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Studies on the Preservation of Korean Rice by Gamma-irradiation (II)
On disinfestation of rice by gamma-ray irradiation

by

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감마선 조사에 의한 쌀 저장에 관한 연구 (제 2 보)

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요 약

1968 년도에 수확한 팔달 벼를 1969년 4월에 현미와 백미를 도정(표준규격)하여 가마니와 kraft paper bag에 넣고 0, 10, 30, 50 krad의 γ-ray를 조사한 후 실온에 저장하면서 해충의 발생 상태, uric acid의 함량, fat acidity, riboflavin의 함량, 식미시험 등을 하여 다음과 같은 결과를 얻었다.

(1) 현미나 백미 모두 kraft paper bag에 포장한 구가 30~50krad의 γ-ray 조사로써 살충효과가 현저하였다. 가마니 포장구는 여름을 지나는 동안 재차 감염에 의한 해충의 발생으로 그 효과가 낮다.

(2) 쌀중의 uric acid 함량의 변화는 해충의 발생 상태와 비례적이다.

(3) Fat acidity는 쌀 저장 중 증가하였고, 30~50 krad 조사구는 대조구보다 다소 증가하는 경향이다.

(4) Riboflavin의 함량은 50krad의 조사로써 대조구와 별 차이가 없다.

(5) 식미시험에 있어서 10, 30, 58 krad의 조사구는 그 맛 · 냄새 · 빛깔 · 점도에 있어서 유의차가 없다.

Introduction

In the previous report[1] we observed the effect of 6~400 krad irradiation of gamma-ray on the disinfestation for hulled Korean rice of Nogkwang and Paldal variety, which were in both polished and unpolished forms, being packed hermitically in polyethylene bags, and stored at room temperature. Also miscellaneous effects of irradiation on water content, amylose, reducing sugar and rancidity of rice were examined

Many researchers reported on the disinfestation by irradiation; Cornwell *et al.*[2] pointed the relation between the insect's living environments and irradiation conditions and reported the distinction in irradiation sensitivity with the species as well. Also the irradiation sensitivity of *Sitophilus* was clarified by Bruel[3]. Cornwell *et al.*[4] described the disinfesation by irradiation differed according to its dose rate and that 10^4~10^6 rad/hr were suitable for disinfestation of grain. By Golumbic *et al.*[5] it was re-

portea that the growth state of insect, such as spawn, pupa, larva and adult determinded the dose necessary for insects sterility or extinction; spawns and pupae would soon die after hatching with 13.2 krad, but they never failed to be extinguished with doses ranging 40~100 krad. Another report by P. Pesson[7] proved that the disinfestation dose for major insects found in stored grains sufficient with 20 krad. In 1964, for the purpose of disinfested storage of wheat and its products, American Authorities permitted the γ-ray irradiation of 20~50 krad.

In connection with the previous report, authors tested and obtained the results on the disinfestation, uric acid content, riboflavin content and panel test of rice irradiated with 10~50 krad γ-ray, the samples of wich being packed in the straw bags and kraft paper bags and stored until October of the year.

Materials and Methods

1. Sampling and packing

Paldal variety, the sample rice, was harvested in 1968 in Suwon region and hulled into polished rice and unpolished one by the common method adopted by the Agricultural Products Inspection Station of Korea.

After being hulled, each 1kg of sample rice was packed into the sealed kraft paper bags and the usual straw bags used in Korea, respectively.

2. Irradiation and storage of samples

On the 18th of April, the samples were irradiated by 700μCi Co^{60}, and the respective doses were 10, 30 and 50 krad. The irradiation and storage of samples were conducted throughout under the room temperature of 10~20°C.

3. Detection of insects

Number of insects occurring in 100 gram of the samples irradiated was counted bimonthly by the naked eye to determine the optimum dose.

4. Determination of uric acid[7]

For the determination, the cyanide free method was adopted, based on a distinct color reaction of sodium carbornate with uric acid.

