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IRRADIATION OF FRUITS AND VEGETABLES

Annual Report, April 16, 1966—April 15, 1967

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Department of Food Science
Michigan State University
East Lansing, Michigan

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Annual Report
IRRADIATION OF FRUITS AND VEGETABLES

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Michigan State University
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INTRODUCTION

This report covers the first year's work under Contract No. AT(11-1)-1592 of the A.E.C. Previous studies on the radiation preservation of fruits and vegetables performed at Michigan State University (MSU) under Contract No. AT(11-1)-1283 have already been reported to the A.E.C.

The accomplishments of the past year are described under two headings: commodity studies and yeast studies. All the irradiations reported herein were performed with the Co-60 source of the Phoenix Memorial Laboratory, University of Michigan (source strength about 10,000 curies). Storage and analysis of samples was performed at M.S.U.

COMMODITY STUDIES

The experiments with fruits and vegetables fall into two general categories: those in which the objective of the irradiation was the delay in the decay of the commodity through reduction of the microbial load, and those in which shelf life extension was expected through retardation of maturation by irradiation.

SUMMARY

Radiation at 30 Krad retarded the elongation of asparagus spears during storage without apparent deleterious effects; however, the analyses have not demonstrated a quality superiority, such as improved texture or less wastage.

Irradiation of strawberries performed at the fully ripe stage did not confirm previous results. This season (1966) there was no increase in shelf life, in contrast to other years when 200 Krad had appreciably extended the shelf life. The inferior quality of this year's berries and their high initial contamination may have caused the poor response.

A dose of 300 Krad materially increased the rate at which blueberries were subsequently freeze-dried.

The untreated sweet cherries of this year kept well for four weeks and irradiation (200 Krad) did not improve their shelf life. The radiation softening of sweet cherries was overcome by soaking the cherries in a CaCl_2 solution, preferably before irradiation.

The post-harvest maturation of sweet corn was not inhibited by irradiation (40 Krad or 300 Krad).

Combinations of heat (120° F, 30 min.) and irradiation (150 Krad) treatment of apple juice did not give a synergistic effect on microbial destruction. However, total counts in reasonably clean juice did approach pasteurization and there was some promise of improved quality.

Irradiation of mushrooms at doses above 10 Krad markedly retarded the opening of the mushroom caps during storage. This effect appears most promising as an application of radiation to foods.

ASPARAGUS

Experiment I

In a preliminary trial, Michigan-grown asparagus harvested the first week of June was packed in wet paper towels and a polyethylene overwrap and exposed to 30 Krad of gamma irradiation. During a two week post-irradiation storage at 32° F the irradiated samples showed a small but statistically significant decrease of spear growth (0.18 in. vs. 0.26 in. for the controls). No significant color difference was observed between irradiated and control samples.

Experiment II

On July 17, 1966, asparagus was harvested in Romulus, Michigan. The spears were cut off below ground level and only "A" grade spears were selected.

The asparagus was sorted to eliminate any bent or feathered spears and each spear was cut to $8.00 \pm .05$ inches. The spears were then placed into 30 bunches, 10 spears per bunch. A paper towel was placed around the base of each bunch, the towel dipped in water, and finally the base of the bunch was placed in a plastic cup containing about one inch of water. The entire unit was then over-wrapped with a perforated plastic bag and placed in a chest containing crushed ice.

These samples were then transported to the Phoenix Memorial Laboratory in Ann Arbor where 3 groups of 10 bunches each received 0, 10, and 100 Krad. Irradiation was performed at ambient temperature (approximately 48°F). After irradiation, the samples were returned to East Lansing and stored at $38 \pm 1^\circ\text{F}$.

Analysis: Growth, color, and texture were used to determine the quality of the spears at 0, 4, 8, 16, and 32 days after irradiation.

Growth: At each time interval, two bunches (20 spears) of each dose were measured to $\pm .05$ inches.

Color: The color of the spears was inspected visually and by colorimetric methods (absorbance of an 80% acetone extract measured at 440 and 660 mμ).

Texture: The shear resistance of the spears was measured with an Allo-Kramer shear press (250 lb. ring, range 200, Lo). The spears were cut in half and measurements taken on 75 grams each of the top and bottom portions.

Results: Table 1 shows the growth of the asparagus spears during the 32 day storage period. It can be seen that irradiation retards the growth of the spears, especially during the first half of the storage period. Also, it should be noted that at each time interval the 30 Krad samples showed less growth than did the 100 Krad samples.

Table I. Length of asparagus (inches), harvested and irradiated on 6-17-66. (Average of 20 spears. Storage temperature 38 $\pm$ 1° F).

Dose - Krad	Storage time - days					Ave.
	0	4	8	16	32 ^a	
0	8.34	8.82	8.75	8.77	8.91	8.72
30	8.35	8.55	8.55	8.60	8.56	8.52
100	8.32	8.61	8.62	8.68	8.68	8.58

^aAt 32 days, a full sample of 20 was not measured due to deterioration of 10 to 20% of the spear tips.

The visual and colorimetric analysis showed no important color differences between irradiated and control samples (data not shown).

The texture analysis showed no significant correlation between shear resistance and irradiation (Table II). Also, it was found that the shear resistance did not differ between the top and bottom of the spear, in spite of the fact that a difference in snap resistance and resilience (between

top and bottom) was easily discernible by handling the spears. The usefulness of the shear press in determining the texture quality of asparagus, and perhaps other products, may seriously be questioned. There is a toughening of the spears up to 4-8 days of storage; however, by 16 days softening had occurred. This effect is independent of dose.

Table II. Shear values^a (micro-amperes) of asparagus harvested and irradiated on 6-17-66 and stored at 38 ± 1° F.

Dose, Krad	Portion of Spear	Days after Irradiation				
		0	4	8	16	32
0	top	33.7	53.0	51.0	38.0	37.0
0	bottom	34.0	50.5	54.5	37.5	38.5
30	top	35.9	52.0	53.0	39.5	36.0
30	bottom	36.1	51.5	53.0	39.0	39.5
100	top	32.0	52.0	54.5	39.0	38.0
100	bottom	32.1	51.0	53.0	38.0	37.5

^aAllo-Kramer shear press with 250 lb. ring, range 100, Lo, on 75g samples.

Conclusion: Irradiation of asparagus at levels of 30 and 100 Krad under the conditions of this experiment did retard growth. Shear press tests, however, did not reveal any concomitant retardation in toughening.

Experiment I

Jersey variety blueberries were purchased on August 2, 1966 from Eldon Knapp of Ionia, Michigan. The berries had been harvested on August 1, 1966. After packing into pint boxes, the berries were transported to the Phoenix Memorial Laboratory in Ann Arbor where they were given doses of 0, 150, and 300 Krad.

On return to East Lansing the samples were weighed into aluminum pans (approximately 300 g per pan, two pans per dose) and frozen at -30°F . Also, three 100 g samples of each dose were used for texture analysis on the Allo-Kramer shear press (250 lb ring, range 50, Lo).

On August 4, the frozen samples were placed in a Stokes freeze-dryer (circulating water at 100°F , 100-125u Hg. psia.) for two hours. At this time the samples were removed and weighed. Then they were again placed in the dryer and weights taken after 4, 6, 10, and 24 hours. It must be noted that the same set of samples was used for all drying periods.

Table III gives the percent weight loss at the end of each drying period (each value is the average of two samples).

Table III. Percent weight loss of Jersey blueberries during freeze drying. Irradiated on 8-2-66, average of two samples.

Dose-Krad	Hours of Freeze Drying				
	2	4	6	10	24
0	7.2	29.7	44.3	66.0	79.2
150	8.3	31.0	44.3	66.8	80.9
300	11.4	34.2	50.9	73.4	81.3

The data shows that the irradiated samples had a faster drying rate. However, the difference is only significant at the early stages. This is due

to the fact that the same sample lot was used for all time intervals. When the vacuum was released, the warm air entering the berries melted the water within. Then, when a vacuum was again pulled on the chamber, the liquid bubbled out of the berries, thus allowing faster drying for all of the samples.

Texture: Table IV gives the shear values for the blueberry samples. It shows a softening effect on the berries at 150 and 300 Krad.

Table IV. Shear values^a (peak readings in micro-amperes) of Jersey variety blueberries harvested on 8-1-66, irradiated 8-2-66.

Dose-Krad	Sample			Average
	1	2	3	
0	46	50	48	48.0
150	39	40	44	41.0
300	40	42	40	40.7

^aAllo-Kramer shear press, 250 lb. ring, range 50, Lo.

