DIRECT AND INDIRECT EFFECTS OF GAMMA RADIATION ON YEAST CELLS

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ABSTRACT

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Saccharomyces cerevisiae var. ellipsoideus (Hansen) Dekker, Candida krusei (Castellani) Berkhout, and Rhodotorula glutinis (Fresenius) Harrison, were irradiated in liquid media and their ability to form colonies after irradiation was measured (direct effects). D values, in Krad, for S. cerevisiae var. ellipsoideus, C. krusei, and R. glutinis when irradiated in a) demineralized water were: 65, 43, and 65 respectively; b) in synthetic growth medium: 95, 60, and 50 respectively; and c) in apple juice: 125, 68, and 73 respectively. When the resistance of the aforementioned yeasts was studied in synthetic medium the pH of which varied in the range of 3.0 to 6.0, it was found that the lower pH favored their radiation resistance. Repeated

exposure of C. krusei to doses of 50-200 Krad resulted in lowering the radiation resistance of this organism.

Synthetic growth media containing either glucose or sucrose as carbon source and sterilized by either heat or filtration were irradiated at the levels of 1, 2, 3, and 4 Mrad. These media were subsequently inoculated with S. cerevisiae var. ellipsoideus, C. krusei, R. glutinis, C. tropicalis, and S. cerevisiae and the outgrowth of these yeasts was determined (indirect effects). A significant reduction of outgrowth was observed for all of these organisms at the dose of 4 Mrad, regardless of the sugar used or the sterilization treatment prior to irradiation. Irradiated media containing sucrose affected more adversely the outgrowth of the yeasts under study. S. cerevisiae var. ellipsoideus ATCC 560 did not grow on sucrose containing media which were exposed even to the level of 1 Mrad. Filter-sterilized fresh apple juice and canned apple juice exposed to 4 Mrad did not support yeast growth to the same extent as juice irradiated at 0, 1, 2, and 3 Mrad.

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By

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To Dr. Robert C. Baker,

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at Cornell University

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INTRODUCTION

The food supply has been of important consideration in the development of civilization. The history of man is the history of his search for food and his attempts to preserve some of his harvest. Before man knew what caused his food to spoil, he was busy trying to prevent it. The principal methods for keeping edible food available are: drying, salting and smoking, pickling and fermentation, refrigeration and freezing, sterilization and pasteurization, sugar concentrates and chemical preservatives.

The possibility of radiation preservation of foods was recognized in general terms in the early 1900's. Research in the mid-1940's revealed the potential of the process. Pasteurization research was undertaken by the Atomic Energy Commission in the 1950's, believing this process would be of benefit to the consumers.

Thus, the use of ionizing radiation became the most recent step in the preservation of foods. When an atom or a molecule is bombarded by radiation having sufficient energy to remove electrons, the neutral atom or molecule is converted to a positively charged particle or ion. This ionization causes an alteration of vital macromolecules which may result in destruction of bacteria and other organisms.

Radiation preservation of foods is accomplished in two ways: Pasteurization, which is achieved by doses from 100 to 600 Kilorads, and sterilization, which requires doses between 2 and 5 Megarads.

The purpose of this study has been to investigate the effects of gamma radiation from a Cobalt-60 source on the yeasts: Saccharomyces cerevisiae var. ellipsoideus, Candida krusei and Rhodotorula glutinis. These yeasts have been involved in the spoilage of fresh fruits, fruit juices, pickles, dairy products, and frozen foods.

S. cerevisiae var. ellipsoideus and C. krusei ferment fruit juices and beverages from apples, grapes, and citrous fruits (mostly orange).

R. glutinis has been reported as one of the chief spoilage agents of frozen peas, and frozen oysters (9).

Sour milk or cream and pickles develop pink spots due to the presence of this yeast.

This study was divided into two parts. In the first part, the yeasts were irradiated in various suspension media and their survival was determined. In the second part, the media were preirradiated, subsequently innoculated with yeast and their growth was measured. The objective of the latter work was to study the possible formation of toxic substances by irradiation, using yeasts as test organisms.

LITERATURE REVIEW

Yeast cells have frequently been used as test objects for the biological effects of ionizing radiation (5, 10, 18, 21, 24, 26-28). A number of studies have been made which illustrate the fact that while most of the microorganisms are destroyed largely by direct action, which is the target theory, indirect effects of radiolysis products are also important in the destruction of most species (1, 6, 8, 12, 25, 29).

James and Werner (18) made an excellent review of the radiobiology of yeasts in which they considered the various effects of radiation and the many factors which modify these effects.

The commercial feasibility of pasteurization of fruit juices through irradiation is of world-wide interest. It is known that, generally, fruit juices are excellent media for the growth of yeasts.

In the last few years attention has been given to irradiation effects which result in abnormal plant, animal

or bacterial cell responses when grown on irradiated material (8, 25, 6, 16, 30).

Choppra et al. (8) observed cytotoxic (chromosomal aberrations) effects in plant materials (barley and onion seeds) grown on orange or apple juice, which had been irradiated with 200 Krad of gamma rays.

Holsten and coworkers (16) have reported inhibition of growth in carrot cells due to cytotoxic substances produced by gamma irradiation of the sucrose of the growth medium. Toxicity to mammalian cells in vitro, as shown by growth inhibition produced by glucose and fructose in the culture media which had been exposed to levels of gamma radiation from 2 to 5 Mrad, was demonstrated by Berry et al. (6). The bactericidal action of gamma irradiated glucose was reported by Molin and Ehrenberg (25) using Pseudomonas species. Chromosomal aberrations in vitro were observed by Shaw and Hayes (30) when human lymphocytes came in contact with sucrose solution preirradiated to 2 Mrad of gamma radiation. The use of irradiated sterilized sucrose as a bactericidal agent in the canning industry has been suggested by Kiss et al. (19).

To the knowledge of the author, no studies have been reported on the effects of irradiated media on yeast cells.

METHODS AND MATERIALS

1. Organisms

The following yeasts were used in this study: *Saccharomyces cerevisiae* var. *ellipsoideus* ATC 560 (Hanson) Dekker, *Candida krusei* ATC 2159 (Castellani) Berkhout, and *Rhodotorula glutinis* ATCC 2527 (Fresenius) Harrison.

2. Growth media

a. Yeast Nitrogen Base--Glucose

The synthetic medium used for the stock cultures was the same used for the survival experiments. This is the medium which Etchells and coworkers (11) called Synthetic Agar B and prepared (33) as follows: Difco yeast nitrogen base (DYNB) solution, to which glucose was added to reach a concentration of 4%, and a 3% agar solution were separately heat-sterilized (121°C. for 17 min.) and mixed at equal volumes just prior to plating. The ready to use medium had a concentration of 2% glucose, 1.5% agar, and a pH of 5.

b. Yeast Nitrogen Base--Sucrose

The same procedure for the preparation of yeast nitrogen base--glucose was followed but sucrose was used instead of glucose.

3. Preparation of the samples

The cultures received from the American Type Culture Collection were transferred twice to the DYNB-glucose slants before starting the irradiation experiments in order to adapt them to the new growth medium.

A third transfer was made on similar agar slants and after three days of growth at room temperature the yeast cells were harvested as follows. Demineralized water containing Triton X-100 was poured into the tubes and the surface of the slant was scraped with a wire loop. The cells were then centrifuged and suspended in similar water. This process was repeated twice more. The suspension contained in a 50 ml round bottom centrifuge tube was shaken with sterile glass beads to disperse any clumps present. A Petroff-Hausser bacteria counter was used for direct microscopic count and to check for uniformity of cell dispersion. The suspension was brought to a final concentration of approximately 10^6 cells per ml.

4. Irradiation

a. Yeast cells suspended in liquid media

Gamma radiation from a 10,000-curie Co^{60} source located at the Phoenix Memorial Laboratory of the University of Michigan in Ann Arbor was used for this study.

The washed suspension containing sterile glass beads was shaken with a Lab-Line super mixer until a uniform cell dispersion was obtained. One ml portions of the suspension were transferred into sterile test tubes containing 2 ml of the liquid medium in which the irradiation was carried out.

Four different media were used for this purpose:

1) Water.

Harvested cells as described previously were irradiated in water demineralized by means of a Bantam demineralizer. Triton X-100 at a concentration of 0.01% was added as a wetting agent.

2) Citrate buffered synthetic medium.

Citrate buffered DYNB--glucose was prepared to obtain a nutritive medium at a definite pH.

The final buffer concentration was 0.02 M.

3) Difco Yeast Nitrogen Base--glucose synthetic medium.

Double strength DYNB--glucose medium was heat sterilized. The pH was 5.0 after the sterilization.

4) Canned apple juice.

The canned apple juice (brand Blossom Queen) used had no sugar or preservatives added to it. The pH was 3.40.