Ten grams of the powdered sample were introduced into a flask containing 100ml of sterile water and was kept stirring for an hour. After five minutes of homogenizing by Waring blender, the mixture was separated by centrifuging, and 9ml of the decanted liquid was then mixed with 0.5ml of 0.66 NH_4SO_4 and 0.5ml of 10% sodium tungstate solution for the complete precipitation of protein. Three of the same test tubes were prepared, each containing 4ml, of the above filtrate, of uric acid, and of sterile water respectively. Addition of 1ml of 14% sodium carbonate solution and 1ml of uric acid reagent into each tube was followed by keeping them stand for 15 minutes and then developing the color reaction. For the optical density (O. D.), a spectrophotometer was used at 680 mμ and sterile water was used as the blank test. In connection with O.D. value the quantity of uric acid was determined by the following formula. mg of uric acid/100g of sample=

$$\frac{\text{O.D. of sample}}{\text{O.D. of standard}} \times 5.76$$

5. Determination of fat acidity[8]

Fat acidity was expressed as mg of potassium hydroxide needed to neutralize free fatty acid involved in 100g of sample.

With 50ml of benzene, 20g of dry powdered sample were put into a 25ml flask and kept stirring for 5 minutes. After filtering through decantation, 25ml of the filtrate were transfered to a 100ml flask and 25ml of 0.1% alcohol-phenolphthalein added to it, being titrated with 0.0356 N-KOH. The color for neutralization point is one that comes out from mixing 2.5ml of 0.001% $KMnO_4$ with the liquid which consists of several drops of 0.5% potassium dichromatesolution and 50ml sterile water, manifesting the same color as that of samples.

6. Dectermination of riboflavin

Flourometry[9] and AOAC official method were combined to determine the quantity of riboflavin.

Three grams of the powdered sample, equivalent to 5μg of riboflavin, and 75ml of 0.1 N-H_2SO_4

were put into a 100ml flask together. In autoclave the hydrolysis was carried out under the pressure of 15 lbs for 15 minutes and then, the resulted cooled, its pH being adjusted to 4.3 with 2.5 mole sodium acetate. When the above suspension was diluted to 100ml and filtered, the first 15ml were discarded and 60 ml of the rest was mixed with 2ml of 4% $KMnO_4$ in a graduated-cylinder, discoloring the excess $KMnO_4$ by adding 3% H_2O_2 solution 3 minutes later. Several drops of acetone could arrest the foams and another filtration was necessary after dilution to 65 ml with full mixing. Into two tubes of 15ml filtrate, 1ml of distilled water, and 1ml of riboflavin standard solution were added respectively, and O.D. of them were measured at 460 mμ through the spectrophotometer (QV-50) attached with a flouresence detector.

The quantity of riboflavin contained is computed from the following formula.

$$\text{ug of riboflavin/g of sample} = \frac{A\text{-}C}{B\text{-}A} \times \frac{6.5}{93G} \times 100$$

A: O.D. of prepared sample plus distilled water

C: O.D. of prepared sample plus distilled water and 20mg of $Na_2S_2O_4$

B: O.D of sample plus standard riboflavin

G: Weight of sample

7. Organoleptic test[10]

The panel test for taste was performed by 20 panels in October, sixth month after irradition. Marks on the discussion paper indicated the scores of the taste, odor, color, and viscosity of the boiled irradiated rice and the cooking method was as usual. The score scales were graduated from 0 to 6 and the standard is 2 point, being alloted to the usual boiled rice.

Results and Discussion

1. General components of samples

The general components of rice of Paldal variety, in their polished and unpolished forms, were as follows.

Sample	Moisture	Crude protein	Crude fat	Crude ash	N-free extract
Unpolished rice	14.3%	8.32%	2.80%	1.34%	72.24%
Polished rice	14.9%	7.56%	1.08%	0.67%	57.79%

2. Insects infestation

For the purpose of preventing infestation during the storage, the sample rice was, hulled into unpolished rice and polished one in April, packed in kraft paper bags and straw bags, irradiated with 10~50 krad and stored at room temperature. The states of infestations detected by naked eyes were illustrated in Fig. 1, 2, 3, and 4.

