Effect of Storage Studies of Microorganisms on Gamma-Irradiated Rice

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ABSTRACT

To observe the effect of storage at 30°C. on microorganisms on rice irradiated by gamma-ray, unpolished and polished rice harvested in Japan were used as samples. The storage life of polyethylene-packaged rice containing 14 to 15% moisture was extended 3 to 4 times by means of 0.2 Mrad irradiation. The number of molds on the irradiated rice grains was not increased for 3 to 4 months under these conditions. When the moisture in the rice sample was 17%, irradiation of more than 0.4 Mrad was necessary to extend its storage life. The only species of molds which can grow on storage-rice grains containing 14 to 15% moisture was *Aspergillus restrictus*. When moisture content was 16%, *A. glaucus* group and *A. restrictus* were observed. At 17% moisture, growths of molds were mainly members of *A. glaucus* group and *Penicillium citrinum* series, whereas *A. restrictus, A. versicolor*, etc. were minor ones. When a sheet of porous material, e.g., kraft-paper bag, was used to pack rice, the storage life of rice containing moisture of less than 15% was extended 3 to 4 times by means of 0.2 Mrad irradiation, the storage condition being 30°C., 75% r.h. In this case, the number of molds on rice was not increased for 3 to 4 months.

Rice is a staple food all over the world, and more than half the population of the world consumes rice as its main food. However, the damage caused by insects or microorganisms is severe in producing areas of East Asia and other parts of the world. Damage is especially severe in Japan because summer humidity is high enough for mold or bacterial growth on rice stored improperly for a long period of time.

Since 1948 Iizuka, one of the authors, has carried out a series of studies (1,2,3) on microflora of normal and damaged rice harvested in Japan, China, Burma, Thailand, etc., and his conclusions have been applied to make improvements on preparation, distribution, and storage of rice in Japan (4,5). In those reports, Iizuka has suggested that for safe storage, unpolished rice should preferably be preserved at low temperature (below 15°C.), that methyl-bromide fumigation decreases infestation, that moisture content must be kept below 15%, and that imported rice must be polished before distribution.

On the basis of the results obtained from such studies, we wanted to investigate the radio-pasteurization of rice. In a previous paper (6), a study on the effect of gamma-irradiation on the microflora of rice has been reported. The present work will report the effects of storage on microorganisms in relation to moisture content, relative humidity, and dosage of gamma-ray, using unpolished and polished new rice harvested in Japan, and will discuss these factors in relation to long-term storage.

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MATERIALS AND METHODS

Materials

Unpolished rice, species of "Koshihikari," and polished rice of "Senshuraku," milled in March 1968 and produced in Gunma Prefecture in 1967, were used for the experiments. Unpolished rice of "Tibaasahi" produced in Gunma Prefecture was also used for the supplementary experiment.

Gamma-Irradiation

The gamma-ray source used was 0.1-Mcurie cobalt-60 equipped in an irradiator. The dose rate at the irradiation position was 0.2 Mrad per hr., according to the determination of a Fricke dosimeter.

The following doses of irradiation were used: 0.02, 0.05, 0.1, 0.2, 0.3, and 0.4 Mrad.

Determination and Adjustment of Moisture Content

Moisture content of rice was determined by the Infrared-Ray moisture meter, and measurement was made at 130°C. The moisture content was adjusted to the desired levels by adding distilled water to the controls, after which the rice was kept at 4°C. for 5 days with occasional shaking to permit uniform distribution of the water.

The original moisture contents of samples were 13.0% for "Koshihikari" (unpolished rice), 14.0% for "Tibaasahi" (unpolished rice), and 13.7% for "Senshuraku" (polished rice), respectively. The adjusted moisture contents of samples were 14.2 to 14.5, 16.0 to 16.2, and 17.0 to 17.2%.

Storage of Samples

To observe the effect of storage on microorganisms in a polyethylene bag, about 400 g. of rice at each moisture level was sealed in a high-density polyethylene bag, 0.08 mm. thick, and irradiated with the various doses described above. Four bags of packaged rice were prepared for each dose level. After irradiation, the samples were stored at 30°C. and 75% r.h. in a Thermo-Hygrostat incubator for 1 to 5 months.

To observe the effect of humidity on a sheet of porous material such as kraft-paper bag, rice containing 15.0% moisture was used. Portions of 100 g. each were placed in sterile 200-ml. glass vials, the open end of the vials was stuffed with sterile cotton, and, after irradiation, stored at 30°C. at 75 and 85% r.h. Some samples were also stored at 30°C. and 70 to 90% r.h. Other samples were stored at 25°C. packed in the polyethylene bag.