The cell suspensions were irradiated at dose levels varying from 0 to 300 Kilorads.

The temperature during irradiation was 48^{o}F. The test tubes were held straight by inserting them in a styrofoam block cut to fit the circumference of the Co^{60} cage. The distance of the tubes from outside the center well in cm was calculated on the basis of the source calibration curve prepared by the University of Michigan personnel and allowed the absorption of the desired dose in one hour.

b. Irradiation of liquid media free of yeast cells

The following media were irradiated before they were inoculated with yeast cells.

filter-sterilized--DYNB--4% glucose

heat-sterilized--DYNB--4% glucose

filter-sterilized--DYNB--4% sucrose

heat-sterilized--DYNB--4% sucrose

filter-sterilized fresh apple juice

canned apple juice

These media were aseptically transferred into 100 ml test tubes and exposed to irradiation for 16 hours at the corresponding distances from the center well in order to absorb 1, 2, 3, and 4 Megrads.

c. Re-irradiation of yeasts surviving certain levels of irradiation

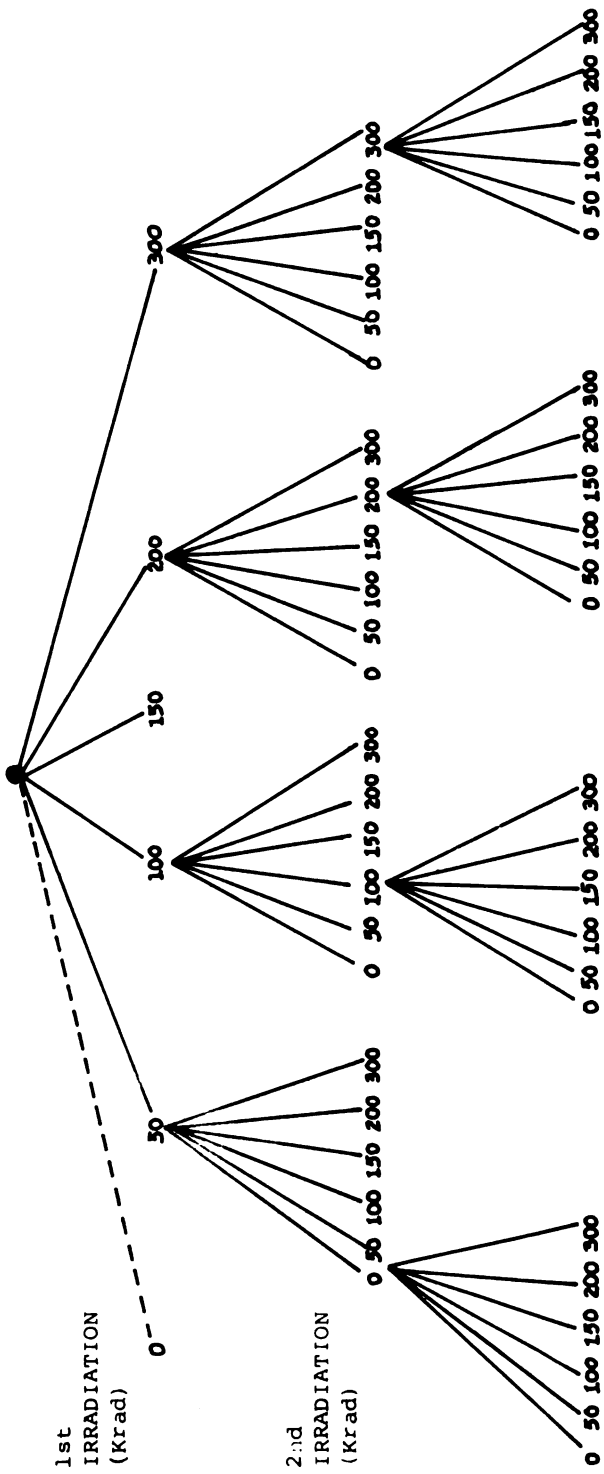
Candida krusei cells grown and harvested as described previously were suspended in DYNB--glucose medium and exposed to 0, 50, 100, 150, 200, and 300 Krads of gamma radiation. A composite inoculum from several survivors' colonies was used to prepare DYNB --glucose agar slants. Survivors of only four of the original six doses were used for this: 50, 100, 200, and 300 Krad. After three days of growth the

cells were harvested and prepared for a second exposure to gamma rays. 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, Per cent survivors was obtained for each dose using the non-irradiated controls for 100% survival.

5. Survival assay

The irradiated samples (either cell suspensions or free-of-cells media) were kept at 38°F overnight. The tubes containing the cells were well mixed with a Lab-Line super mixer. Appropriate water dilutions were made to obtain suitable colony counts per plate. At least triplicate samples were plated on DYNP--glucose agar for each of 2 to 3 dilutions. One ml of cell suspension was mixed with the growth medium before it was solidified in the Petri dish.

The irradiated free-of-cells media corresponding to each dose were mixed with an equal volume of 3.0% agar just before plating, and one ml of unirradiated suspension of approximately 10^2 cells per ml was dispersed in



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

the medium. Unirradiated cell-free-medium was used as control to obtain percent outgrowth. At least four replicate samples were plated for each dose.

The number of colonies on the plate is a measure of the number of viable cells. The ability of a cell to form a visible colony after 5 days of incubation at room temperature for the irradiated yeasts and 7 to 8 days for the irradiated media was the criterion for survival. A Quebec Colony Counter was used.

RESULTS AND DISCUSSION

Although death is a useful indicator of radiation damage, it is well recognized that it can be the manifestation of many different kinds of damage. Included among these are changes in permeability, chromosome breakage, inactivation of cytoplasmic entities and metabolic disturbances. Which of these is decisive may vary from cell to cell within a sample. However, their relative importance may be affected by such factors as dose, dose rate, and quality of the radiation, or by cultural conditions or conditions before, during, and after radiation, genetic constitution and cell development stage (18).

In view of this complexity, the inability of an irradiated cell to produce a visible colony in an agar medium has been chosen as a practical criterion of lethal damage.

The results obtained in this study were expressed as percent survival or percent outgrowth of organism.

$$\% \text{ survival} = \frac{\text{number of colonies after irradiation}}{\text{number of colonies before irradiation}} \times 100$$

Lea (22) reported that generally the biological effects of irradiation are exponential functions of dose. The percent survival is usually plotted on a logarithmic scale (y-axis) and the dose level on an arithmetic scale (x-axis).

A. Direct effects of gamma radiation on yeast cells

In this part of the study the yeast cells were suspended in a liquid medium during irradiation.

1. Sacchromyces cerevisiae var. ellipsoideus ATCC 560

a) Irradiation in demineralized water

The results obtained when S. cerevisiae var. ellipsoideus cells were suspended in demineralized water and then exposed to doses varying from 0 to 300 Krad are given in Table I of the Appendix. A graphical representation of the dose-survival relationship is shown in Figure 1. Two D values were estimated from the survival curve: 65 for the range 0 to 150 Krad, and over 400 for the range 150 to 300 Krad. The survival curve for this yeast strain showed a resistant tail. Different interpretations have been given to this type of a curve. Beam et al.

(5) reported that the resistant tail found with survival curves of haploid yeasts was due to the presence of buds. They concluded that this high radiation resistance may be associated with gene multiplicity or with unique biochemical properties of the nucleus at the time of cell division. Alper and Gillies (3) reported that a population in the logarithmic phase of growth is more sensitive than in the stationary phase; therefore a population containing cells in both stages of growth will be heterogeneous in respect to radiation resistance and likely to display a tail in the survival curve.

b) Irradiation in synthetic medium

A D value of 95 Krad for the range of 0 to 100 Krad and over 400 Krad for the range of 50-300 Krad were calculated for S. cerevisiae var. ellipsoideus cells suspended in a synthetic growth medium and exposed to radiation (Figure 1, Appendix Table I).