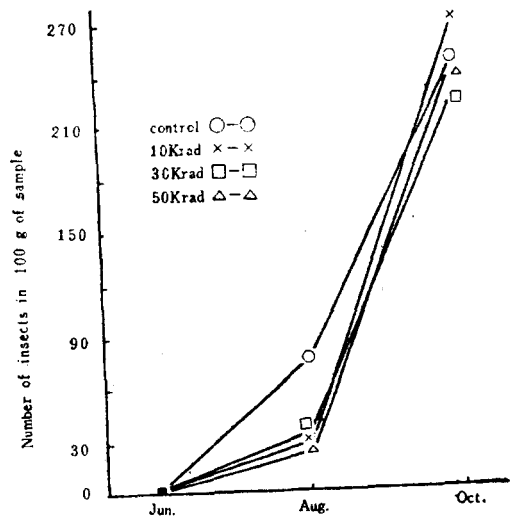

Fig. 1. Comparison with insects infestation in unpolished rice packed with straw bag, by irradiation with low dose during storage

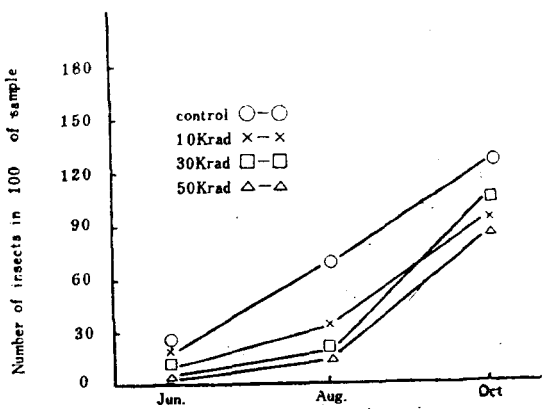

Fig. 2. Comparison with insects infestation in polished rice packed with straw bag, by irradiation with low dose during storage

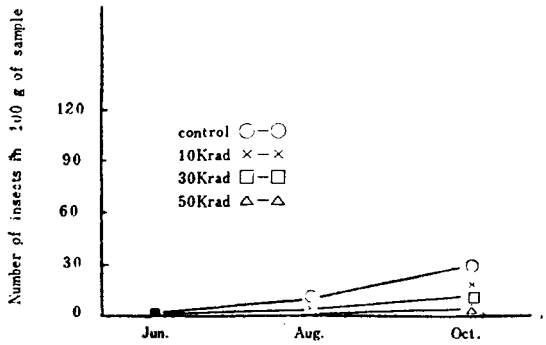

Fig. 3. Comparison with insects infestation in unpolished rice packed with kraft bag, by irradiation with low dose during storage

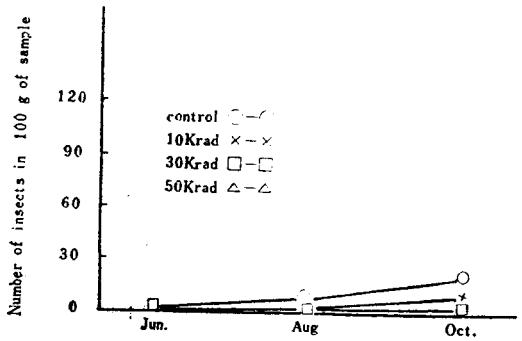

Fig. 4. Comparison with insects infestation in polished rice packed with kraft bag, by irradiation with low dose during storage

As shown in Fig. 1 and 2, the smples in straw bags were slightly infested and there was almost no distinction between the samples in June, but gradual increase in infestation appeared in August, the sultry season here and finally in October vigorous infestation of almost the same level was found both control lot and the irradiated lot. About the second contamination in straw bag packing, we examined in the previous report[1]. As Cornwell[4] reported, with 20 krad dose, insects in grain had or lost their fertility in 5~6 weeks, we can conclude that frequent infestation after August was derived from the second contamination through straw bag, after sufferring the same injuries with 50 krad irradiation. Korean straw bag is made of rice-straw and the texture of the bag is loose enough to allow the change of the water content of rice. Therefore, for the preservation of raw rice, straw bag would do, but in the case of hulled rice, rice-straw could be a good media for insects contamination, only to find the bag an unsuitable packing. The set of kraft paper bags in Fig. 3 and 4 found rarer occurrences of insects in both unpolished control lot and polished than the set of straw bags; up to October, irradiation lot of 10~50 krad nearly effectuated the disinfestation with 30 and 50 krad irradiation. As the authors previously pointed out[1] that both the unpolished rice and polished one hermitically packed in polyethylene bags were not infested, the packing methods and the prevention of second contamination during storage will constitute important factors for disinfestation by irradiation. According to Golumbic and Davis' report,[5] 13, 20, 25 krad irradiation permitted only hatching but no growth to an adult and 40~100 krad dose never failed to be a complete killer. On the other hand, our result was that 30~50 krad was the optimum dose for disinfestation of rice storage.

