potato-dextrose agar in 15 X 100-mm Petri dishes. The cellophane film used was approximately one mil thick and had not received a waterproofing treatment in manufacture (Avisco Cellophane type 215PL). Inoculum consisting of ca. 10^3 conidia in 0.01 ml of a spore suspension was placed on the center of the heat-sterilized cellophane with a capillary pipette. If young mycelium was desired, the resulting colony was permitted to grow near, but not beyond, the edge of the cellophane. To obtain mature mycelium the colony was allowed to grow beyond the edge of the cellophane circle to cover the plate almost completely so that only relatively old hyphae were above the cellophane circle. At this stage no conidia or sclerotia were detected. The cellophane circles supporting mycelia were lifted from the medium in preparation for irradiation treatments. After irradiation the circles were placed on fresh potato-dextrose agar in Petri dishes. To ensure that all portions of the mycelia were in contact with the medium, 5 ml of 1/2-strength potato-dextrose agar cooled to ca. 40°C was poured over the colony. Since post-irradiation incubation periods were usually two weeks or more, the Petri dishes were enclosed in polyethylene bags to prevent desiccation.

Sclerotia were similarly produced from colonies growing on cellophane. Here, the cellophane circles completely covered the surface of the potato-dextrose agar in Petri dishes. After inoculation the dishes were incubated for about seven months at -1°C . Under these conditions abundant and well delimited sclerotia were produced, although under more favorable growing temperatures this isolate forms few sclerotia.

When mycelial colonies were prepared for irradiation treatment, replicate colonies of similar size and appearance were utilized for dry-weight determinations and chemical analyses. For experiments utilizing sclerotia, a preliminary segregation was made for uniformity of sclerotium size. The fresh weight of each sclerotium was determined under aseptic conditions after careful blotting to remove surface moisture. As with mycelia, certain replicates were used for dry-weight determinations and chemical analyses, and the other replicates were irradiated.

For radiation sensitivity comparisons, an end-point technique previously described in detail was used (Sommer *et al.*, 1964a, b). Populations of known size (replicated 20 times) of conidia, mycelia, or sclerotia were irradiated with a selection of doses. Ideally, the lowest dose would inactivate none and the highest dose would inactivate all populations. Obviously, to inactivate a population, every constituent cell capable of unlimited growth must be inactivated, and failure to achieve population inactivation implies the survival of one or more cells. In some cases a variety of population sizes was used. The percentages of populations that failed to grow were plotted against dose, typically yielding a sigmoid curve. From this preliminary plot one could approximate by interpolation the doses causing the inactivation of different percentages of the populations. Used in this study were the doses which inactivated 50% of the populations, called for convenience the "colony inactivation dose 50" (C.I.D._{50}). When the population size was plotted logarithmically against dose, the C.I.D._{50} points were found to lie near a straight line over the range of doses and population sizes studied.

As an alternative method for expressing the results of end-point experiments, radiation D values were computed according to the following equation, suggested by Schmidt and Nank (1960):

$$\underline{D} = \frac{\text{Radiation dose (Megarad)}}{\text{Log } \underline{M} - \text{Log } \underline{S}}$$

where M = the total inoculum, that is, the number of conidia per population or spore equivalents representing the population times the number of populations.

S = the number of populations developing colonies, assuming one surviving conidium per population.

In order to use the equation in connection with populations of sclerotia or mycelia, protein, DNA or dry weight was used as a reference standard and the number of viable units was estimated from the number of conidia yielding the same amount of reference material. Thus, appropriate "spore equivalents" were used in calculating D values for these structures.

Irradiation was performed in an AEC Mark II Co⁶⁰ food irradiator. The gamma dose was absorbed at about 3 Krad/min. All fungus material was moist and in contact with oxygen at the time of irradiation. The containers used for irradiation were 125-ml Erlenmeyer flasks fitted with 2-hole stoppers for hose connections. Conidia were irradiated in aqueous suspensions with about 10² to 1.7 X 10⁹ conidia per ml and 8-14 ml per flask. Folded cellophane circles bearing mycelia, or small paper packets containing sclerotia, were similarly irradiated in the flasks. Air at the rate of 200 cc/min was passed through each flask during irradiation.