The results of the irradiation of S. cerevisiae var. ellipsoideus cells suspended in a synthetic medium and exposed to levels varying

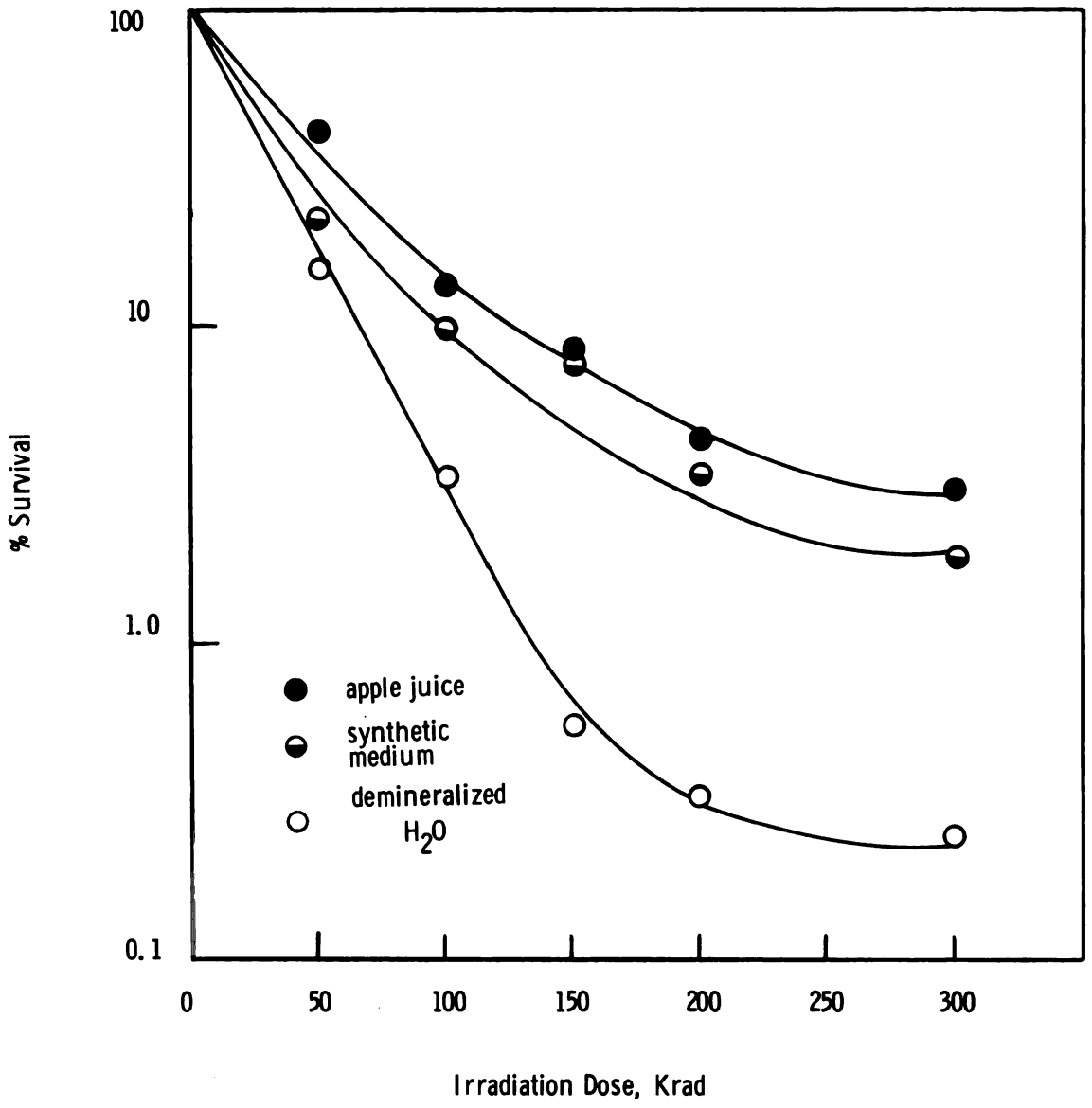


Figure 1. Survival curves of S. cerevisiae var. ellipsoideus irradiated in various suspension media.

from 0 to 300 Krad are presented in Table I of the Appendix. The corresponding survival curve is shown in Figure 1. A D value of 75 Krad for the range 0-100 Krad and over 400 for the range were calculated. A higher resistance to irradiation was observed when the cells were irradiated in synthetic growth medium than in demineralized water.

c) Irradiation in apple juice

A higher resistance to gamma radiation was obtained when this yeast was irradiated in apple juice as compared to demineralized water and synthetic growth medium (Figure 1, Appendix Table I). The calculated D values were 125 Krad for the range of 0-150 Krad and over 400 Krad for the range 150-300 Krad.

d) Irradiation at various pH levels (3.0, 4.5, 6.0)

S. cerevisiae var. ellipsoideus cells were suspended in growth media adjusted to pH 3.0, 4.5, and 6.0 and then exposed to doses varying from 0 to 300 Krad. The results are given in Table II of the Appendix and Figure 2.

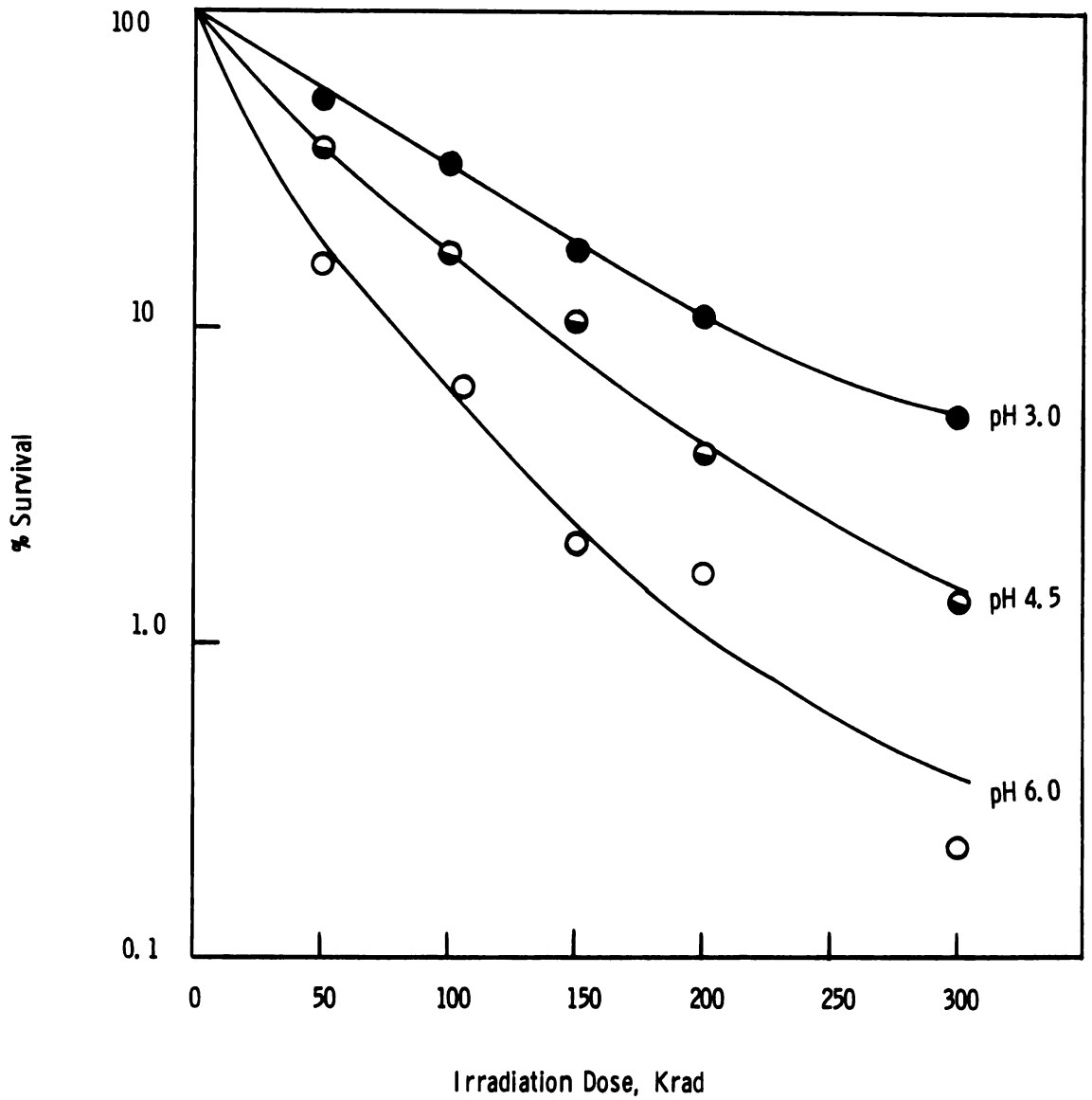


Figure 2. Survival curves of S. cerevisiae var. ellipsoideus irradiated in 0.02 M citrate buffered synthetic growth media at various pH levels.

The lower pH favored the resistance of this yeast to gamma radiation. Similar results were obtained by Alper and Gillies (3) working with E. coli.

2. Candida krusei ATCC 2159

a) Irradiation in demineralized water

The results observed when C. krusei cells were irradiated in demineralized water at doses from 0 to 300 Krad are shown in Table III of the Appendix. The survival curve is presented in Figure 3. An approximate D value of 43 Krad was estimated for the straight portion of the curve. A resistant tail was observed.