3. Changes in uric acid content

In order to supplement the naked eyes detection of insect infestation, a chemical confirmation was applied and resulted as Fig. 5, 6, 7, and 8.

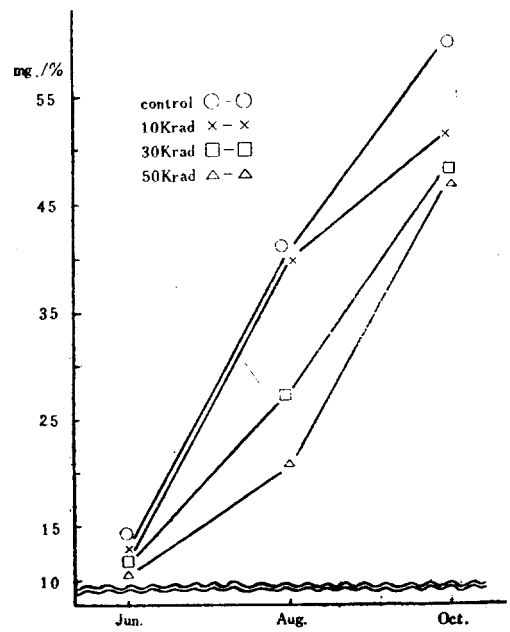

Fig. 5. Changes in uric acid content in unpolished rice packed with straw bag, by irradiation with low dose during storage

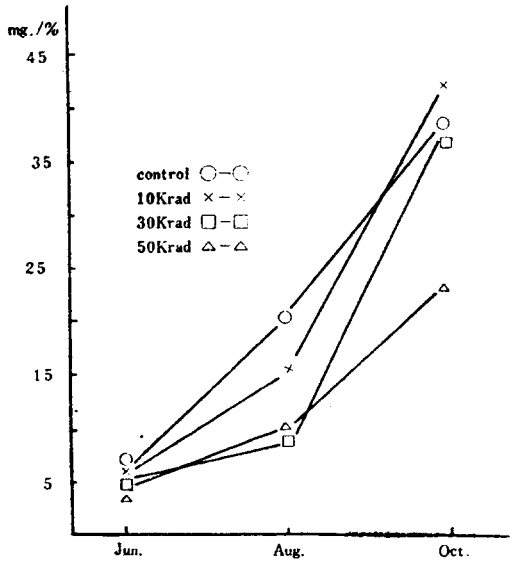

Fig. 6. Changes in uric acid content in polished rice packed with straw bag, by irradiation with low dose during storage

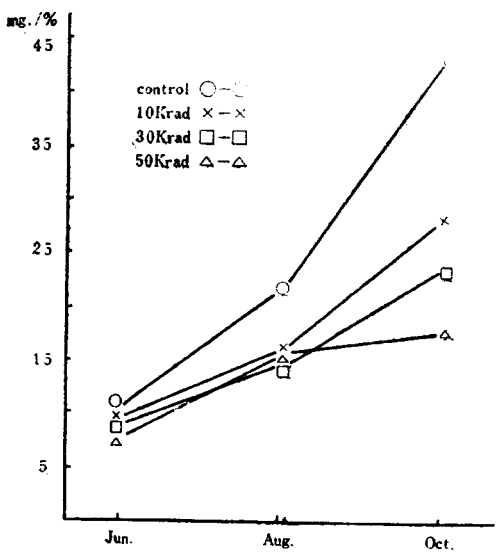

Fig. 7. Changes in uric acid content in unpolished rice packed with kraft bag, by irradation with low dose storage

The amount of uric acid secreted from the increased with the storage interval and had a tendency proportional to the increase of insects infestation. The steep incr ease, since August, of the amount of uric

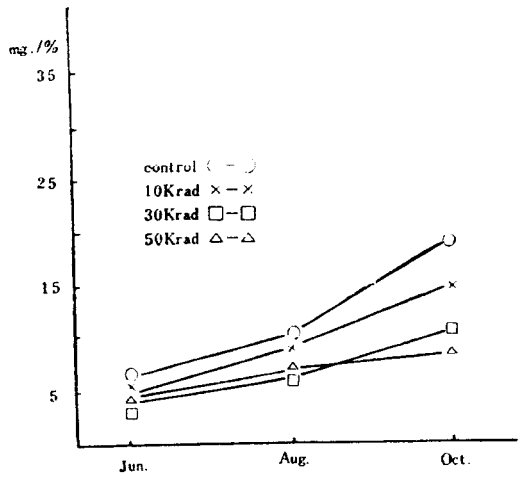

Fig. 8. Changes in uric acid content in polished rice packed with kraft bag, by irradiation with low dose during storage