Protein was determined according to the method of Lowry et al. (1951). Used as the standard material was bovine albumin, crystallized and lyophilized, obtained from Sigma Chemical Company. Readings were made at 540 mμ in 1/2-in. diameter cells in a Bausch and Lomb Spectronic 20 photoelectric colorimeter. Protein was released from spores by sonicating suspensions of 2 to 4 X 10⁷ conidia per ml in 0.1 M phosphate buffer at pH 7.1 containing 0.125% sodium deoxycholate. Ninety-eight per cent or more of the conidia

were disrupted by 20 minutes of sonic treatment at the highest tap setting of a 20-Kc Branson Model S-110 sonifier. For protein from dried mycelial material, a weighed sample was first ground in a mortar with a small amount of deoxycholate-buffer solution. The sample was then transferred to a graduated conical centrifuge tube and brought to a convenient volume, usually 12-15 ml, with rinsings of the same solution. These samples were sonicated for 10 minutes. Sclerotial material was treated similarly except that sonic treatment was for 15 minutes. Ground and sonicated samples were centrifuged to free them from cell debris, and 1.0-ml portions of the clear supernatant solution were used for analyses. The following dry-weight quantities of starting material were found adequate to provide soluble protein solutions of suitable concentrations for analysis by this method: mycelia, 10-20 mg; sclerotia, 20-40 mg; spores, 8-15 mg.

DNA was evaluated by the indole method of Ceriotti (1952) modified according to suggestions of Keck (1956). Fungus cells were disrupted by grinding and sonicating as in the protein determinations. Proteins were then precipitated with sufficient trichloroacetic acid to produce a final concentration of 10%. After centrifugation, the supernatant fluids were removed and residues were subsequently treated in a sequence of 5% trichloroacetic acid, 95% ethyl alcohol, equivolumes of alcohol:acetone, and acetone. Finally they were extracted for 30 minutes with hot 5% trichloroacetic acid to obtain nucleic acid. Interfering pink colors were removed after the indole reaction by using either one extraction with chloroform and one with amyl acetate, or two extractions with chloroform and one with amyl acetate, or two extractions with amyl acetate. Optical density was read on a Beckman Model DB spectrophotometer.

RESULTS

When the C.I.D.₅₀ for conidia were plotted against the log of the population size, the points were seen to lie near a straight line (Figure 1). To compare the sensitivities of mycelia and

Figure 1. Doses required to inactivate 50% of populations (C.I.D.₅₀) of conidia as influenced by the number of conidia comprising the populations.

sclerotia with that of conidia, a similar plot was made in which the population sizes of all structures were expressed as dry weight. In preliminary tests it became evident that the dose required to inactivate mycelial colonies was extremely variable from one experiment to another. Since these differences in sensitivity to inactivation could not be accounted for by variability in colony size, a heterogeneity in the colonies was suspected. One source of variability in the colony could be the proportion of young, actively growing hyphae at the colony edge compared to the more mature and possibly somewhat quiescent hyphae in older portions of the colony.

When compared on an equal dry-weight basis, young hyphae were more resistant than spores, sclerotia, or mature hyphae (Figure 2). However, the differences in resistance might merely reflect the inadequacy of dry weight in equating population sizes. Both conidia and mature mycelia have thickened walls, while the older hyphae may be devoid of protoplasm as well. Young mycelia, in contrast, have thin walls and dense cytoplasm. Consequently, comparable dry weights would represent more protoplasm in young, and less in mature hyphae and conidia.