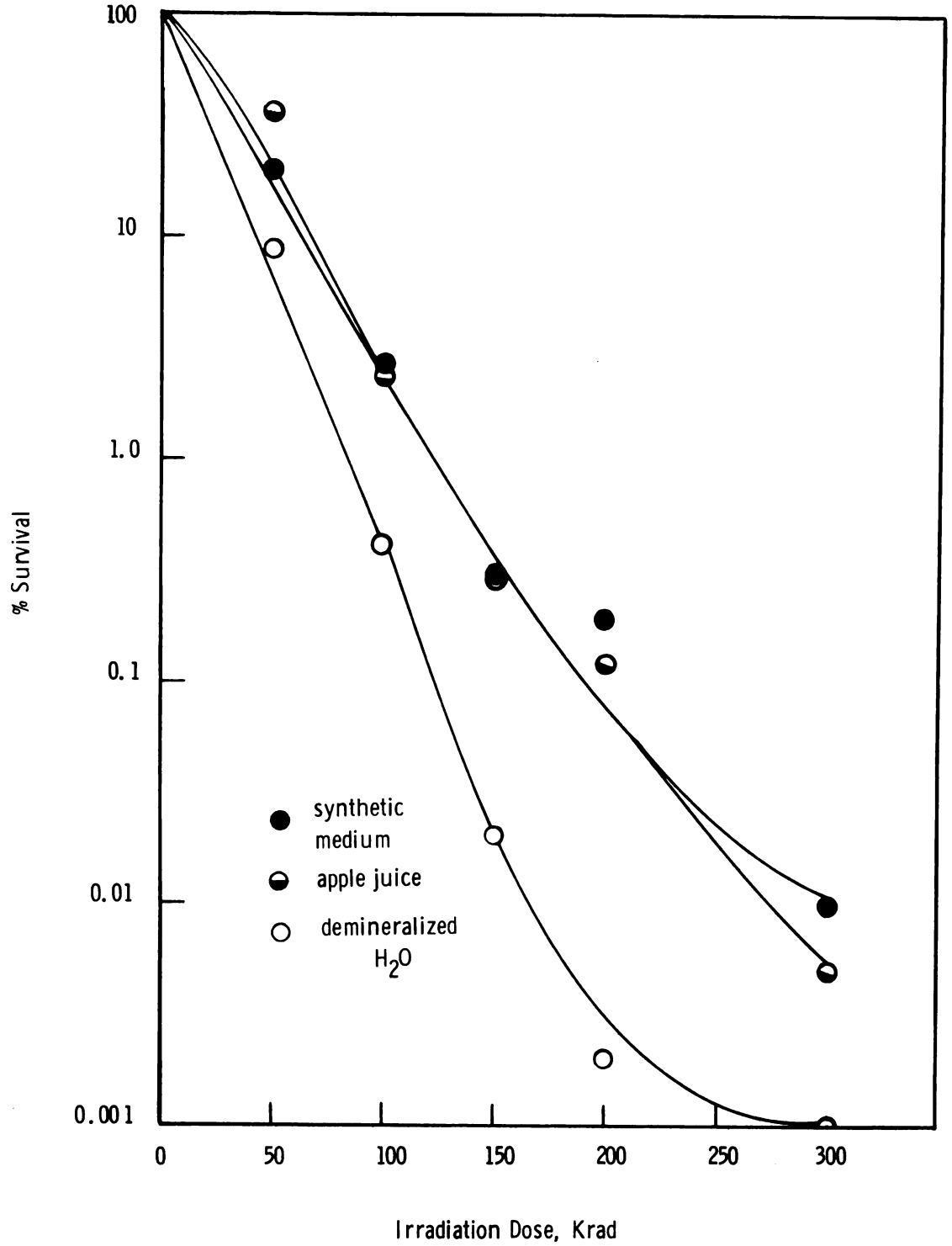


Figure 3. Survival curves of C. krusei irradiated in various suspension media.

b) Irradiation in synthetic medium

Table III of the Appendix summarizes the results obtained when C. krusei was irradiated in a growth medium suspension. A graphical representation of the survival in this medium is shown in Figure 6. The calculated D value was 60 Krad for the range of 0 to 250 Krad.

c) Irradiation in apple juice

No appreciable difference was found between apple juice and synthetic medium when these were compared as suspension media for C.krusei during irradiation. (Table IV of the Appendix, Figure 3) An approximate D value of 63 Krad was calculated for the range of 0 to 250 Krad.

d) Irradiation at various pH levels

The results observed when C. krusei was irradiated at pH levels from 3.0 to 6.0 are presented in Table IV in the Appendix. The survival curves are shown in Figure 4. The pH of 6.0 appears to be the least favorable for the survival of these cells.

3. Rhodotorula glutinis ATCC 2527

a) Irradiation in demineralized water

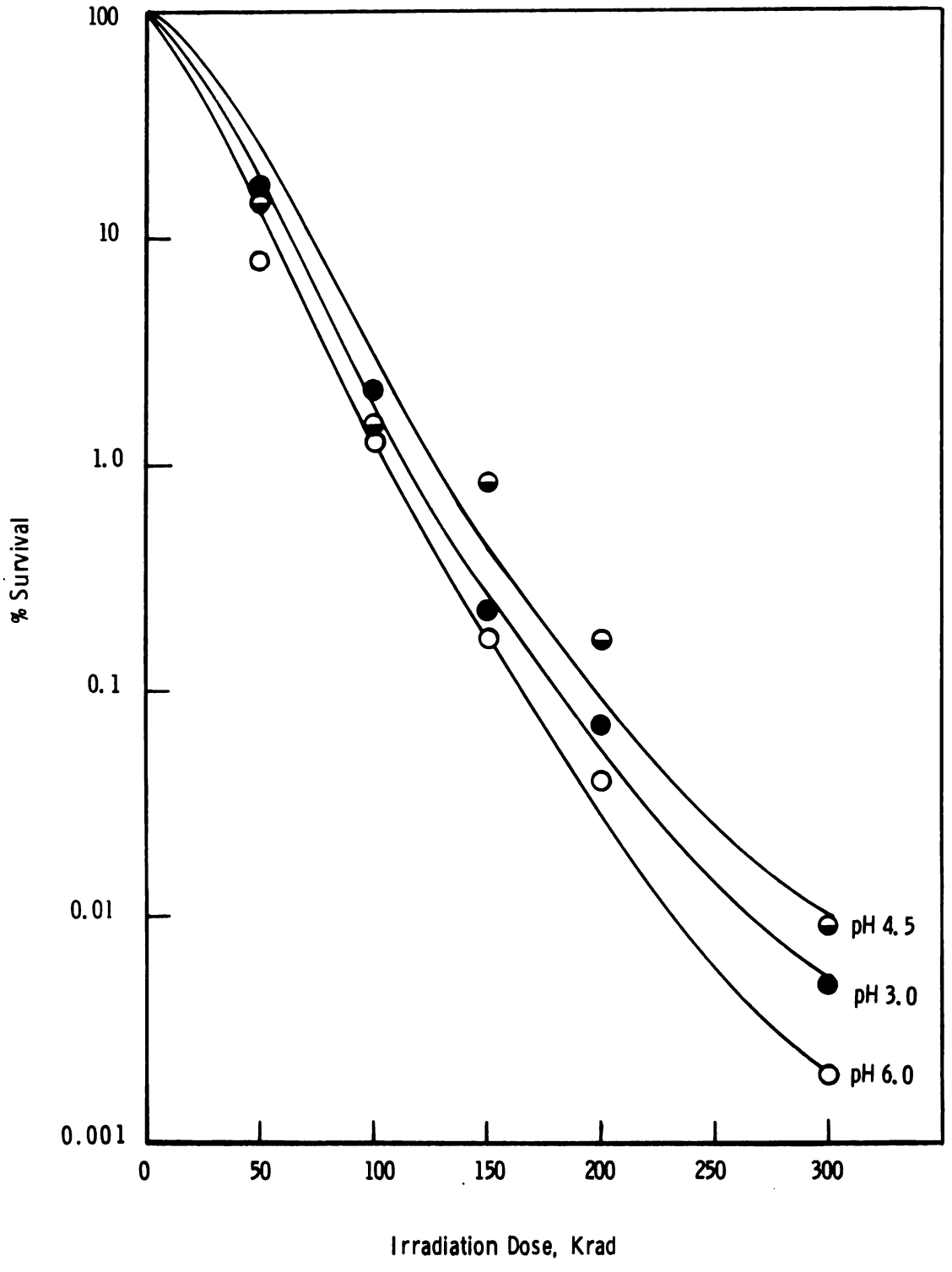


Figure 4. Survival curvesof C. krusei irradiated in 0.02 M citrate buffered synthetic growth media at various pH levels.