Conclusions: Irradiation does increase the freeze drying rate of Jersey blueberries under the conditions of this experiment. However, due to the freeze-drying technique used in this experiment, the full effect is not shown (see Blueberries - Experiment II).

Also, irradiation causes a significant softening of fresh blueberries at levels of 150 and 300 Krad.

Blueberries - Experiment II

On August 16, 1966, Jersey blueberries were purchased from Eldon Knapp of Ionia, Michigan. They were taken to Ann Arbor in one pint boxes and exposed to irradiation doses of 0 and 300 Krad. On return to East Lansing, eight pints of each dose were spread on stainless steel pans and frozen at -5° F. After freezing, the temperature of the berries was lowered to -30° F in preparation

of the freeze-drying operation.

Freeze-drying: For this experiment, a separate sample lot was used for each time interval. The sample lots were dried for 3, 8, 18, and 30 hours in a Stokes freeze-dryer (circulating water at 100° F, 100-125 μ Hg. psia.). The samples were weighed before and after drying. These weights were used to calculate the percent weight loss for each time interval. Duplicate samples were run for each dose at each time interval.

Table V gives the calculated percent weight loss at the designated time intervals. It can be seen from these data that irradiation at 300 Krad greatly increases the rate of water removal during freeze-drying.

Table V. Percent weight loss of Jersey blueberries during freeze-drying. Average of two samples irradiated 8-16-66.

Dose-Krad	Drying time (hours)				
	0	3	8	18	30
0	0	10.3	33.7	61.8	71.8
300	0	14.1	41.8	73.8	83.1

Conclusions and Remarks: Gamma irradiation (300 Krad) could effectively be used to increase the freeze-drying rate of fresh whole Jersey blueberries. Reconstitution of the samples was not attempted because, after 30 hours of drying, the blueberries still did not have enough water removed to give them the structure and characteristics of a freeze-dried fruit. Therefore, reliable reconstitution data must be obtained from a later experiment.

Experiment III

On August 16, 1966, Jersey variety blueberries were purchased from Eldon Knapp of Ionia, Michigan. After being packed into pint boxes, the berries were taken to the Phoenix Memorial Laboratory where they were given doses of 0 and

200 Krad. On return to East Lansing, six pint boxes of each dose were soaked in a 0.5% Ca^{++} solution (from $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) for one hour.

The firmness and texture of the berries, as measured by the Allo-Kramer shear press, were tested at 1, 13, and 27 days following irradiation (storage temperature $38 \pm 1^\circ \text{F.}$). Table VI gives the shear data (microamperes) for the blueberries at the designated storage intervals. Due to malfunction of the shear press in the Food Science Laboratory, the data obtained at 13 days of storage were taken from the shear press (100 lb. ring, range 100 on 40 g. samples) in another department. Therefore, the values at this interval should not be compared to those taken at other storage times. They can only be used to compare the various treatments at 13 days of storage.

Table VI. Peak shear values (micro-amperes) for Jersey variety blueberries irradiated 8-16-66. Average of four to five samples stored at $38 \pm 1^\circ \text{F.}$

Dose - Krad	Treatment	Days after Irradiation			% loss of firmness in 26 days
		1 ^a	13 ^b	27 ^a	
0	none	47.2	100.5	30.0	36
0	Ca^{++}	52.4	127.0	35.2	33
200	none	32.6	61.5	21.5	34
200	Ca^{++}	41.6	108.7	39.8	4

^aAllo-Kramer shear press -- 250 lb. ring, range 50, Lo, on 100 g. samples

^bAllo-Kramer shear press -- 100 lb. ring, range 100, on 40 g. samples

Conclusions:

Gamma irradiation at the level of 200 Krad caused a significant softening of Jersey variety blueberries. The control samples gave a shear value of 47.2 μa one day after irradiation as compared to 32.6 μa for the 200 Krad samples. When the berries were soaked, after irradiation, in a 0.5% Ca^{++} solution for one hour, the softening effect of the gamma irradiation is nearly eliminated after only one day of storage. Then, after 13 days of storage, the soaked-

irradiated samples appeared to be even more firm than the control samples.

After 27 days of storage at $38 \pm 1^\circ$ F., the 200 Krad soaked samples were shown to be firmer than either the control or 0 Krad soaked samples.

STRAWBERRIES

Experiment I

Robinson variety strawberries for this experiment were purchased from the Makielski Berry Farm, Ypailanti, Michigan on June 27, 1966. Picking was delayed by 3 to 4 hours due to an early morning rain.

The berries were hand-sorted, picking out all bruised and spoiled fruit. Even after sorting, the fruit was rather soft and susceptible to damage. Approximately 280 pints of sound berries were packed into one-pint berry boxes, and half of these were over-wrapped with a 5" x 3 1/2" x 13" 1 1/2 mil unperforated polyethylene bag. These samples were then coded and randomly placed in 12 pint master shippers and transported to Ann Arbor. The sorting and packaging was completed about 3:30 p.m. The sorting and transporting temperatures were about 85° F., R. H. 85-90%.

The samples were divided into three equal lots, half of each lot having the over-wrap, and were irradiated at 0, 20, and 200 Krad at the Phoenix Memorial Laboratory. The pint boxes were rotated 180° at half time. The temperature inside the chamber was recorded at 48° F., and control samples were kept at a similar temperature. Upon completion of the irradiation the samples were transported to East Lansing and stored, one layer high, at $38^{\circ} \pm 1^{\circ}$ F.

The berries for this experiment were analyzed at 12 hours, 5 days, and 10 days after irradiation. It had originally been planned that the berries also be checked at 15 and 20 days, but after 10 days most of the berries were spoiled and testing was useless.

Percent Sound Fruit: Ten pints of each treatment were sorted and any spoiled or moldy fruit was removed. The total weight and number of the

berries in each pint box was recorded, along with the weight and number of the sound fruit in that box. From these figures, the percentage of sound fruit by weight was calculated and appears in Table I. A pint weighed about 280 grams and contained 18-20 berries.

Texture: The texture of the berry samples was measured with an Allo-Kramer shear press using a 250-lb ring, range 50, and Lo setting. One hundred grams of sound berries were used for each sample, one sample from each of five pints. Since only sound berries were tested, the firmness figures obtained are greater than those of unsorted berries would have been. The shear values as a function of storage time, dose, and wrapping are given in Table II.

Table I. Keeping quality of irradiated strawberries, Robinson variety picked 6-27-66, stored at $38 \pm 1^\circ\text{F}$. Figures are % sound fruit by weight.

Treatment		Storage time, days			
Dose, Krad	Wrapping	0	5	10	Ave.
0	Wrapped	93	37	11	47
0	Not wrapped	94	66	14	58
20	Wrapped	93	42	21	52
20	Not wrapped	99	73	16	63
200	Wrapped	95	29	26	50
200	Not wrapped	94	75	13	61
Ave.		95	54	17	55

Treatment	Ave.
All wrapped samples	49.6
All not wrapped samples	60.6
All 0 Krad samples	52.5
All 20 Krad samples	57.5
All 200 Krad samples	55.5

Table II. Shear resistance^a (ma/100 grams) of Robinson variety strawberries^b picked 6-27-66, stored at 38° F (average of five samples)

Dose Krad	Treatment Wrapping	Storage time, days			
		0	5	10	Ave.
0	Wrapped	40	46	42	43
0	Not wrapped	40	48	49 ^c	46
20	Wrapped	48	45	41	45
20	Not wrapped	43	44	46 ^c	45
200	Wrapped	39	46	37	40
200	Not wrapped	38	36	47	40
Ave.		41	44	43	43

Treatment	Ave.
0 Krad	44.5
20 Krad	45.0
200 Krad	40.0

^aThe Allo-Kramer shear press was used with the 250 lb. ring, range 50, setting Lo - values given are peak values.

^bBerries tested were only remaining sound fruit.

^cAdequate samples were not available for five trials.

There was no significant advantage in the use of ionizing radiation at doses of 20 and 200 Krad under the conditions of this experiment. The very moist condition of the strawberries made them more susceptible to bruising and microbial degradation. The variety, Robinson, used in this experiment is known as soft and highly susceptible to bruising.

The results also show that the over-wrapped berries did not keep as well as those that had no over-wrap, probably because of the excessive moisture on the berries at the time of packing. This surface water provided ideal conditions for microbial growth and the over-wrap prevented any evaporation of

this water. The percentage of sound berries by number was higher (data not shown), evidently because the larger berries are more easily bruised.

Table II gives the average peak shear values for five 100 g samples of berries. There is, essentially, no softening with 20 Krad irradiation and about a 10% change at 200 Krad.