acid in the straw bag lot was well coincided with Fig. 1 and 2. In the early stage, with increased dosage, the infestation ratios in irradiated lot were less than those of control lot, but in the late stage little difference between the two lots suggested the second contamination as described above. With the suggested contamination, the infestation in the straw bag lot increased by far during August through October, though there was little distinction between two samples in the kraft bags and straw bags in June. More uric acid was detected in the unpolished rice than in the polished one and continued to increase throughout the storage period. The typical results that appeared in the kraft bag group, the irradiated lot contained less uric acid than the control lot supported the concept of insects infestation being suppressed so much.

4. Changes in fat acidity

The changes of fat acidity in γ-irradiated samples, which were packed in the straw bags and the kraft bags respectively and stored at room temperature, are shown in Fig. 9, 10, 11, and 12.

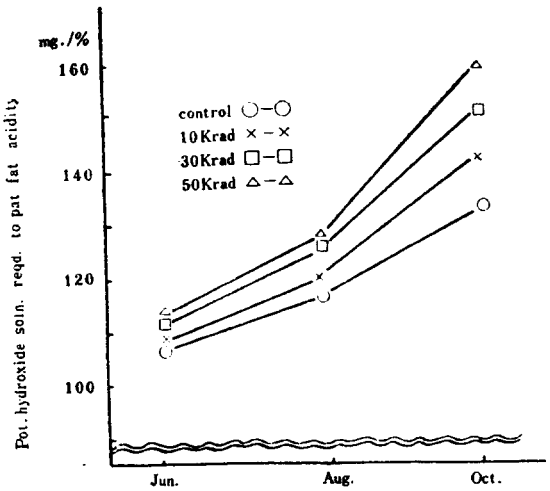

Fig. 9. Changes in fat acidity of unpolished rice packed with straw bag, by irradiation with low dose during storage

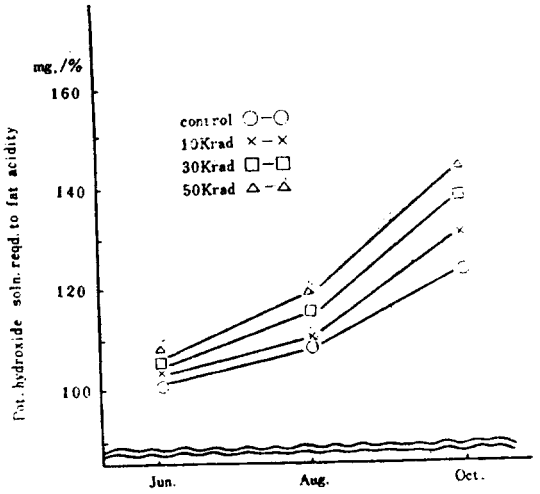

Fig. 11. Changes in fat acidity of unpolished rice packed with kraft bag, by irradiation with low dose during storage

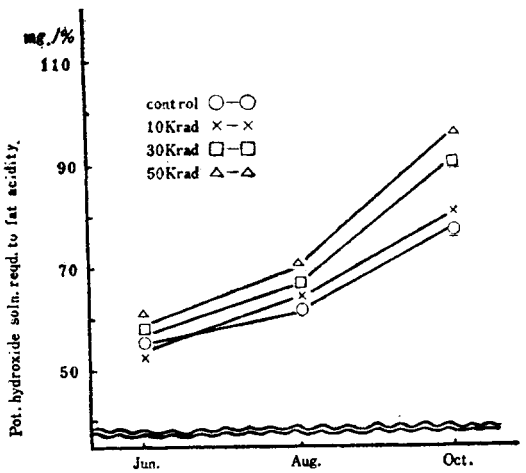

Fig. 10. Changes in fat acidity of polished rice packed with straw bag, by irradiation with low dose during storage