A D value of 65 Krad was calculated for the dose range of 0 to 300 Krad. The survival curve is approximately a straight line (Figure 5, Table V of the Appendix). Alper (2) explains this straight line relationship as resulting from one or more monotopic (single site) effects.

b) Irradiation in synthetic growth medium

The results obtained when R. glutinis cells were exposed in synthetic growth medium to dose levels from 0 to 300 Krad are indicated in Table V in the Appendix. The dose-survival curve is presented in Figure 5. Its shape reveals a resistant tail at levels from 200 to 300 Krad. The calculated D value was 50 Krad for the straight line portion of the curve.

c) Irradiation in apple juice

The results of the irradiation of R. glutinis suspended in apple juice and then exposed to doses varying from 0 to 300 Krad are shown in Table V of the Appendix. The survival curve is depicted in Figure 5. A D value of 75 Krad was calculated from the curve. Table VI of the Appendix shows the D values observed for the three species studied.

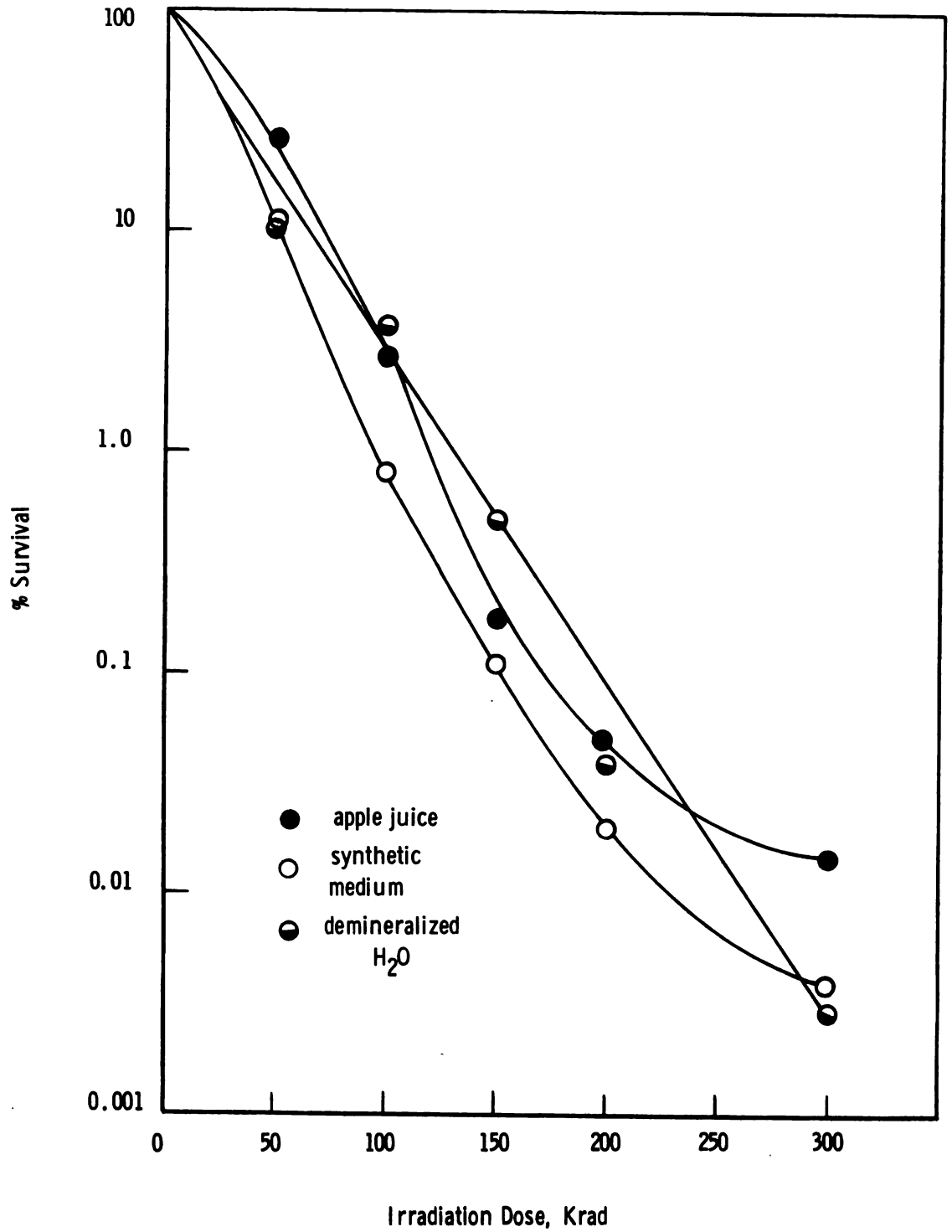
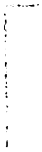


Figure 5. Survival curvesof R. glutinis irradiated in various suspension media.



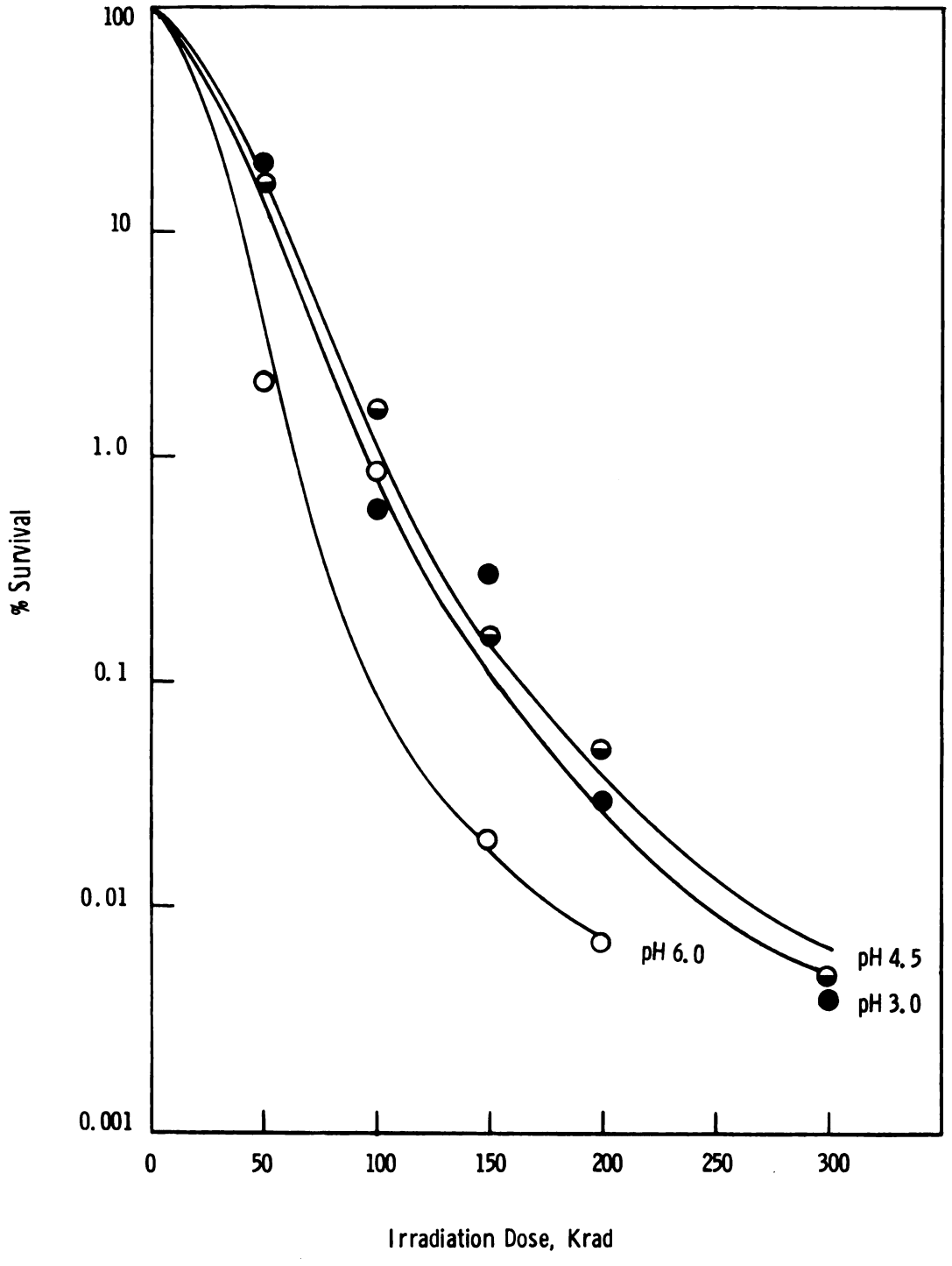


Figure 6. Survival curvesof R. glutinis irradiated in 0.02 M citrate buffered synthetic growth media at various pH levels.

d) Irradiation at various pH levels

Table VI summarizes the results obtained when R. glutinis was irradiated at various pH levels at doses varying from 0 to 300 Krad. Dose-survival curves are shown in Figure 6. There was no appreciable difference in the percent survivors of this yeast when it was irradiated at pH levels of 3.0 to 4.5. This yeast was less radiation resistant at pH 6.0. A summary of D values observed is presented in Table VI of the Appendix.