Experiment II

Robinson variety strawberries, additional from Experiment I, were tested for microbial contamination of June 28, 1966. The following procedure based on past experiments was used. Three medium-to-small berries of each treatment were weighed and placed in a 500 ml Erlenmeyer flask with enough distilled water to make a mixture of about 10 water to 1 fruit. This mixture was then shaken in a reciprocal shaker for 30 minutes. Dilutions of 10^1 , and 10^2 , and 10^3 were then made from the liquid portion. These dilutions were plated in triplicate on plate count agar and acidified potato dextrose agar. The results are given in Table III.

Table III. Organisms per gram of fruit for Robinson variety strawberries picked and irradiated on 6-27-66 and plated on 6-28-66.

<u>Treatment</u>		Total Count	Total Yeast and Mold
Krad	Wrapping		
0	Wrapped	7,800	46,000
0	Not Wrapped	1,500	28,000
20	Wrapped	21,000	0
20	Not Wrapped	2,300	74,000
200	Wrapped	320	35,000
200	Not Wrapped	2,400	20,000

Interpretation of these results is rather difficult because the large variability among individual samples. From one 3-berry sample to another, the counts differed by as much as a factor of 10.

Experiment III

On June 30, 1966 strawberries of the Midway variety were obtained from the Makielski Berry Farm. The berries were hand-sorted, put into pint baskets, and covered with an unperforated plastic bag. The samples were divided into five lots and each lot received one of the following doses of irradiation: 0, 100, 200, 300, and 400 Krad.

On return to East Lansing, two pints of each dose were separately weighed and blended with 400 ml of distilled water for two minutes. Fifty ml of the slurry were then mixed with 150 ml of distilled water in a 500 ml Erlenmeyer flask. Dilutions of 10^1 , 10^2 , and 10^3 were made from this mixture and these dilutions were plated in triplicate on plate count agar and acidified potato dextrose agar. The results appear in Table IV.

Table IV. Organisms per gram of fruit for Midway variety strawberries picked and irradiated on 6-30-66 and plated on 7-1-66.

Dose, Krad	Total Count, 1000's		Total Yeast and Mold, 1000's		Ratio of Yeast and Mold to Total Count	
	Sample		Sample		Sample	
	A	B	A	B	A	B
0	8.8	10.	71.	70.	8:1	7:1
100	5.3	250.	38.	50.	7:1	0.2:1
200	---	3.7	12.	32.	---	9:1
300	2.7	5.1	25.	74.	9:1	15:1
400	4.3	3.8	17.	7.4	4:1	2:1

Again, the high variability among samples obscured any effect of dosage on the microbial contamination. The initial contamination of each pint sample was very sensitive to the contamination on an individual berry. Therefore, if a pint box contained one moldy or otherwise badly infected berry, its contamination could easily be many times greater than a box containing all sound berries.

Experiment IV

Strawberries of the Sparkle variety were picked on the morning of July 8, 1966, and taken to Ann Arbor for irradiation. The berries were hand-sorted so that only sound fruit remained, and special attention was paid to remove highly infected berries.

Two 700-800 g lots of these berries were disintegrated in a blender for three minutes and placed in a 1000 ml Erlenmeyer flask. Each lot was then divided into 5 large test tubes (cap. 100 ml) and given the following doses of irradiation: 0, 100, 200, 400, and 800 Krad.

On return to East Lansing, 75 g of each sample were diluted 2 to 1 with distilled water, yielding a slurry containing 0.33 g berries/g slurry. This slurry was then used to make dilutions of 10^1 and 10^2 which were plated in duplicate on plate count agar and acidified potato dextrose agar. Table V summarizes the results.

Table V. Organisms per gram of fruit for Sparkle variety strawberries picked, irradiated, and plated on 7-8-66.

Dose Krad	Sample ^a	Total count	Total yeast and mold	% Survival Total count	% Survival Yeast and mold
0	A	8.2×10^4	6.2×10^4	100	100
	B	10.1×10^4	6.1×10^4	100	100
100	A	3.7×10^4	3.7×10^4	39.	59.
	B	3.9×10^4	3.4×10^4	40.	56.
200	A	1.6×10^4	1.4×10^4	19.	23.
	B	1.7×10^4	1.4×10^4	17.	23.
400	A	6.7×10^2	4.2×10^2	0.81	0.69
	B	$12. \times 10^2$	3.9×10^2	1.2	0.64
800	A	2.7×10^2	0	0.33	0.10
	B	1.8×10^2	0	0.18	0

^a All sample A's or B's are subsamples of the same master sample.

The results show that with a homogenized sample, ionizing radiation decreases the microbial contamination in Sparkle variety strawberries.

Also, it seems that yeasts and mold are somewhat more resistant than bacteria at low (100 and 200 Krad) levels of irradiation.

SWEET CHERRIES

Experiment I

On July 20, 1966 sweet cherries of the Schmidt variety were purchased from Lewis R. Gray of Traverse City, Michigan.

Cherries for the fresh market are picked with the stems attached. This is to prevent bleeding and microbial infection at the point where the stem would normally be pulled from the cherry. The 1966 growing season was rather dry, but the cherries were of excellent quality. However, they were a little smaller than in previous years. There had been no rain two or three days prior to picking.

Directly after picking, the cherries were subjected to the following operations: 1) a decluster separated the cherries with merging stems, 2) workers removed spoiled and immature fruit, 3) the cherries were sized by rollers that removed cherries of diameter less than 22mm. The cherries were then packed into paperboard shipping cartons (19 lbs/box) and transported to East Lansing (180 miles).

In East Lansing, the cherries were sorted (spoiled, immature, and cherries without stems were removed) and packed in pint boxes which were placed in 12-pint master shippers. The samples were stored overnight at $38 \pm 1^{\circ}\text{F}$.

On July 21, 50 pints of cherries (25 for 0 Krad and 25 for 200 Krad) were soaked for one hour in a 0.5% Ca^{++} (from $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) solution. The following doses and treatments were each given to 25 pints of cherries at the Phoenix Memorial Laboratory in Ann Arbor.

<u>Dose-Krad</u>	<u>No Ca^{++} Soak</u>	<u>Soak Before</u>	<u>Soak After</u>
0	25 (pints)	25 (pints)	25 (pints)
20	25	--	--
200	25	25	25

Also, to test for the significance of leaving the stems on the cherries, 0 and 200 Krad were given to two 10-pint lots of cherries without stems (destemmed).

On completion of irradiation, the samples were returned to East Lansing and 25 pints of each of the 0 and 200 Krad samples were soaked for one hour in a 0.5% Ca^{++} solution. The samples were stored at $38 \pm 1^\circ \text{F}$.

Testing: The cherries were tested at 1, 7, 14, 21, and 28 days after irradiation for the following characteristics:

- A. Percent sound fruit. Five pints of each treatment were inspected at each time interval. Each pint was separated into sound and spoiled (moldy or rotten) cherries and each portion weighed.
- B. Texture. The shearing characteristics of the cherries was tested with an Allo-Kramer shear press (250 lb. ring, range 50, Lo) using 100 g samples.
- C. Color. The color of the different treatments was compared visually at each testing period.

Results:

Percent sound fruit. Table I gives the percent sound fruit at the various testing periods. Each figure is the average of five samples. The results show that there is no important difference in the keeping quality of the irradiated and non-irradiated or soaked and non-soaked samples. However, the values given for the destemmed cherries at 28 days of storage show that destemmed cherries do not keep as well as those with the stems on, and that irradiated destemmed cherries are more susceptible to spoilage than control destemmed cherries.

Table I. Percent sound fruit of Schmidt variety sweet cherries harvested 7-20-66 and irradiated 7-21-66. Average of five samples, stored at $38 \pm 1^\circ\text{F}$.

Treatment		Days Storage					
Krad - Soak		1	7	14	21	28	Ave.
0	None	99.4	99.1	93.9	92.2	80.7	93.1
0	Before	99.3	98.9	93.1	90.2	79.5	92.2
0	After	100.0	98.4	91.3	90.7	83.5	92.8
20	None	100.0	99.2	94.5	85.0	81.4	92.1
200	None	100.0	97.1	93.4	92.5	80.1	92.6
200	Before	99.3	97.5	93.9	88.0	82.3	92.2
200	After	99.2	98.8	89.9	90.1	83.3	92.3
0	Destemmed	-	-	-	-	60.9	-
200	Destemmed	-	-	-	-	50.7	-

Texture: The shear values for the Schmidt variety cherries at the given time periods are given in Table II. Each value is the average of five trials using the Allo-Kramer shear press (250 lb ring, range 50, Lo). The values show that for each dose, soaking the fruit in the $0.5\% \text{Ca}^{++}$ solution significantly increased its shear resistance. Irradiation at the level of 200 Krad tends to soften the cherries, but if the Ca^{++} soak is applied to these samples, they are returned to the same shear level as the controls after a few days of storage.