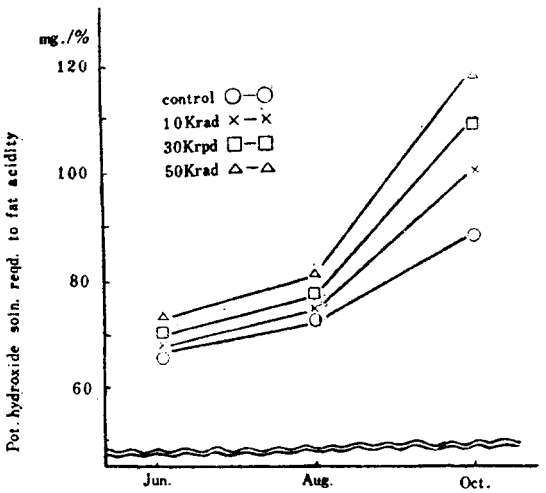

Fig. 12. Changes in fat acidity of polished rice packed with kraft bag, by irradiation with low dose during storage

On the second month after irradiation, the unpolished samples, levelled at 100~110mg%, and polished ones, levelled at 50~66%, marked difference in fat acidity and both of them attained additional 50~60 % from August to October, but in their increase ratios, any notable distinction was not found between the kraft packing lot and the straw packing lot. Along with the increased doses of 10, 30 and 50 krad, some proportional increasings of fat acidity were inclined to appear above the control level.

The rancid odor peculiar to the stored rice originates from the deteriorative changes in rice and has a close relation with the rancidity of fat, which was also reported by Zelamy[11]. Fat in rice is found variously; namely, 2% in unpolished rice, under 1% in polished rice and about 2% in rice-bran and embryo. This small amount of fat constitutes a cause of rancidity in the unpolished rice and there has been established that the rancid odor in rice is caused by free fatty acid or carbonyl compound formed by

self-oxidation of fat[12]. As mentioned above, fat in rice highly affects the storage and taste of rice, so Yasumatsn's table seems very imformative.

Changes of fat in highly polished, stored rice[12]

	Six months storage	%Fat in 100g of rice	% in 100g of fat			
			Phosphatdie	Free fatty acit	Neutral fat	Unsaponifable matter
Koshifiwase	9°C	0.35	1.5	30.6	49.9	5.7
	room temp.	0.34	0.4	51.6	30.8	6.0
Asahi	9°C	0.35	1.5	32.0	54.9	5.6
	room temp.	0.36	0.6	51.6	33.0	4.9

The free fatty acid increased during rice storage is assumed to accelerate the rancidity of rice and the mechanism involved is that since free fatty acid, being enveloped by the spiral structure of straight chain in starch, disturbs the water transition necessary for gelatinization, which raises the temperature for gelatinization and also makes starch particles mechanically hard[13] Although the rice rancidity is developed with increased fat acidity in the aged rice and also the fat acidity increases slightly with 50 krad of suitable disinfestation dose, more close examination will be required in determining the expected uniqueness of free fatty acid in rice rancidity

5. Changes in riboflavin content

For the changes in riboflavin contents of Samples, irradiated with 10~50 krad, packed in the straw bags and stored at room temperature, Fig. 13 and 14 were plotted to show in June 0.12mg with the unpolished rice but about 40% of the former, that is, 0.05mg % with the polished one.

In August through October about 30% of riboflavin content was reduced in the control lot while no significant effects were introduced by 10~50 krad irradiation. The unpolished rice wich contains relatively much vitamin B group has thiamine of 0.4 mg% level and this thiamine has been proved to decrease below 0.25mg% through the summer storage of rice[13]. A similarity was noted in the case of riboflavin.

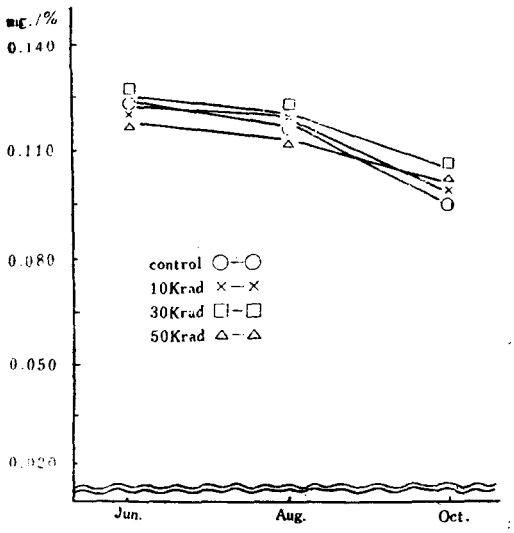

Fig. 13. Changes in riboflavin content in unpolished rice packed with straw bag, by irradiation with low dose during storage