These experiments were carried out mainly from April to September 1968, and some were done the following year.

Measurement of Storage Effect

The storage effect was measured by counting the surviving microorganisms by the following methods.

Method 1. Five grams of grain was pulverized in a homogenizer in 50 ml. of sterile water. Successive dilutions were made with this same medium; replicate 0.5-ml. portions of one or more dilutions were spread and cultured on malt agar plates containing 7.5% sodium chloride, while replicate 0.2-ml. portions of one or

more dilutions were cultured on a Difco nutrient agar plate. Colonies were counted and identified after 5 to 7 days of incubation at 30°C. The malt agar containing 7.5% sodium chloride supports the growth of molds, and the nutrient agar supports the growth of bacteria.

Method 2. One hundred grains of rice were washed by shaking well 3 times with 0.1% Tween 20, and then with sterile water 7 times. The washed grains were then cultured on malt agar containing 7.5% sodium chloride to detect molds.

Ability of unpolished rice to germinate was measured by incubating 100 grains on Czapek agar plates.

RESULTS

Effect of Moisture Content on Stored Rice in Polyethylene Bag

(a) *14% Moisture level.* The moisture levels of 14.2% for unpolished rice and 14.5% for polished rice corresponded to that of rice in Japanese markets. These samples were sealed in polyethylene bags and, after irradiation, were stored at 30°C. and 75% r.h. For unirradiated rice stored at a 14.5% moisture level, the spoilage by molds was high after 1 or 2 months of storage, as shown in Table I; these growths of molds on unpolished rice are shown in Fig. 1. The volume of gas in the bag diminished after 4 months of storage; this may be due to the growth of molds in the sample. The growth delay of molds on the irradiated rice was observed clearly at this moisture level as compared with the higher moisture levels, as will be seen later. Rice irradiated with a dose of 0.1 Mrad extended the storage life from 2 to 3 months. At a dose of 0.2 Mrad, storage life was extended for more than 3 to 4 months.

The only species of mold detected at this moisture level was an osmophilic type of *Aspergillus restrictus.*

In Table II is shown the extent of mold contamination after 4 months of storage (14.2% moisture) of surface-disinfected polished rice grains (cultured on malt agar-sodium chloride plates) as affected by several levels of irradiation.

TABLE I. NUMBER OF COLONIES OF MOLD FROM IRRADIATED RICE STORED AT 30° C. IN A POLYETHYLENE BAG

Storage months	0 Mrad	0.02 Mrad	0.05 Mrad	0.10 Mrad	0.20 Mrad
	A. Mold count per g. of unpolished rice (14.5% moisture content)				
0	26	26	7	...	...
1	34	...	20	...	...
2	2.2×10^3	1.3×10^4	6.5×10^2	4.4×10^2	10
3	3.0×10^4	2.1×10^5	4.7×10^4	6.2×10^2	40
4	1.4×10^6	1.4×10^6	2.2×10^6	8.3×10^5	9.2×10^4
	B. Mold count per g. of polished rice (14.2% moisture content)				
0	22	17	7	...	...
1	86	26	66	...	...
2	2.2×10^3	1.3×10^4	6.5×10^2	4.4×10^2	10
3	3.0×10^4	2.1×10^5	4.7×10^4	6.2×10^2	40
4	4.1×10^5	3.1×10^5	2.0×10^5	5.8×10^4	1.0×10^2

TABLE II. GERMINATION AND SURFACE-DISINFECTED GRAINS YIELDING MOLD WHEN USING MALT AGAR MEDIUM CONTAINING 7.5% SODIUM CHLORIDE FOR THE DETECTION

	0 Mrad %	0.02 Mrad %	0.05 Mrad %	0.10 Mrad %	0.20 Mrad %
A. Unpolished rice stored 4 months at 30°C. in a polyethylene bag (14.5% moisture content)					
Surface-disinfected grains yielding mold	100	100	100	100	85
Germination	9	22	30	46	48
B. Polished rice stored 4 months at 30°C. in a polyethylene bag (14.2% moisture content)					
Surface-disinfected grains yielding mold	78	80	0	0	0

During storage, germination of unpolished rice was not affected at a 14.5% moisture level for 2 months, but it was retarded greatly for longer periods of storage, as shown in Fig. 2. Furthermore, this retardation was correlated with mold propagation.