Table II. Shear values (micro-amperes) for Schmidt variety cherries harvested 7-20-66 and irradiated 7-21-66. Average of five samples stored at $38 \pm 1^\circ\text{F}$.

Treatment		Storage time (days)					
Krad - Soak		1	7	14	21	28	Ave.
0	None	52.2	52.6	63.8	62.2	60.4	58.2
0	Before	54.4	57.6	65.8	67.6	63.2	61.7
0	After	54.6	63.6	63.2	69.2	60.0	62.1
20	None	51.8	55.0	62.4	62.0	60.2	58.3
200	None	39.8	40.4	49.0	55.4	64.0	49.7
200	Before	45.8	52.2	56.4	63.2	71.8	57.9
200	After	48.2	55.4	64.6	64.0	65.6	59.6

Conclusions: Under the conditions of this experiment, irradiation at levels of 20 and 200 Krad did not increase the shelf life of fresh Schmidt variety sweet cherries.

A one-hour soak in a 0.5% Ca^{++} solution (from $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) caused a substantial firming of cherries. The softening effect of irradiation was overcome by this treatment within a few days.

The shelf life of cherries was greatly increased when the stems were left on the fruit.

Experiment II

Schmidt variety cherries harvested on July 21, 1966 (see Experiment I) were treated and irradiated on July 26, in the following manner.

- A. Approximately 600 g of whole cherries (including pits and stems) were blended in a sterile Waring blender and divided into five large (approx. 75 cc.) sterile test tubes. These samples were given irradiation doses of 0, 200, 300, 400, and 800 Krad. On July 27, these samples were diluted 3 to 1 with water and plated on total plate count agar and potato dextrose (acidified) agar in dilutions of 10^0 and 10^1 .
- B. Three tubes each of whole, pitted and macerated cherries (pits and stems removed; blended in Waring blender) were prepared and irradiated at 0, 300 and 600 Krad. These samples were then tested for color differences visually and by use of a Gardner color difference meter.

Results: Table III gives the total count and total yeast and mold (organisms per gram) for the cherry samples. The results show a definite reduction of organisms with increase in dose. It appears that the cherries were not highly contaminated. The usually dry season and absence of brown rot are probably factors which would explain this light microbial load.

Table III. Organisms per gram of Schmidt variety sweet cherries harvested July 20, 1966, irradiated July 26, 1966.

<u>Dose - Krad</u>	<u>Total Count</u>	<u>Total Yeast and Mold</u>
0	1200	580
200	720	-
300	240	100
400	480	80
800	40	30

Table IV gives the Gardner color difference values for the whole, pitted and macerated samples. Whole cherries were not affected appreciably by 300 Krad, but turned slightly darker by 600 Krad. Pitted and macerated cherries turned darker with some loss of redness and increase in yellowness upon irradiation.

Visual inspection agreed with the results of the Gardner values.

Table IV. Gardner color difference values for Schmidt variety cherries harvested 7-20-66, irradiated 7-26-66.^a

<u>Krad</u>	<u>Treatment</u>	<u>L-value</u>	<u>a-value</u>	<u>b-value</u>
0	Whole	19.9	12.7	4.6
0	Pitted	15.2	7.8	4.5
0	Macerated	17.5	3.4	4.7
300	Whole	18.8	12.0	5.7
300	Pitted	18.3	5.8	5.4
300	Macerated	19.0	5.0	6.0
600	Whole	22.2	9.9	8.2
600	Pitted	19.8	11.5	6.1
600	Macerated	27.2	6.6	10.7

^a +a = redness; -a = greenness; +b = yellowness; -b = blueness; L = 0 to 100 (white to black) standard plate. L = 24.8; a = +20.4; b = +6.0.

Conclusions: Gamma irradiation (up to 800 Krad) of Schmidt variety sweet cherries reduced their microbial contamination. The 1966 sweet cherries showed a light microbial load. 300 Krad had no appreciable effect on the color of whole cherries, but turned pitted and macerated cherries darker.

MUSHROOMS

Mushrooms - Experiment I

On November 16, 1966, mushrooms (Agaricus campestris) were obtained from the Great Lakes Mushroom Co-op, Warren, Michigan. They were transported to Ann Arbor where they were randomly sorted into 40 one-pint wooden containers (12 to 15 mushrooms per container). Only closed mushrooms of good quality were used. Doses of 0, 10, 15, 20, and 50 Krads were each given to 8 boxes of mushrooms. At the end of the irradiation treatment (1-1/2 hours), the mushrooms were transported to East Lansing and placed in storage at $38 \pm 1^\circ$ F.

Testing Procedure: At the end of four storage periods (2, 7, 15, and 21 days after irradiation), two pints of each treatment were removed from storage and tested for three quality factors, reflectance, degree of opening, and opening ratio.

L_D values obtained from a Gardner color difference meter, was measured on all mushrooms having a diameter large enough to cover the opening on the meter. This, however, only allowed for measurement of the larger mushrooms in the samples. Also, mushrooms with varying diameters protruded into the diaphragm to different depths. This caused a large variance in readings.

Each mushroom was also graded according to its degree of opening. The degree to which a mushroom was said to have opened depended on how much of the velum had broken away from the annulus and pileus, thus exposing the lamellae (see Fig. 1). The degree of opening was described as 0 (completely closed), 0.1, 0.2,, 0.9, and 1.0 (lamellae exposed over entire circumference).

A second method of measuring the extent of opening was also used. Each mushroom was cut in half and three measurements were made with a vernier caliper on the newly exposed internal surface. These measurements were used to calculate the opening ratio. Figure 1 shows a sketch of a divided mushroom which illustrates where the three measurements a, b, and c, were made. The ratio, $\frac{b + c}{a - (b + c)}$, was then calculated. As the mushroom opens, the values which measure the distance between the tip of the lamellae and the stipe become larger (values b and c). The a value is the overall cap diameter at each time of measurement; and $a - (b + c)$ is an effective diameter--that is, the cap diameter adjusted for the separation between the lamellae tip and the stipe. The ratio $\frac{b + c}{a - (b + c)}$ would then represent the ratio of the total opening gap to the "effective" closed-cap diameter. As the mushroom cap continues to open, then, the value of the opening ratio becomes increasingly larger. Theoretically, if the mushroom were completely closed, the opening ratio would be zero. On the other hand, as the mushroom continued to open, the ratio would become increasingly larger.

Fig. 1. Mushroom showing measurements d , e , and D from which the opening ratio was calculated.

Results and Conclusions: Table 1 gives the L_D values for the mushrooms as measured by a Gardner color difference meter. Each value is the average of from 7 to 10 mushrooms. These values are not very precise as measurements of reflectance because of the great variability among mushrooms. The readings varied greatly, depending largely on the diameter of the mushroom and the position in which it was placed over the diaphragm. The Gardner L_D values or visual comparison of the samples do not show a highly significant difference in reflectance among treatments. If anything, gamma irradiation shows a slight tendency to increase the browning rate of mushrooms. It should be noted that all samples collectively show a decrease in L_D value upon storage. This indicates a browning or darkening of the mushrooms during storage. Further experimentation using a larger sample size and a more refined method for determining reflectance is necessary, however, before a definite conclusion can be reached.

Table 1. L_D values (determined by a Gardner color difference meter) for mushrooms irradiated 11-16-66. Average of from 7 to 10 mushrooms stored at $38 \pm 1^\circ\text{F}$.

Dose-Krad	Storage time (da)			
	2	7	15	21
0	81.2	78.9	70.9	66.5
10	76.5	80.1	68.5	62.1
15	81.8	69.8	69.2	61.0
20	78.8	74.7	69.5	64.9
50	<u>80.2</u>	<u>71.9</u>	<u>66.6</u>	<u>65.0</u>
Ave.	79.7	75.1	68.9	63.9

Table 11 gives the degree of opening and opening ratio data for the mushrooms at the four storage intervals (2, 7, 15, and 21 days). At 2 and 7 days, the opening ratio seemed dependent only on the size of the mushrooms in a particular sample box. The larger mushrooms, on the average, yielded a considerably higher opening ratio than did the smaller ones. At 15 days storage, however, the effect of the gamma irradiation treatment on the rate of cap opening becomes evident. At this interval, the control samples had an average opening ratio of 0.683 and an average degree of opening of 0.30. The combined (all doses

except zero rad) irradiated samples, however, had an average opening ratio of 0.498 and an average degree of opening of 0.01. At 21 days of storage, the effect became even more evident. At this interval, the control samples had an opening ratio and degree of opening of 0.785 and 0.42, respectively. When compared to average values of 0.486 and 0.01 for all irradiation treatments, it is evident that gamma irradiation does effectively retard the rates of certain metabolic processes which cause the mushroom cap to open.