The following observations were made under the conditions of these experiments:

1) C. krusei showed the lowest survival when it was irradiated in demineralized water as compared to R. glutinis and S. cerevisiae var. ellispoideus.
2) R. glutinis showed the lowest percent survival of the three species when exposed to radiation in synthetic medium.
3) All three species exhibited lowest survival at pH 6.0.
4) S. cerevisiae var. ellipsoideus was found

to be the most radiation resistant of these yeasts in all the media employed.

4. Re-irradiation of C. krusei ATCC 2159 cells surviving certain levels of gamma radiation

The re-irradiation of C. krusei was described in Methods and Materials of this manuscript (Scheme 1). The results are summarized in Tables 1, 2, 3, 4, and 5. It was observed that repeated irradiation of C. krusei survivors at doses from 50 to 200 Krad resulted in lowering the resistance of this organism to gamma radiation. This might be explained by assuming irreparable non-lethal damages from each previous irradiation. These damages may render the cells more sensitive to subsequent irradiations. However, an increase in the radiation-resistance was observed when C. krusei was exposed repeatedly to 300 Krad. This may indicate a selection of radiation-resistant cells.

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	100.00
50	5.10×10^5	10.00
100	1.30×10^5	2.60
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	100.00	6.40×10^6	100.00
50	1.29×10^6	9.50	1.98×10^5	3.10
100	2.09×10^5	1.50	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	100.00	2.07×10^6	100.00
50	4.85×10^5	6.20	1.03×10^5	5.00
100	1.04×10^5	1.30	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	100.00	1.45×10^6	100.00
50	1.89×10^5	4.30	1.19×10^5	8.20
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	100.00	4.02×10^7	100.00
50	9.00×10^5	9.90	1.47×10^7	37.00
100	3.45×10^4	0.38	1.83×10^6	4.60
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

A. Indirect effects of gamma radiation on yeast cells

1. C. krusei ATCC 2159

a) Irradiation of synthetic media

The results of the experiments in which the carbon source (glucose or sucrose) of the synthetic medium and the sterilization treatment were compared concerning the outgrowth of yeasts in irradiated media will be presented on a species basis. Similar procedure for the presentation of the results obtained with irradiated apple juice will be used.

The percent outgrowth observed when C. krusei cells were grown on synthetic media pre-irradiated at 1, 2, 3, and 4 Mrads are summarized in Tables 6 and 7 and statistically analyzed in Tables 8 and 9. The following conclusions were reached after analyzing these results:

1) The irradiation of the media results in highly significant suppresion of the growth of C. krusei.
2) The effect of 4 Mrad although not significantly different from that at 3 Mrad, resulted in 87% outgrowth of C. krusei in irradiated medium.
3) There is also a significant interaction between the sterilization treatment and the media. The filter-sterilized-glucose medium resulted in the lowest outgrowth (Table 6).

Berry et al. (6) found toxic effects to mammalian cells in vitro when a 1% solution of glucose or fructose irradiated at 100 Krad was added to the growth medium. Molin and Ehrenberg (25) also reported toxicity of irradiated glucose solutions for mammalian cells and bacteria.

TABLE 6.--Percent outgrowth of C. krusei grown on both irradiated heat-sterilized and filter-sterilized yeast nitrogen base-glucose.

Dose Mrad	Filter-sterilized YNB-glucose		Heat-sterilized YNB-glucose	
	Cells/ml	% Survivors	Cells/ml	% Survivors
0	9.15×10^7	100	10.40×10^7	100
1	9.53×10^7	104	10.20×10^7	98
2	8.70×10^7	95	9.33×10^7	90
3	8.25×10^7	90	9.53×10^7	92
4	7.48×10^7	82	9.34×10^7	90

TABLE 7.--Percent outgrowth of C. krusei grown on both irradiated heat-sterilized and filter-sterilized yeast nitrogen base-sucrose.

Dose Mrad	Filter-sterilized YNB-sucrose		Heat-sterilized YNB-sucrose	
	Cells/ml	% Survivors	Cells/ml	% Survivors
0	9.80×10^7	100	9.85×10^7	100
1	9.90×10^7	101	10.90×10^7	110
2	9.58×10^7	98	10.10×10^7	102
3	9.15×10^7	93	9.30×10^7	94
4	8.65×10^7	88	8.65×10^7	88

TABLE 8.--Analysis of variance of C. krusei outgrowth on YNB-glucose and YNB-sucrose media.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F
Total	80	717,559.00		
Mean	0	705,189.01		
Adj. Total	79	12,370.01		
A*	1	324.01	324.01	2.583
B	4	2,614.17	653.54	5.210**
C	1	308.12	308.12	2.457
AB	4	301.94	75.49	0.602
AC	1	1,102.61	1,102.61	8.791**
ABC	4	145.33	36.33	0.290
ERROR	60	7,525.77	125.43	

*A=sterilization treatment; B=radiation; and C= media.

**Significant to the 1% probability level.

TABLE 9.--Radiation treatment means of actual counts/ml comparison for C. krusei grown on irradiated media.*

Dose-Mrad	0	1	2	3	4
Means, all treatments	98	101	94	91	81
Means, Glucose	98	99	90	89	84
Filtered	104	95	87	83	75
Heated	92	102	93	95	94
Sucrose	99	104	98	92	92
Filtered	99	109	100	93	87
Heated	99	100	96	92	86
Dose-Mrad	4	3	2	0	1

*Duncan's Multiple Range Test.

b) Irradiation of apple juice

Apple juice was chosen as representative of a natural medium for the growth of the yeasts species under study. In order to investigate the effect of the sterilization treatment prior to irradiation fresh, filter-sterilized juice obtained in two different weeks was compared to

canned juice irradiated at 1, 2, 3, and 4 Mrad. Unirradiated juice from each sterilization treatment was used to obtain the percent outgrowth for each dose. The results are shown in Tables 10 and 11.

No significant difference in percent outgrowth of C. krusei was found between fresh, filter-sterilized apple juice and canned apple juice at the 0.05 probability level.

A significant suppression of growth of C. krusei was found at the 4 Mrad dose level in both filter-sterilized and canned apple juice. Choppra et al. (8) reported cytotoxical effects in plant material grown in orange and apple juice which had been irradiated at 200 Krad of gamma rays.

TABLE 10.--Percent outgrowth of C. krusei grown on both irradiated filter-sterilized and canned apple juice.*

Dose Mrad	Filter-sterilized Apple Juice		Canned Apple Juice	
	Cells/ml	% Survivors	Cells/ml	% Survivors
0	10.80×10^7	100	10.70×10^7	100
1	9.75×10^7	90	9.70×10^7	91
2	9.45×10^7	88	8.85×10^7	82
3	8.00×10^7	74	8.60×10^7	80
4	6.43×10^7	60	8.30×10^7	77

TABLE 11.--Percent outgrowth of C. krusei grown on both irradiated filter-sterilized and canned apple juice.**

Dose Mrad	Filter-sterilized Apple Juice		Canned Apple Juice	
	Cells/ml	% Survivors	Cells/ml	% Survivors
0	5.88×10^7	100	10.8×10^7	100
1	5.75×10^7	98	10.4×10^7	96
2	5.85×10^7	99	9.70×10^7	90
3	4.73×10^7	80	9.83×10^7	91
4	4.43×10^7	75	8.33×10^7	77

*Fresh apple juice was obtained on October 8.

**Fresh apple juice was obtained on October 15.

2. S. cerevisiae var. ellipsoideus ATCC 560

a) Irradiation of synthetic media

The results obtained when S. cerevisiae var. ellipsoideus cells were grown in glucose-synthetic media irradiated at doses from 0 to 4 Mrad are summarized in Table 12 and statistically analyzed in Tables 13 and 14. The following observations were made:

1) The irradiation of the media resulted in highly significant reduction of outgrowth of S. cerevisiae var. ellipsoideus.

2) Heat sterilization showed to be the least favorable for the outgrowth of this yeast on the irradiated medium containing glucose as compared to the filter-sterilized medium.

3) The effect at 4 Mrad, although not significantly different from that at 3 Mrad, resulted in 83% outgrowth of this yeast.

S. cerevisiae var. ellipsoideus did not grow on sucrose containing media which were exposed even to 1 Mrad level of gamma radiation.

TABLE 12.--Percent outgrowth of S. cerevisiae var. ellipsoideus grown on both irradiated filter-sterilized and heat-sterilized yeast nitrogen base-glucose.