Infestation by insects of storage rice was observed after 4 months of storage only in the unirradiated rice.

(b) *16% Moisture level.* At a moisture level of 16.0 to 16.2%, the aerial gas volume in the bag diminished after about 1 month of storage when the sample was irradiated with less than 0.05 Mrad. The delay in growth of molds on both unpolished and polished grains was not observed at a dose of less than 0.05 Mrad. As shown in Table III, this delay was observed at doses of more than 0.1 Mrad. When rice was irradiated with a dose of 0.2 Mrad, the mold growth on and in grains was not observed for more than 1 month of storage, and storage life of grains was

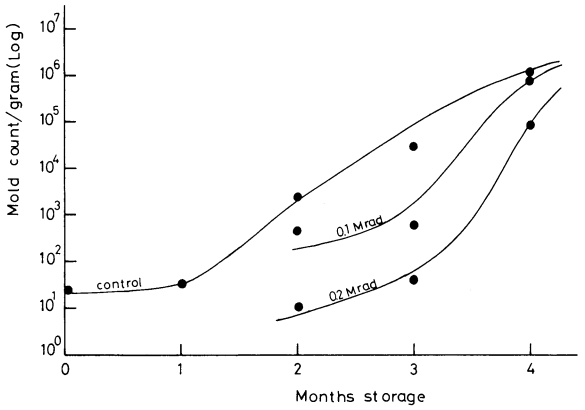


Fig. 1. Number of colonies of mold from irradiated unpolished rice stored at 30°C. in polyethylene bag (14.5% moisture content).

TABLE III. NUMBER OF COLONIES OF MOLD FROM IRRADIATED RICE STORED AT 30°C. IN A POLYETHYLENE BAG

Storage months	0 Mrad	0.05 Mrad	0.10 Mrad	0.20 Mrad	0.30 Mrad
	A. Mold count per g. of unpolished rice (16.0% moisture content)				
0	26	7	...	...	...
1.0	7.3×10^5	5.5×10^5	6.5×10^3	...	...
1.5	6.8×10^6	6.8×10^6	1.1×10^6	...	...
2.0	1.0×10^7	1.1×10^7	1.1×10^7	2.7×10^5	...
	B. Mold count per g. of polished rice (16.2% moisture content)				
0	22	7	...	...	...
1.0	2.2×10^5	1.4×10^5	4.8×10^3	...	...
1.5	8.0×10^5	8.0×10^5	1.1×10^6	4.6×10^2	...
2.0	6.7×10^5	1.4×10^6	3.8×10^6	2.6×10^7	1.1×10^3

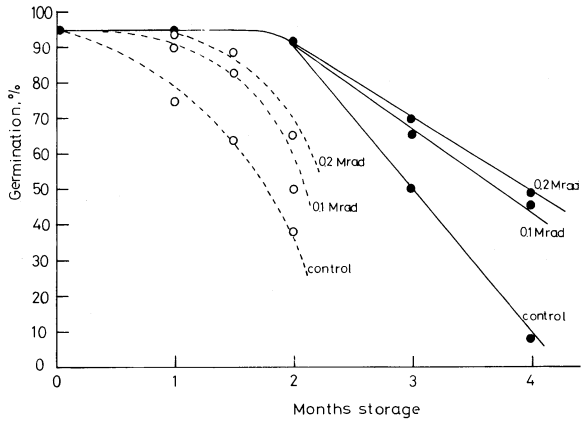


Fig. 2. Loss of germination of irradiated unpolished rice (stored at 30°C.). Solid circles, 14.5% moisture content; open circles, 16.0% moisture content.

extended 2 times as compared with unirradiated rice. At a dose of 0.3 Mrad, storage life was extended by more than 3 times. These growth curves of molds on unpolished rice are shown in Fig. 3.

The molds propagated on the stored grains were members of the *A. glaucus* group and osmophilic strains of *A. restrictus*.

Germination of unpolished rice was maintained for less storage time compared with rice containing 14.2 to 14.5% moisture when irradiated with the same dose (Fig. 2). No increase was observed in the number of bacteria on the rice during this 2 months of storage.