Table II. Opening ratio and degree of opening values for mushrooms irradiated 11-16-66. Average of 25 to 30 mushrooms stored at $38 \pm 1^\circ$ F.

Dose-Krad	Storage Time (days)							
	2		7		15		21	
	Ratio	Degree	Ratio	Degree ^b	Ratio	Degree	Ratio	Degree
0	0.343	0.00	0.386	--	0.683	0.30	0.785	0.42
10	0.310	0.00	0.443	--	0.436	0.01	0.503	0.02
15	0.281	0.00	0.410	--	0.537	0.01	0.435	0.02
20	0.399	0.00	0.378	--	0.487	0.01	0.469	0.00
50 ^a	0.343	0.00	0.417	--	0.533	0.01	0.538	0.00

^a The 50 Krad samples seemed to be somewhat more desiccated than the others.

^b Degree of opening measurements were not taken at 7 days.

Experiment II

On January 12, 1967, mushrooms (*Agaricus bisporus*) were obtained fresh (harvested that morning) from the Great Lakes Mushroom Co-op, Warren, Michigan. These mushrooms were transported to Ann Arbor where they were sorted (only mushrooms of medium size and tightly closed veils were used) and ten mushrooms placed in each of 75 wooden pint boxes. Doses of 0, 10, 20, 50, and 100 Krad were each given to 15 of these boxes. After completion of the irradiation treatment, the mushrooms were taken to East Lansing where they were stored overnight at 33° F.

On January 13, 1967, the mushrooms were taken to the Horticulture Building where they were prepared for respiration rate measurement over a period of 4 1/2 days. Quantities of 10, 15, and 20 mushrooms were each

placed into 45 tared plastic pails and weighed (three pails at each dose level). The pails were then sealed and 15 pails containing the ten mushrooms, each were placed in a 15° C storage room, the 15 pails containing the 15 mushrooms each were placed in a 10° C room, and the 15 pails containing the 20 mushrooms each were placed in a 5° C room. Two plastic tubes were then connected to each pail in preparation for CO₂ and O₂ analysis, with a Beckman analyzer, at 12-hour time intervals. The respiration rates, as measured by the intake of O₂ and release of CO₂, were measured for 4 1/2 days. On completion of the respiration experiment, the mushrooms were removed from the plastic pails and returned to storage at 38° F. On the following day, seven days after irradiation, the samples were measured for calculation of the Opening Ratio and the Degree of Opening. For this experiment, however, two new measurements were made. The stripe diameter, d, at the annulus was measured, as well as e, the distance between the two tips of the lamellae. With these new measurements, the quantity (e - d) is obtained which is equal to the quantity (b + c). Also, a scaler, a radially ruled disc with a hole cut in the center, was used to determine the Degree of Opening for this experiment.

Results: A portion of the respiration experiment, CO₂ exchange vs. time, is reported in Fig. 1. Each point on the curves is an average of three samples. The curves of the samples held at 10° C (not shown) lie between the curves obtained at 5° and 15° C. The 20 Krad samples (not shown) are similar to the 10 and 100 Krad curves. The curves show that at 5° C the gamma ray dose has little or no affect on the rate of respiration of fresh mushrooms. However, at 15°C, the rate of respiration is greatly affected by irradiation treatment. This affect is more evident at increased storage time. The reduction in respiration in samples given a dose of 10 Krad was nearly as great as that at the higher levels, 50 Krad having the greatest affect.

Table I gives the average measurements of the opening values of these mushrooms seven days after irradiation. Irradiation causes a marked reduction in the Opening Ratio and the Degree of Opening at all temperatures, especially in samples stored at 15°C. It must be noted, however, that this difference is also present in the samples held at 5° C.

It can be concluded that irradiation at dose levels of 10 to 100 Krad has a significant affect on the respiration rate of mushrooms when they are stored at 15°C. However, this affect is very small or nonexistent when the mushrooms are held at 5° C. Gamma irradiation affects the Opening Ratio and Degree of Opening of mushrooms whether held at 5, 10, or 15° C. Respiration rate alone is not a good predictor of quality degradation as measured by Degree of Opening or by Opening Ratio.

Table 1. Opening Ratio and Degree of Opening measurements^a (inches) of mushrooms (*Agaricus bisporus*) irradiated 1-12-67, held at 5, 10 and 15°C for seven days. Average of 20 mushrooms per temperature and gamma ray dose.

Storage Temp.	Dose-Krad	a	e	d	e-d	a-(e-d)	Opening Ratio ^b		Degree of Opening ^c
							$\frac{b+c}{a-(b+c)}$		
5°C	0	1.59	1.20	.51	.69	.90	.76		.57
	10	1.49	1.01	.50	.61	.89	.68		.06
	20	1.52	1.01	.48	.53	.99	.54		.06
	50	1.51	1.04	.50	.54	.97	.56		.02
	100	1.55	1.02	.50	.52	1.03	.51		.01
10°C	0	1.74	1.48	.53	.95	.79	1.21		.98
	10	1.49	1.10	.48	.61	.88	.70		.83
	20	1.46	1.02	.45	.57	.89	.64		.67
	50	1.55	1.05	.44	.60	.95	.63		.32
	100	1.49	0.99	.44	.55	.94	.59		.11
15°C	0	1.90	1.88	.53	1.35	.55	2.49		1.00
	10	1.53	1.11	.50	.61	.91	.67		.90
	20	1.48	1.11	.47	.64	.84	.76		.86
	50	1.58	1.13	.43	.70	.88	.79		.69
	100	1.52	0.97	.42	.55	.97	.57		.11

^a a = total cap diameter
d = stipe diameter
e = distance between lamellae tips

^b See MPL No. 6, December, 1966.
e-d = b+c (see sketch)
a-(e-d) = a-(b+c)

^c For this experiment, an objective scaler was used for the measurement of the Degree of Opening.
See MPL No. 6.

Fig. 1. CO₂ gas exchanged (cc/100 gm-hr) vs. storage time (days) for mushrooms irradiated 1-27-67. Average of three replicate samples.

Mushroom Experiment No. III

On March 7, 1967, fresh mushrooms (Agaricus bisporus) were obtained from the Great Lakes Mushroom Co-op, Warren, Michigan. The mushrooms were taken immediately to the Phoenix Memorial Laboratory, Ann Arbor, Michigan, where they were sorted (only whole mushrooms of good quality were used) and packed into 72 containers of four types:

1. A commercial, one quart, polyethylene cottage cheese tub (5.5" high, 4.0" inside top diameter) with no cover.
2. The same tub as in (1), but covered with 1 1/2 mil polyethylene film having four 1/4 inch perforations.
3. The same tub as in (1), only covered with an unperforated 1 1/2 mil polyethylene film.
4. Commercial (5" x 7" x 2 1/2") one-lb mushroom box coated with wax film and having ten 7/16 inch diameter perforations (4 on both top and bottom and one on each side).

Eighteen containers of each type were filled and 6 containers of each were given irradiation doses of 0, 10, and 100 Krad in a one hour period with a temperature of 48° F. in the irradiation chamber.

On return to East Lansing, all containers were weighed and 3 containers of each treatment were placed into two cabinets with controlled temperature and air flow. One cabinet was held at 5° C. and the other at 15° C. Both had an approximate air flow of 0.6 ft./sec. over the containers.

The containers in the 15° C. cabinet were weighed at 1, 2, 4, 6 and 7 days after irradiation, and those in the 5° C. cabinet were weighed at 1, 2, 4, 6, 8, and 10 days. The percent weight loss calculated from these weighings is given in Table I.

At the end of the two storage periods, the mushrooms were subjected to a visual quality examination and the degree of opening was estimated.

Results:

Table I gives the percent weight loss of the mushrooms during storage. This table shows that under the conditions of this experiment, the irradiation dose has little or no affect on the rate of transpiration in fresh cultivated mushrooms. However, the type of container in which the mushrooms are stored and the temperature of storage has a marked affect on the rate of weight (water through transpiration) loss. These findings, however, are not surprising since the same characteristics have been reported for most other fresh fruits and vegetables.