Dose Mrad	Filter-sterilized YNB-glucose		Heat-sterilized YNB-glucose	
	Cells/ml	% Survivors	Cells/ml	% Survivors
0	4.43×10^7	100	4.40×10^7	100
1	4.63×10^7	104	4.14×10^7	94
2	4.77×10^7	107	4.23×10^7	96
3	4.17×10^7	94	4.13×10^7	94
4	4.00×10^7	90	3.67×10^7	83

TABLE 13.--Analysis of variance of S. cerevisiae var. ellipsoideus outgrowth data.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	F
Total	40	19,383.00		
Mean	1	18,619.23		
Adj. Total	39	763.77		
A	1	198.02	198.02	31.23**
B	3	302.67	100.89	15.91**
AB	3	60.28	20.09	3.17*
ERROR	32	202.80	6.34	

**1% probability level

* 5% probability level

TABLE 14.--Radiation treatment means of actual counts/ml comparison for S. cerevisiae var. ellipsoideus grown on irradiated media.

Dose-Mrad	1	2	3	4
Means, Glucose (both treatments)	26	22	20	19
Means, Filtered	30	24	20	21
Heated	22	21	19	16

*Duncan's Multiple Range Test.

b) Irradiation of apple juice

The results of the experiment in which S. cerevisiae var. ellipsoideus cells were grown in irradiated apple juice are presented in Table 15.

The irradiation treatment of the apple juice caused a significant reduction of the outgrowth of this yeast. The lowest outgrowth (74%) was obtained when the juice was exposed to 4 Mrad.

3. R. glutinis (ATCC 2527

a) Irradiation of synthetic media

The effect of irradiation at doses from 0 to 4 Mrad on a synthetic medium containing either sucrose or glucose and on the sterilization

treatment prior to irradiation was investigated. The percent outgrowth of R. glutinis grown in irradiated synthetic media are presented in Tables 16 and 17 and the data is statistically analyzed in Tables 18 and 19.

TABLE 15.--Percent outgrowth of C. krusei, S. cerevisiae var. ellipsoideus and R. glutinis grown on irradiated canned apple juice.

Dose Mrad	C. krusei % outgrowth	S. cerevisiae var. ellipsoideus % outgrowth	R. glutinis % outgrowth
1	93	77	98
2	87	79	97
3	88	76	83
4	75	74	70

TABLE 16.--Percent outgrowth of R. glutinis grown in both irradiated filter-sterilized and heat-sterilized yeast nitrogen base-glucose.

Dose Mrad	Filter-sterilized YNB-glucose		Heat-sterilized YNB-glucose	
	Cells/ml	% outgrowth	Cells/ml	% outgrowth
0	4.50×10^5	100	1.08×10^4	100
1	3.80×10^5	80	8.80×10^4	81
2	3.30×10^5	73	7.50×10^4	69
3	3.15×10^5	70	7.20×10^4	67
4	2.75×10^5	61	7.10×10^4	66

TABLE 17.--Percent outgrowth of R. glutinis grown on both irradiated filter-sterilized and heat-sterilized yeast nitrogen base-sucrose.

Dose Mrad	Filter-sterilized YNB-sucrose		Heat-sterilized YNB-sucrose	
	Cells/ml	% survivors	Cells/ml	% survivors
0	2.65×10^5	100	3.15×10^5	100
1	2.32×10^5	88	2.70×10^5	87
2	2.20×10^5	83	2.50×10^5	81
3	1.97×10^5	74	2.20×10^5	71
4	1.72×10^5	65	1.40×10^5	45

TABLE 18.--Analysis of variance of R. glutinis outgrowth on YNB-glucose and YNB-sucrose media.

Source of Variation	Degree of Freedom	Sum of Squares	Mean Square	F
Total	80	187,104.00		
Mean	1	130,411.25		
Adj. Total	79	56,692.75		
A*	1	13,056.05	13,056.05	483.92**
B	4	3,766.88	941.72	34.90**
C	1	25,276.05	75,276.05	936.84**
AB	4	621.32	155.33	5.76**
AC	1	11,281.25	11,281.25	418.13**
BC	4	767.07	191.77	7.11**
ABC	4	305.13	76.28	2.83**
ERROR	60	1,619.00	26.98	

*A=sterilization treatment; B=radiation; C=media.
**Significant to the 1% probability level.

TABLE 19.--Radiation treatment means of actual counts comparison for R. glutinis grown on irradiated media.*

Dose-Mrad	0	1	2	3	4
Means, all treatments	53	42	38	36	33
Means, Glucose	77	59	54	52	49
Filtered	46	30	33	32	28
Heated	107	89	75	72	71
Sucrose	29	25	23	21	16
Filtered	27	23	22	20	14
Heated	31	27	24	22	17

*Duncan's Multiple Range Test.

The following conclusions are apparent:

1) The least favorable media for supporting growth after irradiation were those containing sucrose.

2) No significant difference was found between sucrose filter-sterilized and sucrose-heat sterilized and subsequently irradiated media.

3) The lowest percent outgrowth was obtained when the cells were grown on media irradiated at 4 Mrad regardless of the carbon source (glucose or sucrose) or the sterilization treatment before irradiation.

b) Irradiation of apple juice

The results of irradiation of apple juice at levels from 0 to 4 Mrad and subsequently inoculated with R. glutinis are given in Table 15.

Preliminary work done on irradiation of synthetic media containing sucrose as carbon source and irradiated at 4 Mrads is presented in Table 20. The yeast species used and their origin were the following: Saccharomyces cerevisiae var. ellipsoideus was obtained from a Michigan winery, Saccharomyces cerevisiae from a Fleischmann's baker's yeast cake, Candida tropicalis and Rhodotorula mucilaginosa were provided by Dr. E. S. Beneke from the Biology Research Center of Michigan State University.

The fact that Mrad levels of gamma radiation resulted in the suppression of growth of certain yeasts does not necessarily indicate unwholesomeness of the irradiated foods or toxicity for humans or higher animals.

TABLE 20.--Percent outgrowth of yeasts grown on heat-sterilized yeast nitrogen base-sucrose medium exposed to 4 Mrad of gamma radiation.

Yeast	Counts/ml		% Outgrowth
	Control	Irradiated	
S. cerevisiae var. *ellipsoideus*			
Expt. 1	2.83×10^7	1.80×10^6	6.4
Expt. 2	1.25×10^7	2.50×10^5	2.0
C. tropicalis			
Expt. 1	3.61×10^7	2.07×10^7	57.
Expt. 2	2.00×10^6	1.20×10^6	60.
Expt. 3	9.95×10^6	5.75×10^6	58.
S. cerevisiae			
Expt. 1	1.82×10^7	8.32×10^6	46.
Expt. 2	1.21×10^7	5.40×10^6	45.
R. mucilaginosa			
Expt. 1	8.75×10^6	6.90×10^6	79.

From this table it is apparent that the reduction of outgrowth on the heat-sterilized and irradiated sucrose medium was statistically significant ($P=0.001$) for all organisms tested. *S. cerevisiae* var. *ellipsoideus* was particularly sensitive to the combined treatment of the growth medium.

SUMMARY AND CONCLUSIONS

The direct and indirect effects of gamma radiation on the survival of several food spoilage yeasts were investigated. This study consisted of two parts. In the first part, the direct effects were investigated by exposure of yeast cells to gamma radiation up to 300 Krad in the following suspension media:

1. demineralized water,
2. synthetic growth medium (yeast nitrogen base double strength),
3. canned apple juice,
4. synthetic media (YNB) adjusted to pH levels of 3.0, 4.5, and 6.0 with a citrate buffer.

The yeasts studied were: Saccharomyces cerevisiae var. ellipsoideus ATCC 560, Rhodotorula glutinis ATCC 2527, and Candida krusei ATCC 2159. The D values in Krad were calculated from the survival curves in demineralized water, in synthetic growth medium, and in apple juice and were: 65, 95, and 125 for S. cerevisiae var. ellipsoideus; 43, 60, and 68 for C. krusei; 65, 50, and 73 for R. glutinis, for the

three media respectively. Survival curves showed a resistant tail for the three species. The higher percent survival was observed when the yeasts were irradiated suspended in apple juice. Demineralized water was the poorest suspension medium for the survival of the cells. S. cerevisiae var. ellipsoideus showed higher resistance to irradiation in all the media used as compared to R. glutinis and C. krusei. The lower pH (3.0 - 4.5) favored the survival of all these species. Thrice repeated irradiation of C. krusei survivors at the dose range of 50 to 200 Krad resulted in lowering of the radiation resistance of this organism. At 300 Krad re-irradiation resulted in increase of the resistance of this organism to radiation.

In the second part of this study media without cells were exposed to doses of 1, 2, 3, and 4 Mrad of gamma radiation and subsequently tested for the support of growth of six different yeasts.

The following observations were made:

1. Irradiated media containing sucrose affected more adversely the outgrowth of most of the yeasts under study. This was significant to the 1% probability level.

2. Heat sterilization prior to irradiation of the media accentuated the inhibition of outgrowth of most of the yeasts studied.