(c) *17% Moisture level.* When unirradiated rice adjusted to high moisture levels of 17.0% was stored for more than 15 days, the aerial gas volume in the bag decreased considerably; at 20 days of storage, the shape of the bag changed as if it

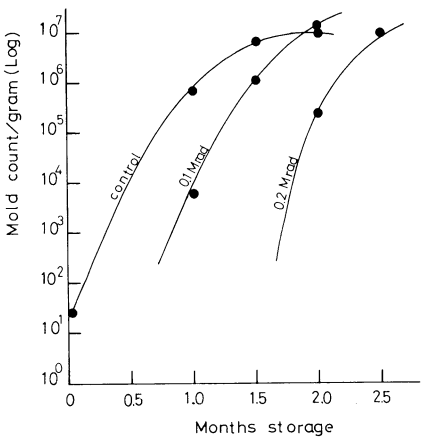
had been packaged under a vacuum. The aerial gas volume in the bags irradiated with 0.05 to 0.1 Mrad also decreased markedly after 15 days of storage. At 0.2 Mrad, this decrease of the aerial gas volume was observed after 20 days of storage; at 0.3 Mrad, it was observed after 30 days.

When aerial gas volume in the bags of storage samples decreased, the growth of molds on the rice could be observed with the naked eye, and these sealed samples emitted the odor of molds. The molds, propagated on the storage-rice grains, were mainly members of the *A. glaucus* group capable of forming perithecia, and of the *Penicillium citrinum* series; *A. restrictus, A. versicolor*, and others were also detected in minor quantities. The maximum number of propagated molds for the unpolished grains were from 1.0 to 3.0×10^6 per g., and from 1.0 to 9.0×10^5 per g. for the polished grains (Table IV). The growth curves of molds on storage grains are shown in Fig. 4. It can be seen from the results regarding mold growth that more than 0.4 Mrad is necessary to extend storage life for 1 to 2 months at 30°C.

Germinability of the unpolished rice, irradiated with less than 0.2 Mrad, decreased rapidly in 1 month of storage, and this may be due to the propagation of molds on the germ. No increase was observed in the number of bacteria on the rice during storage.

Storage Effect at Various Levels of Humidity in Relation to Irradiation

The rice containing 14.0% moisture was packed both in kraft-paper and polyethylene bags, irradiated with various doses, and stored at 30°C. at humidities of 70 to 90%. In the case of the paper bag stored under 90% humidity, rapid growth of molds on rice was found, and it was not affected for 20 days of storage by dosage of less than 0.1 Mrad. The rice grains which were stored for that period were covered with bluish-green molds that could be observed with the naked eye.



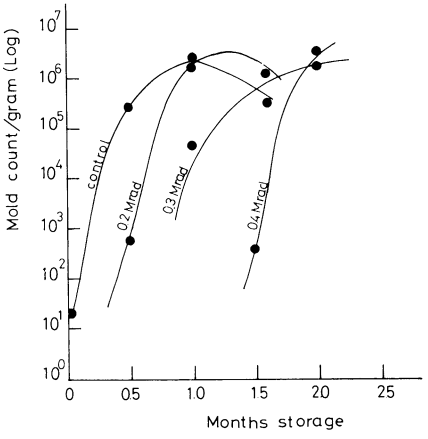


Fig. 3 (left). Number of colonies of mold from irradiated unpolished rice stored at 30°C. in polyethylene bag (16.0% moisture content).

Fig. 4 (right). Number of colonies of mold from irradiated unpolished rice stored at 30°C. in polyethylene bag (17.0% moisture content).

TABLE IV. NUMBER OF COLONIES OF MOLD FROM IRRADIATED RICE STORED AT 30°C. IN A POLYETHYLENE BAG (17.0% moisture content)

Storage months	0 Mrad	0.05 Mrad	0.10 Mrad	0.20 Mrad	0.30 Mrad	0.40 Mrad
	A. Mold count per g. of unpolished rice					
0	20	17	20	...	...	...
0.5	2.7×10^5	2.4×10^5	9.4×10^4	5.8×10^2	...	...
1.0	1.8×10^5	2.6×10^6	4.2×10^6	2.9×10^6	5.2×10^4	...
1.5	2.9×10^5	2.5×10^5	3.1×10^5	1.1×10^6	1.0×10^3	4.2×10^2
	B. Mold count per g. of polished rice					
0	1.8×10^2	40	...	...	...	...
0.5	1.8×10^5	1.9×10^5	9.0×10^4	3.6×10^2	...	...
1.0	1.1×10^5	5.0×10^5	1.0×10^5	8.2×10^4	3.4×10^4	60
1.5	1.4×10^5	9.1×10^5	1.4×10^5	7.9×10^5	...	33

TABLE V. NUMBER OF COLONIES OF MOLD FROM IRRADIATED RICE STORED AT 30°C., 85% r.h.