The apparent quality of the mushrooms, however, was greatly affected by the gamma dose as well as the type of container and storage temperature. Table II contains brief descriptions of the resulting mushrooms at the end of the storage periods. These descriptions, predominantly subjective, suggest a marked effect of the investigated variables on the market life of fresh mushrooms.

The mushrooms near the top of the open tubs were extremely dry and withered and showed considerable browning at the 0 and 10 Krad levels. The tubs covered with perforated polyethylene allowed free moisture to collect on the mushrooms, thus supporting the growth of a slime mold and the production of an obnoxious odor at the 0 and 10 Krad level.

The tubs covered with unperforated polyethylene caused suffocation of the mushrooms. This resulted in no opening of the caps but a production of an off odor and extreme decay of the flesh (especially of the stipes). The 100 Krad samples were not as affected by this suffocation as were the 0 and 10 Krad samples.

The commercial box permitted extreme desiccation of the mushrooms. This drying was more uniform than in the open tubs, but still the mushrooms were very withered and unmarketable.

Table 1. Percent weight loss of Mushrooms during storage, irradiated 3/7/67.
(Each item is the average of 3 replicates; air flow over the container
0.6 ft/sec.)

	Temp.	Dose, Krad	Storage Time (days)						
			1	2	4	6	7	8	10
1. Open Tub (1 qt. polyethylene, no cover)	5° C. ^a (41°F)	0	6.45	11.59	24.10	32.36	-----	43.58	49.74
		10	6.06	11.07	20.50	29.57	-----	37.97	45.31
		100	5.56	11.15	25.48	31.65	-----	39.92	46.61
2. Tub with 4 1/4" Perforations in 1 1/2 mil poly- ethylene top.		0	0.63	0.81	2.58	3.84	-----	6.92	7.71
		10	0.86	1.76	3.32	4.34	-----	5.93	7.30
		100	0.76	1.38	3.12	4.32	-----	5.71	7.05
3. Tub Closed With 1 1/2 mil polyethylene		0	0.06	0.12	0.16	0.31	-----	0.50	0.65
		10	0.07	0.15	0.31	0.42	-----	0.54	0.88
		100	0.05	0.11	0.28	0.35	-----	0.43	0.57
4. Commercial Box, 1 lb. cap.. waxed inside, 10 7/16 "" perforations.		0	6.30	12.43	27.02	37.34	-----	47.26	55.63
		10	6.60	12.26	27.19	35.94	-----	45.59	55.18
		100	6.82	13.73	25.39	33.92	-----	43.77	53.37
1. Open Tub	15° C. ^a (59°F)	0	6.60	15.45	38.54	52.50	55.80	-----	-----
		10	8.25	17.92	38.87	48.91	54.01	-----	-----
		100	12.44	20.20	37.38	47.00	52.15	-----	-----
2. Tub with Perforations		0	0.91	1.95	4.73	6.15	7.06	-----	-----
		10	0.97	2.10	4.20	5.44	6.49	-----	-----
		100	0.87	1.78	3.85	5.19	6.22	-----	-----
3. Tub without Perforations		0	0.09	0.23	0.54	0.75	0.90	-----	-----
		10	0.11	0.23	0.53	0.68	0.82	-----	-----
		100	0.13	0.25	0.50	0.64	0.72	-----	-----
4. Comm. Pack		0	8.38	16.76	36.80	49.23	55.32	-----	-----
		10	8.25	17.00	35.05	47.86	54.13	-----	-----
		100	9.52	17.72	37.09	50.31	56.59	-----	-----

Table II. Quality of Mushrooms Irradiated 3-7-67, Seven and Ten days after Treatment.

Container ^a	Storage Temp. C		Dose, Krad 10	100
1	15 ^b	Degree of Opening (D.O.) = 100% Top mushrooms very shriveled Mold mycelia visible Much browning	D.O. = 100% Top mushrooms very shriveled Mold mycelia visible Moderate browning	D.O. = 5% Top mushrooms shriveled Slight browning No apparent mold
2	15 ^b	D.O. = 100% Free water within container Slime mold on surface Pronounced browning Obnoxious odor	D.O. = 95% Some free moisture Slime mold on surface Moderate browning Moderate off-odor	D.O. = 7-10% Some free moisture Some mold mycelia Very slight browning No off-odor
3	15 ^b	D.O. = 1-2% Some extreme browning Very moldy Extreme free moisture Musty (mold) odor	D.O. = 1-2% Moderate to slight browning Very moldy Some free moisture Musty odor	D.O. = 1-2% Slight browning Very little mold Slight free moisture Very little odor
4	15 ^b	D.O. = 100% (extremely open) Mushrooms very withered Extreme browning Stipes very small and dry	D.O. = 98% Much withering Moderate browning Stipes very small and dry Some mold	D.O. = 2% Some withering Slight browning No apparent mold Stipes small and dry

Table II continued

Container ^a	Storage Temp. C	0	Dose, Krad 10	100
1	5 ^c	D.O. = 15% Top mushrooms very shriveled Some very brown, others only slightly	D.O. = 0% Top mushrooms very shriveled Some very brown, others only slightly	D.O. = 0% Top mushrooms very shriveled Some very brown, others only slightly
2	5 ^c	D.O. = 15% Moderate browning Unpleasant odor Free moisture	D.O. = 5-8% Slight browning Some unpleasant odor Some free moisture	D.O. = 0% Very slight browning No unpleasant odor No free moisture on mushrooms Excellent quality
3	5 ^c	D.O. = 0% Moderate browning Evident decay of flesh Unpleasant odor	D.O. = 0% Slight to moderate browning No evident decay Slight odor	D.O. = 0% Slight odor No evident decay Little or no odor
4	5 ^c	D.O. = 0% (too dry to open) Extremely brown Extensively shriveled	D.O. = 0% (too dry to open) Extremely brown Extensively shriveled	D.O. = 0% (too dry to open) Extremely brown Extensively shriveled

^a See Table 18.

^b Seven days after treatment

^c Ten days after treatment

APPLE JUICE

Experiment I

Fresh apple juice pressed from a blend of three Michigan apple varieties was purchased from Corda West of Okemos, Michigan. The juice was irradiated at levels of 0, 100, 200, 300, and 800 Krad at the Phoenix Memorial Laboratory in Ann Arbor, Michigan and returned to East Lansing where viscosity and microbial examinations were run. The results (data not included) showed that the juice was highly contaminated with bacteria as well as yeasts and molds. Viscosity measurements with an Ostwald viscometer revealed a definite decrease in viscosity with increasing gamma dose levels.

Experiment II

Fresh apple juice was again purchased from Corda West of Okemos, Michigan on September 28, 1966. Two gallons of juice were mixed and divided into 30 small (4 oz.) bottles. Of these, 15 bottles were placed in a vacuum chamber (30 min at 29-30 in Hg) and the vacuum was released with nitrogen gas. This procedure was repeated once to insure removal of most of the oxygen.

The lids were tightened and the samples were taken to Ann Arbor where they were given doses of 0, 100, 200, 300 and 400 Krad. After irradiation, the samples were returned to East Lansing and stored overnight at $38^{\circ} \pm 1^{\circ}\text{F}$.

On the following day, the samples were used to make appropriate dilutions and plated on plate count and acidified potato dextrose agars. The results of these plate counts are given in Table I.

Table I. Organisms per milliliter of apple juice treated and irradiated September 28, 1966, plated^a September 29.

Dose-Krad	Atmosphere	Total Count	Total Yeast and Mold
0	N ₂	4.22×10^5	8.63×10^5
	Air	10.1×10^5	9.09×10^5
100	N ₂	1.01×10^5	1.68×10^5
	Air	1.06×10^5	2.02×10^5
200	N ₂	1.77×10^4	3.71×10^4
	Air	3.09×10^4	4.25×10^4
300	N ₂	6.38×10^3	8.38×10^3
	Air	7.56×10^3	9.00×10^3
400	N ₂	1.47×10^3	TNTC ^b
	Air	TNTC ^b	TNTC ^b

^aThe samples were plated in duplicate on plate count agar and acidified potato dextrose agar.

^bA high enough dilution was not plated to give an accurate count for these samples.

The data show : 1) After storage overnight at $38^\circ \pm 1^\circ\text{F}$, the N₂ packed control samples showed less contamination, especially in total count, than did the control samples with an air atmosphere. 2) Gamma irradiation reduced the total count and total yeast and mold contamination in fresh apple juice. However, this reduction was only 100 fold at 300 Krad. 3) Packing atmosphere had little influence on the percent reduction caused by gamma irradiation.