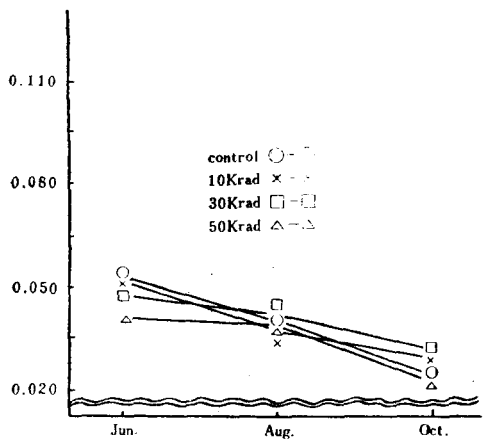

Fig. 14. Changes in riboflavin content in polished rice packed with straw bag, by irradiation with low dose during storage

6. Results of organoleptic test

Korean Paldal variety rice was polished in April and packed in the kraft paper bag, irradiated with 10, 30, 50 krad γ-ray, and stored at room temperature until it was cooked as usual.

Following the test, the scored marks were resulted statistically by the multiple comparison difference analysis.

a) Taste

Analysis of variance

Source of variance	df	SS	MS	F	
Sample	5	1.75	0.35	6.53	<2.37
Panelist	12	41.2	3.43		
Error	60	39.42	0.66		
Total	77	82.37			

b) Flavor

Analysis of variance

Source of variance	df	SS	MS	F	
Sample	5	0.62	0.12	0.34	0<2.37
Panelist	12	16.87	1.40		
Error	60	21.05	0.35		
Total	77	38.54			

c) Color

Analysis of variance

Source of variance	df	SS	MS	F	
Sample	5	0.75	0.15	0.63	<2.37
Panelist	12	43.73	3.64		
Error	60	14.42	0.24		
Total	77	58.9			

d) Viscosity

Analysis of variance

Source of variance	df	SS	MS	F	
Sample	5	2.56	0.51	1.13	<2.37
Panelist	12	42.0	3.50		
Error	60	26.77	,.45		
Total	77	71.33			

Although the above sample rice had passed the summer season, the taste, flavor, color and viscosity of the cooked rice showed no significant difference comparing with the control lot. Hayakawa[14] reported that one could feel the irradiation odour even at the cooked rice of 5 krad irradiation and concluded that the oriental's habits of eating rice provided them such a keen sense about unusual taste and odour experienced in the cooked rice. However, contrary to the above statement, the results we obtained gave no trace of sensing unusual taste and odour in the cooked irradiated rice. Although Hayakawa *et al.*[14] also asserted some improvement in viscosity and hardness of cooked rice with 30 krad irradiation, Brownell *et al.*[15] and Bouman[16] couldn't find any effects on the cake mix with same dose irradiation. Moreover, Cropsey[17] reported that no effects were detected in the appearance, taste and flavor of Hard-red Spring variety wheat which contained 12~14.6 % water content and was irradiated with 900 krad electronic ray irradiation, as Cropsey *et al.*[18] put it, the high water content resisted against high dose irradiation, in case of electronic ray, lower energy caused less effects on taste and flavor. In this test we are doubtful of the weakness of Co source for no significant difference at cooked rice irradiated with 50 krad.

Summary

Korean Paldal variety rice, harvested November and hulled into polished and unpolished forms in April, 1969, packed in the straw bags and the kraft paper bags. Being irradiated with 10, 30 and 50 krad γ-ray, the samples were stored at room temperature for testing infestation, uric acid content, fat acidity, riboflavin content and panel test. The results are as follows.

1. The kraft bag lot with polished rice or unpolished rice manifested the apparent disinfestation with 30~50 krad irradiation. On the other hand,in the straw bag lot the irradiation effects on disinfestation were weakened through summer on account of the second contamination.
2. The changes of uric acid content in rice were proportional to the state of infestation.
3. Fat acidity, which has a close relation with the rancidity of fat in rice, increased during storage and showed some increasing tendency in the irradiated lot with 30~50 krad.
4. In comparison with the control lot, riboflavin content was not affected by 50 krad irradiation.
5. With 10, 30 and 50 krad dose, the irradiated lot should have no significant difference in taste, odour, color and viscosity for the panel test.

Acknowledgements

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