3. S. cerevisiae var. ellipsoideus ATCC 560 did not grow on either heat or filter-sterilized sucrose containing media which were exposed even to the level of 1 Mrad of gamma radiation.

4. No significant difference was found between irradiated filter-sterilized apple juice and irradiated canned apple juice as growth media for C. krusei.

5. A significant suppression of growth was observed when C. krusei was grown on either canned or filter-sterilized apple juice pre-irradiated to 4 Mrad.

6. No significant difference in outgrowth was found between R. glutinis, C. krusei, and S. cerevisiae var. ellipsoideus when they were grown on irradiated apple juice.

7. A significant outgrowth reduction was repeatedly observed when the yeasts were grown on media pre-irradiated to 4 Mrad regardless of the species

grown, the nature of the media (synthetic or natural) or the sterilization treatment prior to irradiation.

APPENDIX

TABLE I.--Percent survival for S. cerevisiae var. ellipsoideus irradiated in various suspension media.

Dose Krad	Demineralized Water	Synthetic Growth Medium*	Canned Apple Juice
	% Survivors		
0	100	100	100
50	15	22	41
100	3.3	10	13
150	0.53	8.3	7.8
200	0.34	3.4	4.3
300	0.28	1.8	3.0

*Yeast nitrogen base double strength.

TABLE II.--Percent survival of S. cerevisiae var. ellipsoideus irradiated in 0.02M citrate buffered synthetic growth media at various pH's.

Dose Krad \ pH	3.0	4.5	6.0
	% Survivors		
0	100.	100.	100.
50	52.06	36.59	15.75
100	34.33	17.43	6.43
150	17.47	10.53	2.06
200	10.85	3.96	1.68
300	5.23	1.33	0.27

TABLE III.--Percent survival of C. krusei irradiated in various suspension media.

Dose Krad	Demineralized Water	Synthetic Growth Medium	Canned Apple Juice
	% Survivors		
50	8.6	20.	37.
100	0.42	2.4	2.5
150	0.02	0.28	0.35
200	0.002	0.19	0.12
300	0.001	0.01	0.005

TABLE IV.--Percent survival of C. krusei irradiated in 0.02 M citrate buffered synthetic growth medium at various pH's.

Dose Krad	pH 3.0	pH 4.5	pH 6.0
	% Survivors		
50	17.	15.	8.0
100	2.2	1.50	1.3
150	0.23	0.86	0.17
200	0.07	0.10	0.04
300	0.005	0.009	0.002

TABLE V.--Percent survival of R. glutinis irradiated in various suspension media.

Dose Krad	Demineralized Water	Synthetic Growth Medium	Canned Apple Juice
	% Survivors		
50	12.	11.	26.
100	3.7	0.84	2.7
150	0.15	0.11	0.18
200	0.04	0.02	0.05
300	0.003	0.004	0.02

TABLE VI.--Percent survival of R. glutinis irradiated in 0.02 M citrate buffered synthetic growth medium at various pH's.

Dose Krad	pH 3.0	4.5	6.0
50	20.	16.	2.2
100	0.58	1.6	0.87
150	0.30	0.16	0.02
200	0.03	0.05	0.007
300	0.004	0.005	-

TABLE VII.--D values in Krad for S. cerevisiae var. ellipsoideus, C. krusei, and R. glutinis irradiated in various suspension media.

Yeast	Demineralized Water	Media Synthetic Medium	Apple Juice
S. cerevisiae var. ellipsoideus	65	95	125
C. krusei	43	60	68
R. glutinis	65	50	73

REFERENCES

1. Alper, T. Hydrogen peroxide and the indirect effect of ionizing radiation, Nature, 162, 615 (1948).

2. _____. Effects on subcellular units and free-living cells. In: Errera, M. and Forssberg, A. (Eds.). Mechanisms in Radiobiology. Academic Press, N. Y., pp. 353-412 (1961).

3. _____, and Gillies, N. E. Restoration of Eschirichia coli Strain B after irradiation. Its dependence on suboptimal growth conditions, J. Gen. Microbiol., 18, 461 (1958).

4. Bacq, Z. and Alexander, P. Fundamentals of Radiobiology. Pergamon Press, N. Y., 1961.

5. Beam, C. A., Mortimer, P. K., Wolfe, R. G., and Tobias, C. A. The relation of radioresistance to budding in Saccharomyces cerevisiae, Arch. Biochem. Biophys., 49, 110 (1954).

6. Berry, R. J., Hills, P. R., and Trillwood, W. Demonstration of a cytotoxic agent in gamma-irradiated carbohydrate solutions, Int. J. Rad. Biol., 9:6, 559 (1965).

7. Bridges, A., Olivo, J. P., and Chandler, V. Relative radioresistances of microorganisms to cathote rays. II. Yeasts and molds, Appl. Microbiol., 4, 147 (1956).

8. Choppra, V. L., Natarajan, A. T., and Swaminathan, M. S. Cytological effects observed in plant material grown on irradiated fruit juices, Radiation Botany, 3, 1 (1963).

9. Cook, A. H. (Ed.). The Chemistry and Biology of Yeasts. Academic Press, N. Y., 1958.

10. Elkind, M. M. and Beam, C. A. Variations of the biological effectiveness of X-rays and alpha particles in haploid S. cerevisiae, Radiation Res., 3, 88 (1955).

11. Etchells, J. L., Bell, T. A., and Jones, I. D. Morphology and pigmentation of certain yeasts from brines and cucumber plants, Farlowia, 4:3, 265 (1953).

12. Frey, H. E., and Pollard, E. C. Ionizing radiation and bacteria. Nature of the effect of irradiated medium, Radiation Res. 28, 668 (1966).

13. Goldblith, S. A. Wholesomeness of irradiated foods: Past history, present status, international aspects and future outlook, Food Technol. 20:2, 93 (1966).

14. _____. Radiation Sterilization of Food, Nature, 210, 433 (1966).

15. Hollaender, A. (Ed.). Radiation Protection and Recovery. Pergamon Press, N. Y., pp. 1-392 (1963).

16. Holsten, R. D., Sugii, M., and Steward, F. C. Direct and indirect effect of radiation on plant cells: their relation to growth and growth induction, Nature 208, 850 (1965).

17. Ingram, M. Yeasts in food spoilage. In: Cook, A. H. (Ed.), The Chemistry and Biology of Yeasts. Academic Press, N. Y., pp. 603-631 (1958).

18. James, A. P. and Werner, M. M. The radiobiology of yeast, Radiation Botany, 5, 359 (1965).

19. Kiss, I., Falkas, J., Andrassy, E., and Beczassy, K. The sterilization of sugar by ionizing radiations and investigations related to the use of sterilized sugar in the canning industry. A paper presented in the 2nd International Congress on Food Science and Techbology, Warsaw, August 1966.

20. Korogodin, V. I. Forms of inactivation of yeast cells by ionizing radiations, Biofizika 3:2, 206 (1958).

21. Laser, H. Some observations on irradiation effects in yeast, Radiation Res., 16, 471 (1962).

22. Lea, D. E. Actions of Ionizing Radiations or Living Cells. Cambridge University Press, N. Y., 1955.

23. Löfloth, G. Toxic effects of irradiated foods, Nature, 211, 302 (1966).

24. Miller, J. J., Kingsley, V. V., and Ramirez, C. Comparative sensitivity to gamma radiation of yeast mitosis and meiosis, Radiation Botany, 5, 383 (1965).

25. Molin, N., and Ehrenberg, L. Anti-bacterial action of irradiated glucose, Int. J. Rad. Biol., 8:3, 223 (1964).

26. Mortimer, R. K. Factors controlling radiosensitivity of yeast cells. Fundamental aspects of radiosensitivity, Brookhaven Symp. Biol., 14, 62 (1961).

27. O'Brien, R. T. Radiation sensitivity studies on related fermenting and respiring yeasts, Radiation Botany, 1, 61 (1961).

28. Patrick, M. H., Hayes, R. H., and Uretz, R. B. Dark recovery phenomena in yeasts, Radiation Res. 21, 144 (1964).

29. Pollard, E. C., Ebert, J. M., Miller, C., Kolacz, K., and Barone, T. F. Ionizing radiations: Effect of irradiated medium in synthetic processes, Science 147, 1045 (1965).

30. Shaw, M. W. and Hayes, E. Effects of irradiated sucrose on the chromosomes of human lymphocytes in vitro. Nature 211, 1254 (1966).

31. Stapleton, G. E. Factors modifying sensitivity of bacteria to ionizing radiations, Bacteriol. Rev. 19, 26 (1955).

32. Stuart, B. O., and Stannard, J. N. Radioprotection of yeast by cystamine, Radiation Res. 28:3, 609 (1966).

33. Wickerham, L. J. Taxonomy of yeasts, U. S. Dept. Agr. Tech. Bull. 1029, 1 (1951).