Table II gives the relative viscosities and absorbances of the apple juice samples. The viscosities were obtained by measuring the time required for the apple juice to flow through an Ostwald viscometer (size 100). The

absorbances were obtained by measurement on a Spectronic 505 from wavelengths 380 to 680 μ . The values in the table are those recorded at 440 μ .

Table II. Viscosity and absorbance of apple juice treated and irradiated September 28, 1966, tested September 30.

Dose-Krad	Atmosphere	Absorbance at 440 μ	Time (sec) of flow through Ostwald viscometer ^a
0	N ₂	.900	92.8
	Air	.883	97.4
100	N ₂	.639	95.2
	Air	.620	100.0
200	N ₂	.388	96.7
	Air	.390	97.5
300	N ₂	.302	94.5
	Air	.338	95.3
400	N ₂	.287	93.6
	Air	.313	94.8

^a Average of from 2 to 5 tests.

The data show that there is a significant decrease in absorbance with increasing gamma dose. Also, the N₂ packed samples had a higher absorbance at 0 and 100 Krads and a lower absorbance at 300 and 400 Krads than did the air packed samples. As gamma dose increased, the viscosity of the apple juice decreased. Also, it was found that the N₂ packed samples were less viscous than were the air packed samples.

Apple Juice - Experiment III

On October 26, fresh apple juice was purchased from the Centennial Farm Market, DeWitt, Michigan. This juice was filtered through several layers of cheese cloth and divided into 25 jars (cap. 6 oz.). The following treatments were given to these samples.

Table III. Treatment given to apple juice on October 26, 1966.

Number of Jars	Dose-Krad	Heat Treatment at 120°F (min)
5	0	0
5	0	30
5	150	0
5	150	30 ^a
5	150	30 ^b

^a The heat treatment preceded the irradiation.

^b The irradiation preceded the heat treatment.

Irradiation was accomplished at the Phoenix Memorial Laboratory in Ann Arbor. The samples were heated in a constant temperature water bath at 120°F for 30 minutes and cooled directly afterwards in an ice bath at 32°F. When heat and irradiation were used in combination, approximately 1-1/2 hours elapsed between the two treatments.

Analysis

Plating: At one and nine days after irradiation the samples were diluted and plated in triplicate on plate count and acidified potato dextrose agars. Colony counts were made four to five days after plating.

Percent soluble solids: Percent soluble solids was read directly from a refractometer at two and nine days following irradiation. The measurements were made on paper-filtered juice.

Absorbance: The absorbance of filtered juice was measured on a Spectronic 505 from wavelengths 380 to 680 μ .

Acidity: Acidity of the apple juice was measured by titration of 10 ml of juice to pH 8.1 with 1/10 N NaOH using phenolphthalein as an indicator, and calculated as percent malic acid.

Viscosity: Viscosity measurements were made at 32°C with an Ostwald viscometer (size 100). The time (sec.) of flow was measured at two and nine days following irradiation.

Flavor: At two and nine days following irradiation samples were given to seven untrained panelists and graded according to a Hedonic scale (1 to 9, poorest to best).

Results

Table IV gives the total count and total yeasts and molds at one and nine days following irradiation.

Table IV. Organisms per milliliter of apple juice treated on October 26, 1966, plated (in triplicate) at one and nine days following treatment.^a

Dose-Krad	Heat (min. at 120°F)	Total Count		Total Yeasts and Molds	
		1 da	9 da	1 da	9 da
0	0	2.38×10^4	1.3×10^7	3.0×10^5	2.7×10^5
0	30	1.80×10^3	3.7×10^5	8.7×10^3	4.2×10^5
150	0	1.06×10^2	5.7×10^3	2.1×10^4	4.8×10^4
150 ←	30	4.0	2.2×10^2	6.8×10^1	5.3×10^2
150 →	30	3.7	5.5×10^2	2.4×10^1	8.9×10^2

^a Samples stored at 38° ± 1°F.

Irradiation was accomplished at the Phoenix Memorial Laboratory in Ann Arbor. The samples were heated in a constant temperature water bath at 120°F. for 30 minutes and cooled directly afterwards in an ice bath at 32°F. When heat and irradiation were used in combination, approximately 1-1/2 hours elapsed between the two treatments.

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Dose-Krad	Heat (min. at 120°F)	Total Count		Total Yeasts and Molds	
		1 da	9 da	1 da	9 da
0	0	2.38x10 ⁴	1.3x10 ⁷	3.0x10 ⁵	2.7x10 ⁵
0	30	1.80x10 ³	3.7x10 ⁵	8.7x10 ³	4.2x10 ⁵
150	0	1.06x10 ²	5.7x10 ³	2.1x10 ⁴	4.8x10 ⁴
150 ←	30	4.0	2.2x10 ²	6.8x10 ¹	5.3x10 ²
150 →	30	3.7	5.5x10 ²	2.4x10 ¹	8.9x10 ²

^a Samples stored at 38° + 1°F.

The acidity of the apple juice, as was determined by titration to pH 8.1 with 1/10 N NaOH, showed little or no change due to treatment or to storage at $38^{\circ} \pm 1^{\circ}\text{F}$.

Table VI gives the absorbance and soluble solids data for the apple juice at two and nine days after treatment.

Table VI. Percent soluble solids and absorbance of filtered apple juice treated October 26, 1966 after two and nine days of storage at $38^{\circ} \pm 1^{\circ}\text{F}$.

Dose-Krad	Heat at 120°F (min)	Absorbance at 480 μ		% Soluble Solids	
		2 da	9 da	2 da	9 da
0	0	.713	.693	12.0	12.1
0	30	.650	.553	12.0	12.5
150	0	.405	.362	12.0	12.3
150 ←	30	.350	.272	12.2	12.1
150 →	30	.346	.330	12.0	12.5

The absorbance (480 μ) of the juice was significantly reduced by all treatments. The heat treatment did not have as much effect as did the irradiation, but both treatments caused a significant lightening of the juice. Also, a decrease in absorbance occurred during the storage period. The difference in color between samples was easily detected by visual inspection. The irradiated samples were much lighter in color than were the control or heated samples. This, however, is not necessarily an undesirable change.

The soluble solids content of the juice was not influenced measurably by treatment or by storage. Cruess suggests 12.0% or above as the desirable soluble solids content of fresh apple juice. All samples were in this range so that the treatments given had no detrimental effects on this quality factor.

Table VII gives the average flavor values given to the juice samples by seven untrained panelists. They were asked to give each sample a score of from one to nine with nine being the best. A score of five would mean that the panelist neither liked nor disliked the flavor of the juice.

Table VII. Flavor values (Hedonic scale), at two and nine days, for apple juice treated October 26, 1966.^a

Dose-Krad	Heat at 120°F (min)	2 days	9 days
0	0	6.9	4.3
0	30	6.9	4.4
150	0	6.5	6.4
150 ←————	30	5.1	5.9
150 —————→	> 30	5.6	4.0

^aSamples stored at 38° ± 1°F.

At two days following treatment, the control and heated samples were preferred over the other treatments, but no sample was, on the average, disliked. After nine days of storage, the preference was for the irradiated juice with the control, heated, and irradiated-then-heated juices showing scores below five. In weighing this flavor data, however, it must be remembered that the panel consisted of only seven untrained members.

SWEET CORN

Experiment I

On August 24, 1966, sweet corn of the Northern Bell variety was harvested at the M.S.U. Horticulture farm. Two dozen ears were shucked, placed in plastic cups (large end down), and transported to Ann Arbor where they were given doses (8 ears per dose) of 0, 30, and 300 Krad. They were then returned to East Lansing and stored at $38 \pm 1^\circ \text{ F}$.

On August 25, every fourth row of kernels was removed from three ears of each dose. These kernels (approximately 20 g per sample) were used for a moisture analysis using a vacuum oven. The ears were then placed in labeled plastic bags and returned to storage. On September 7, the same sampling and testing procedure was used to determine the percent moisture after 14 days of storage. Table I gives the results in percent water (wet basis).

Table I. Percent moisture (wet basis) for Northern Bell sweet corn harvested and irradiated 8-24-66.

Dose-Krad	Sample	<u>Days storage</u>		Difference	Average Difference
		1	14		
0	A	79.0	59.4	19.6	19.6
	B	78.2	58.6	19.6	
	C	73.3	53.7	19.6	
30	A	77.6	57.7	19.9	20.3
	B	76.1	54.2	21.9	
	C	73.9	54.9	19.0	
300	A	77.6	56.4	21.2	20.8
	B	75.1	56.9	18.2	
	C	78.5	55.5	23.0	

The data show no better retention of moisture with the use of gamma irradiation. Since moisture content is directly related to quality in sweet corn (higher moisture indicates less conversion of sugar to starch), gamma irradiation shows no desirable influence under the conditions of this experiment.