Storage months	0 Mrad	0.05 Mrad	0.10 Mrad	0.20 Mrad
	A. Mold count per g. of unpolished rice			
0	26	7	...	...
0.5	2.3×10^2	7	7	...
1.0	1.5×10^4	2.0×10^4	8.9×10^3	13
1.5	8.5×10^6	1.2×10^7	9.9×10^5	60
	B. Mold count per g. of polished rice			
0	7	...	...	...
0.5	13	...	...	...
1.0	7.9×10^3	6.0×10^3	7	7
1.5	1.5×10^6	5.4×10^6	1.8×10^6	1.7×10^2

TABLE VI. NUMBER OF COLONIES OF MOLD FROM IRRADIATED RICE STORED AT 30°C., 75% r.h.

Storage months	0 Mrad	0.05 Mrad	0.10 Mrad	0.20 Mrad
	A. Mold count per g. of unpolished rice			
0	26	7	...	...
1	20	20	...	...
2	2.3×10^3	2.3×10^3	10	...
3	1.6×10^5	1.2×10^5	8.6×10^3	4.0×10^2
	B. Mold count per g. of polished rice			
0	7	...	...	...
1	46	...	...	...
2	1.0×10^4	5.5×10^2	10	...
3	8.0×10^4	3.4×10^4	1.4×10^4	33

However, rice packed in the polyethylene bag and stored in this manner did not exhibit mold growth for 3 months. As shown in Fig. 5, the total number of bacteria comprising the characteristic microflora on the newly harvested unpolished rice (1,2,3,6) decreased rapidly in the kraft-paper bag at 90% r.h., and this decrease may be due to growth of molds on the rice. However, for rice packaged in the polyethylene bag, the total number of bacteria decreased slowly in comparison with rice packed in the kraft-paper bag at 90% r.h. At first, there was a rapid decrease of bacteria characteristic of newly harvested rice, such as chromogenic *Pseudomonas* and fluorescent *Pseudomonas*, and after that predominant microflora consisted of so-called "red *Pseudomonas*" (6).

To observe the growth effect of molds on the rice (15.0% moisture content) under various humidity levels, glass vials stuffed with sterile cotton were used. As shown in Table V, the preservation life of rice at 85% r.h. was extended more than 2 to 3 times by means of 0.2 Mrad irradiation, and propagation of the molds was not observed for longer than 1.5 months. When rice was stored at 75% r.h., mold growth on grains was not observed for 3 to 4 months by means of 0.2 Mrad irradiation (Table VI). The growth curve of molds differed with humidity levels as shown in Fig. 6. At 85% r.h. species of *A. glaucus* and *A. restrictus* were detected; at 75% r.h. only the osmophilic strain of *A. restrictus* was grown.

To observe the storage effect at room temperature, the polyethylene-packaged rice samples were stored for 5 months at 25°C. and 80% r.h. Here polished rice containing 15.0 and 13.7% moisture and unpolished rice containing 14.5% moisture were used for samples. After storage for 5 months, mold growth on the

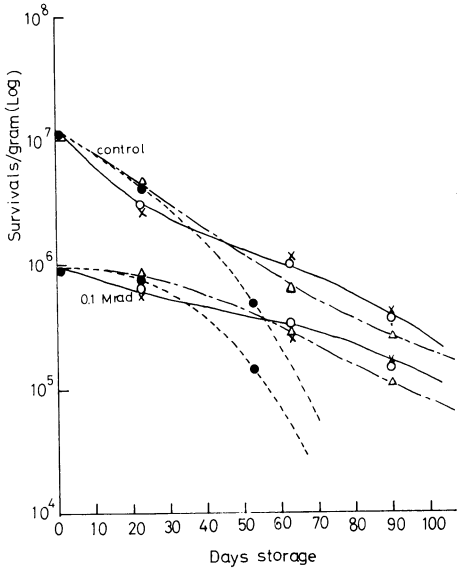


Fig. 5. Number of survivors of bacteria on unpolished rice stored at 30°C. The sample was not crushed before incubation. X, in polyethylene bag, 70% r.h.; open circles, in polyethylene bag, 90% r.h.; triangles, in kraft-paper bag, 70% r.h.; solid circles, in kraft-paper bag, 90% r.h.