Equilibration of "populations" of fungus structures by protein analyses revealed that even on this basis young hyphae were still inordinately resistant to inactivation by radiation (Figure 3). It remained to be considered if radiation resistance might be more closely correlated with the numbers of nuclei present. The concentration of nuclei in young, growing hyphae would impart resistance if cell inactivation required irreparable damage to virtually all nuclei. To test the possibility that the resistance of young hyphae was associated with an abundance of nuclei, DNA analyses were made. After examination of Figure 4, however, it was apparent that the young mycelia had greater resistance than could be explained by the DNA content.

Sclerotia are formed among mature mycelia in older portions of the colony. In sensitivity, however, these structures are seen to approximate young hyphae more nearly than either mature hyphae or conidia (Figures 2, 3, 4).

Figure 2. Doses required to inactivate 50% of populations (C.I.D.₅₀) of conidia, sclerotia, and mycelia when population sizes were compared by dry weight.

Figure 3. Doses required to inactivate 50% of populations (C.I.D.₅₀) of conidia, sclerotia, and mycelia when population sizes were compared by protein analyses.

Figure 4. Doses required to inactivate 50% of populations (C.I.D.₅₀) of conidia, sclerotia, and mycelia when population sizes were compared by DNA analyses.

Figure 5 provides D values obtained for conidia, mycelia, and sclerotia when the fungus structures were equated by dry weight, protein, or DNA contents. When the significance of differences was evaluated by the "t" test, results were as follows: young mycelia were more resistant ($P = 0.01$) than mature mycelia or conidia regardless of the method used for equating populations; young mycelia were more resistant ($P = 0.01$) than sclerotia when populations were compared on the basis of dry weight and DNA; sclerotia were more resistant than mature mycelia and conidia when equated by protein content ($P = 0.01$); and, sclerotia were more resistant than conidia when compared on a dry weight basis ($P = 0.05$).

The relatively low water content of sclerotia (35-55%) presumably might contribute to their resistance. The water content of young mycelia (83%) and mature mycelia (77%), however, would appear to be too similar for that to be of consequence.

DISCUSSION

The greater resistance to irradiation of young hyphae than of mature hyphae and conidia has not been adequately explained. When populations were equated by dry weight it was expected that mature hyphae, with their thickened walls and large vacuoles, would be less resistant than young hyphae. It was anticipated, however, that resistance would be similar if populations were equated by DNA content. Here, different populations presumably contained approximately equal numbers of nuclei if we assume that the nuclei were of equal size. That populations of young hyphae, although containing no more DNA, were still more radiation resistant suggests several possible reasons which deserve future attention. Possibly, nuclei in young, actively growing hyphae are smaller than in mature mycelia, so that equal DNA content does not represent equal numbers of nuclei. On the other hand, young hyphae might contain radio-protective substances which are less abundant in mature hyphae.

As colonies increase in size, the relative proportions of the component structures vary. New colonies would consist almost entirely

Figure 5. D values obtained for inactivation of conidia, sclerotia, and mycelial populations. Vertical lines represent one standard deviation above and below the mean. ▲ = young mycelia, △ = mature mycelia, ● = spores, and ○ = sclerotia.

of young mycelia. As colonies enlarge, the amount of young hyphae would decrease relative to the amount of mature mycelia present. In time, the presence of relatively radiation-resistant sclerotia would influence the dose required for colony inactivation. Sporulation resulting in the presence of conidia would similarly influence the colony inactivation dose. The heterogeneity of Botrytis colonies in radiation resistance is thus abundantly clear.

Careful studies of the inactivation of Botrytis lesions in vivo obviously require comparable colonies. These could probably be best achieved by inoculating with a uniform number of conidia and standardizing the incubation period as to time and temperature.

Although an "end-point" technique, as here used, is required for studies of the radiation sensitivity of filamentous fungi, an important limitation is inherent in this method. Since the inactivation of every cell in a population is determined, the dose required reflects the sensitivity of the most resistant individuals. For this reason, conditions which might influence the radiation sensitivity of a large portion of the population might not be detected.

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