YEAST STUDIES

Summary

Three yeasts, Saccharomyces cerevisiae var. ellipsoideus, Candida krusei and Rhodotorula glutinis were irradiated in liquid media and their ability to form colonies after irradiation was taken as a measure of their viability. The respective D values were 65, 43 and 50 when the yeasts were irradiated in demineralized water; 95, 60 and 50 in synthetic growth medium and 125, 68 and 73 in apple juice. When the pH of the synthetic medium was varied in the range of 3.0 to 6.0 it was found that at pH 6.0 the resistance to radiation of all three yeasts was the lowest: ellipsoideus was more sensitive at pH 4.5 than 3.0, but the other two yeasts were slightly more sensitive at pH 3.0 than 4.5. Repeated irradiation of the survivors of C. krusei at doses of 50-100 Krad lowered the radiation resistance of this yeast; re-irradiation at 300 Krad raised this resistance.

IRRADIATION OF YEASTS

Experimental

The yeasts, Saccharomyces cerevisiae var. ellipsoideus ATCC 560, Candida krusei ATCC 2159 and Rhodotorula glutinis ATCC 2527 were obtained from the American Type Culture Collection and grown on agar slants of Difco yeast nitrogen base (YNB) and glucose. The cells were harvested from the slants with demineralized water containing Triton X-100 and the aid of a wire loop. After centrifugation and washing twice with water the cells were suspended in each of the four following liquids:

1. Demineralized water
2. Synthetic growth medium made of liquid YNB and containing 2% glucose (pH 5.0)
3. The same synthetic medium as above mixed with citrate buffers to obtain the final pH levels of 3.0, 4.5 and 6.0 (0.02M in citrate)
4. Canned apple juice (pH 3.4)

These media were autoclaved (121°C, 17 min) in 50 ml test tubes containing glass beads and mixed with the washed yeast cells to reach a concentration of approximately 10^6 cells per ml. The tubes were exposed to gamma radiation at the temperature of 48°F. The distances from the source were calculated so that all suspensions received the desired dose in 1 hour. The irradiated suspensions were kept overnight at 38° F and plated the following morning on YNB-glucose agar after suitable dilutions were made. At least triplicate plates were made for each of the 2 or 3 dilutions. The colonies were counted after 5 days of incubation at room temperature.

In another experiment, the effect of repeated exposure to irradiation of survivors of previous irradiation was investigated. Candida krusei cells, grown, and harvested as described previously, were suspended in YNB-glucose medium and exposed to 0, 50, 100, 150, 200 and 300 Krad of gamma radiation. A composite inoculum from several colonies of survivors of the same dose was used to prepare YNB-glucose slants. Survivors of only four of the original six doses were used: 50, 100, 200, and 300 Krad. After three days of growth the cells were harvested and prepared for a second exposure to gamma radiation. The suspensions from the survivors of each of the selected doses were then exposed to the whole range of 0 to 300 Krad. The same procedure was repeated once more as indicated in Scheme 1. Percent survivors were obtained for each dose using the count for non-irradiated controls as 100% survival.

Figures 1 to 6 depict the effect of irradiation dose on the colony-forming ability of the three yeasts studied under various conditions of irradiation. Some tailing is apparent in the survival curves. From the straight line portion of the curves, which extends to the first 100 or 200 Krad, the following D values were derived for S. cerevisiae var. ellipsoideus, C. krusei and R. glutinis: in demineralized water, 65, 43 and 50 respectively; in synthetic medium, 95, 60 and 50 and in canned apple juice

125, 68 and 73 respectively. Raising the pH of the synthetic medium to 6.0 resulted in decrease of the radiation resistance of all three yeasts. At pH 3.0 ellipsoideus was more radiation resistant than at pH 4.5, but the other two yeasts showed the opposite response, although the difference between these two pH levels was not great.

The results from the re-irradiation experiment of C. krusei are summarized in Table 1, 2, 3, 4 and 5 and indicate that repeated exposure to irradiation in the range of 50 to 300 Krad of survivors of the 50 Krad or the 100 Krad doses results in increasing sensitivity of this organism to irradiation. On the contrary, repeated exposure of the 300 Krad survivors results in decreasing sensitivity. No definite change in sensitivity is apparent for 200 Krad survivors.

The increased sensitivity of the lower dose survivors may be due to irreparable non-lethal damages accumulating with successive exposures to irradiation and rendering them more vulnerable to the next exposure. On the other hand, the higher the resistance of the higher dose survivors may be interpreted as selection of radiation-resistant forms.

Scheme 1. Re-irradiation of Candida krusei survivors of certain doses of gamma radiation

Figure 1. Survival curves of *S. cerevisiae* var. *ellipsoideus* irradiated in various suspension media.

Figure 2. Survival curves of *S. cerevisiae* var. *ellipsoideus* irradiated in 0.02 M citrate buffered synthetic growth media at various pH levels.

Figure 3. Survival curves of *C. krusei* irradiated in various suspension media.

Figure 4. Survival curves of *C. krusei* irradiated in 0.02 M citrate buffered synthetic growth media at various pH levels.

Figure 5. Survival curves of *R. glutinis* irradiated in various suspension media.

Figure 6. Survival curves of *R. glutinis* irradiated in 0.02 M citrate buffered synthetic growth media at various pH levels.

TABLE 1.--Percent survival of C. krusei after first exposure to gamma radiation in yeast nitrogen base growth medium.

Dose Krad	Cells/ml	% Survivors
0	5.00×10^6	100.
50	5.10×10^5	10.
100	1.30×10^5	2.6
150	1.62×10^4	0.32
200	1.58×10^4	0.32
300	7.42×10^2	0.01

TABLE 2.--C. krusei survivors from 50 Krad gamma radiation in yeast nitrogen base growth medium.

Dose Krad	1st Exposure of 50 Krad Survivors		2nd Exposure of 50 Krad Survivors	
	Cells/ml	% Survivors	Cells/ml	% Survivors
0	1.36×10^7	100.	6.40×10^6	100.
50	1.29×10^6	9.5	1.98×10^5	3.1
100	2.09×10^5	1.5	2.63×10^4	0.41
150	1.73×10^4	0.13	- - - -	- - - -
200	3.55×10^3	0.03	1.50×10^2	0.002
300	3.70×10^1	0.003	1.20×10^1	0.0002

TABLE 3.--C. krusei survivors from 100 Krad gamma radiation in yeast nitrogen base growth medium.

Dose Krad	1st Exposure of 50 Krad Survivors		2nd Exposure of 50 Krad Survivors	
	Cells/ml	% Survivors	Cells/ml	% Survivors
0	7.85×10^6	100.	2.07×10^6	100.
50	4.85×10^5	6.2	1.03×10^5	5.0
100	1.04×10^5	1.3	6.47×10^4	0.31
150	1.11×10^4	0.14	1.42×10^3	0.07
200	1.67×10^3	0.02	1.00×10^1	0.0005
300	1.50×10^1	0.0002	0	0

TABLE 4.--C. krusei survivors from 200 Krad gamma radiation in yeast nitrogen base growth medium.

Dose Krad	1st Exposure of 200 Krad Survivors		2nd Exposure of 200 Krad Survivors	
	Cells/ml	% Survivors	Cells/ml	% Survivors
0	4.35×10^6	100.	1.45×10^6	100.
50	1.89×10^5	4.3	1.19×10^5	8.2
100	2.43×10^4	0.56	1.26×10^4	0.87
150	5.75×10^3	0.13	1.57×10^3	0.11
200	5.70×10^2	0.01	9.66×10^1	0.007
300	6.50×10^0	0.0001	1.15×10^1	0.0008

TABLE 5.--C. krusei survivors from 300 Krad gamma radiation in yeast nitrogen base growth medium

Dose Krad	1st Exposure of 300 Krad Survivors		2nd Exposure of 300 Krad Survivors	
	Cells/ml	% Survivors	Cells/ml	% Survivors
0	9.05×10^6	100.	4.02×10^7	100.
50	9.00×10^5	9.9	1.47×10^7	37.
100	3.45×10^4	0.38	1.83×10^6	4.6
150	2.88×10^4	0.32	3.26×10^5	0.81
200	1.32×10^3	0.01	3.68×10^4	0.09
300	1.15×10^1	0.0001	9.21×10^3	0.02