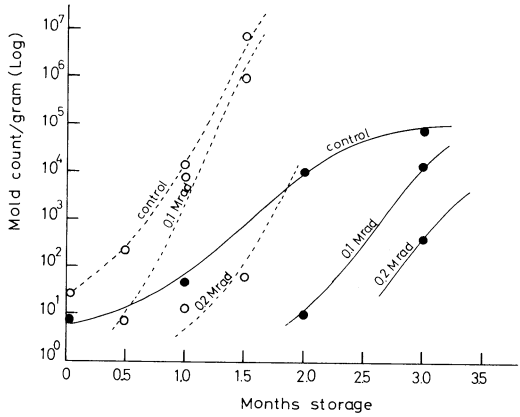


Fig. 6. Number of colonies of mold from irradiated unpolished rice stored at 30°C. Solid circles, at 75% r.h.; open circles, at 85% r.h.

TABLE VII. NUMBER OF COLONIES OF MOLD AND SURFACE-DISINFECTED GRAINS YIELDING MOLD WHEN USING MALT AGAR MEDIUM CONTAINING 7.5% SODIUM CHLORIDE FOR THE DETECTION (polished rice stored 5 months at 25°C. in a polyethylene bag; 15.0% moisture content)

Dose	Number of Colonies	Surface-Disinfected Grains Yielding Mold
0 Mrad	2.1×10^6	93%
0.02 Mrad	1.7×10^5	50%
0.05 Mrad	1.6×10^6	65%
0.10 Mrad	3.4×10^5	12%
0.20 Mrad	3.2×10^2	2%

grains of polished rice at the 15.0% moisture level was high at dosages of less than 0.1 Mrad (Table VII). The molds detected in this case consisted mainly of osmophilic strains of *A. vitricolae* which grew best at 20° to 25°C. and showed poor growth at 30°C. In addition to this species, some *A. glaucus* and *A. restrictus* strains were isolated. In the case of polished rice with 13.7% moisture, slight growth of molds was detected only in the unirradiated case; for unpolished rice with 14.0% moisture, irrespective of irradiation, mold growth was not detected and germination was not retarded after 5 months of storage.

DISCUSSION

When rice was stored in a polyethylene bag, the moisture content showed the primary effect on mold growth, and relative humidity was of minor importance. The storage life of polyethylene-packaged rice containing 14 to 15% moisture can be extended 3 to 4 times by means of 0.2 Mrad irradiation. When the moisture content of the rice is greater than 16%, a dose of 0.3 to 0.4 Mrad is necessary to extend its storage life. By keeping the moisture content of rice stored in a polyethylene bag below 15%, a dose of 0.2 Mrad is probably enough to extend storage life safely for more than 1 year, under summer climate conditions in Japan.

Furthermore, irradiated unpolished rice stored at 30°C. retains its germinability for a longer time than unirradiated controls. Loss of germinability appears to be correlated with irradiation dose and mold growth.

Growth of molds on rice containing 13.7 to 14.0% moisture was slow compared to rice containing 14.2 to 14.5% moisture, and only a dose of 0.05 Mrad can extend the storage life of rice for more than 1 month at 30°C. without increase of molds.

When the rice containing 15.0% moisture was packed in a polyethylene bag and stored for 5 months at 25°C., some species of molds grew on the rice grains, and aerial gas volume in a bag decreased considerably. At a dose of 0.2 Mrad, growth of molds on rice was negligible after 5 months of storage. Microflora on the rice stored at 25°C. were different from those of rice stored at 30°C. Mold growth on unpolished rice containing 14.0% moisture was negligible whether irradiated or unirradiated, and no loss of germination potential was observed in 5 months of storage.

Storage life of rice (containing less than 15% moisture) packed in a sheet of porous material (e.g., kraft-paper bag) can be extended 3 times by means of 0.2 Mrad irradiation, and the number of molds on the rice would not be increased for 3 to 4 months at 30°C. and 75% r.h. However, for rice stored at 85% r.h., storage life is extended 2 to 3 times by means of 0.2 Mrad irradiation, and growth of molds on the grains observed only after 1 month of storage.

For the safe preservation of rice by gamma-irradiation, biochemical and organoleptic tests must be investigated. Okanoue et al. (7) reported that the dose for processing rice harvested in Japan without causing change in its color, odor, flavor, and taste must be kept below 0.1 Mrad. Nishimura and Takaoka (8) investigated the change of quality after gamma-irradiation of Burma rice, and reported that dose level below 0.35 Mrad must be irradiated on the rice for practical use. In this investigation, a dose of 0.2 to 0.3 Mrad extended the storage life of rice containing moisture levels below 15% by 3 to 4 times. However, further investigations must be carried out on the wholesomeness and acceptability of irradiated rice.

Acknowledgment

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