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

by

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감마선 조사에 의한 쌀 저장에 관한 연구(제 3 보)

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요 약

한국산 벼(팔달)를 현미와 백미로 도정하여 kraft paper bag에 넣고 살균하여 저장할 목적으로 비교적 높은 선량인 500, 800, 1,000 krad의 감마선 (Co^{60})을 조사하여 실온에 저장하면서 그 품질변화에 관하여 몇가지 결과를 얻었기에 보고하는 바이다.

(1) 1,000 krad의 감마선 조사로 곰팡이는 현저하게 살균되었다.
(2) Fat acidity는 조사량의 증가에 따라 계속 증가하였다.
(3) Riboflavin의 함량은 500~1,000 krad 조사로 현저하게 파괴되었다.
(4) 식미시험에 있어서 밥의 빛깔이 황색을 띠고 조사취가 현저하였다.
(5) 3,000 krad를 조사한 쌀 전분은 bacterial amylase에 의해서 분해가 저해되었고, diastase에 의해서는 분해가 촉진되는 경향이었다.

Introduction

Irradiated Korean rice of Paldal variety and Nongkwang variety, which were hulled into polished and unpolished forms, packed in polyethylene bags, stored at room temperature. As to the above samples authors reported[1] previously that 400 krad dose was insufficient for disinfection. Iizuka *et al.*[2] reported accordingly that, in case of red Pseudimonas, 800 krad was sufficient for complete sterilization of purely cultured one but even with 1,200 krad dose the one attached on the surface of rice could be exempted from extinction.

In this experiment, for the purpose of preventing the microbes loss in polished and unpolished rice under storage, we irradiated samples with 500~1,000 krad γ-ray and gained some results about the occurrence of microorganisms, fat acidity, riboflavin content, organoleptic test and irradiation effects on

the hydrolysis of rice starch by amylase.

Materials and Methods

1. Sampling and packing

Paldal variety rice produced in Suwon region was hulled, in April, into unpolished rice and polished one according to the common methods adopted by the Agricultural Products Inspection Station of Korea. Each 1.5kg of samples as then packed in three fold kraft paper bags.

2. Irradiation and storage of samples

The irradiation source was 700 Ci-Co^{60} and its doses were varied as 0, 500, 800, and 1,000 krad, and the samples were stored at the room temperature.

3. Detection of molds

Among the microorganisms such as bacteria, mold and yeast which are found in polished rice and unpolished one under storage, the genus *Penicillium* in yellow changed rice[3] produces a toxic material. So we examined in this experiment the sterilization of mold and malt agar medium was used as a medium. Opening the packings in a sterilized room, a certain amount of rice was taken from and washed continuously 15 times by sterile water. On a petridish contained mold agar medium, 10 grains of rice were inoculated and they were cultured in an incubator of 37°C for 5 days. The generated colonies of mold were then checked by naked eyes.

4. Determination of fat acidity[4]

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

With 50 ml 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 transferred to a 100ml flask and 25ml of 0.1% alcohol-phenolphthalein was 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 dichromate solution and 50ml distilled water, manifesting the same color as that of samples.

5. Determination of riboflavin

Flourometry[5] 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 an 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 2.5mole-sodium acetate. When the above suspension was diluted to 100ml and filtered, the first 15ml were discarded and 60ml of the rest were 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 460mμ through the spectrophotometer (QV-50) attached with a fluoresence detector.

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

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

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

6. Organoleptic test[6]

The panel test for taste was performed by 20 panels on October, sixth month after irradiation. 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 0 to 6 and the standard is 2 point, being alloted to the usual boiled rice.

7. Hydrolysis of irradiated rice starch by amylase

a. Preparation of rice starch[7]

About 150g of rice powder and 300ml of 0.2% NaOH were mixed into a 2 *l* flask and then it was kept standing for one day, decanting the liquid. Starch residue at the bottom of the flask was washed with 300ml of 0.2% NaOH solution and the liquid was decanted again, which procedure was repeated until the decanted liquid gave negative Biuret test. The starch residue was then washed 5 times with 200ml of 95% ethanol to remove fats and lipids by heating the mixture to boiling followed by centrifuging at 2,500rpm for 20 minutes. Ethanol in the starch preparation was evaporated in a vaccum oven.

b. Purification of amylases

For a sample amylase, we used an industrial bacterial amylase, which is a liquefying enzyme, and medical diastase, purifying them by the method modified by Akabori[8]. First, 50g of sample enzyme were extracted with 500ml of 2% copper acetate solution and filtered. Second, the filtrate was saturated with $(NH_4)_2SO_4$ to be precipitated into α-amylase and diastase. The former was extracted from between 2/5 saturation and 3/4 saturation, and the latter was from 0.8% saturation. After centrifuging, the extracted precipitate was dialyzed in the running water for 48 hours and then stored in a refrigerator of 5±1°C for 24 hours and finally was treated by another dialysis with large amount of running water. When considered into the cases of α-amylase and diastase, 3ml of 1% rivanol solution for this and 14ml of same solution for that were added to each 100ml of 1~3% enzyme solution and they were treated with successive procedures of agitation, keeping them stand for 10 minutes and then centrifuging. Into the decanted of the above solutions, with agitation, 20ml and 15ml of 1% rivanol solutions were added respectively to form yellow precipitates. By centrifuging after 20 minutes of keeping still, the precipitate was collected and then washed with distilled water, and it was dissolved into 30ml of 0.5 M-acetic acid buffer solution of pH 6.0. The solution was stirred, being added with about 10g of acid clay, and was kept still for 20 minutes to remove rivanol, being filtered by succion and obtained as a transparent liquid which will be cooled in a refrigerater. The first precipitate caused by adding cold acetone of half as much as the above solution was removed, and also the second by another addition equivalent to 60% of the solution was separated by centrifuging. Finally, the drying in a vaccum oven of 40°C produced a crystal.

c. Conditions of purified enzyme action

The conditions of pH and temperature adequate for the actions of purified α-amylase and diastase upon starch were determined by comparing colors, which were manifested by iodine reaction according to the degree of dialysis.

d. The actions of amylases on rice starch

After purified samples of rice starch were irradiated with 50, 500, 1,000 and 3,000 krad doses, 20ml of distilled water was put into 100mg of the sample each. 0.2, 0.3 and 0.4ml of 0.1% α-amylase solution adjusted to pH 6.5, and 4ml of 1% diastase adjusted to pH 5.5 were added to the above liquid respectively, being shaked for 30 minutes at 70°C, and were cooled to stop reaction after boiling in a water bath. With the color of 10ml of above solution reacted with a drop of iodine solution, O.D. was measured at 600mμ with OV-50 spectrophotometer. The quantity of reducing sugar was also determined by Somogi method with 5ml of the reacted solution.

Results and Discussions

1. General components of samples

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

Samples	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%	75.79%

2. Disinfection

In rice preservation, mold is a major cause for deterioration of rancid rice, and among molds some of *Penicilliums* produce such toxic materials as are re-

quired of exclusion even in the international rice circulation. The results of mold inoculation to polished rice and unpolished rice, which were stored in malt agar medium of pH 5.6~5.7 are shown in Fig.1 and 2.

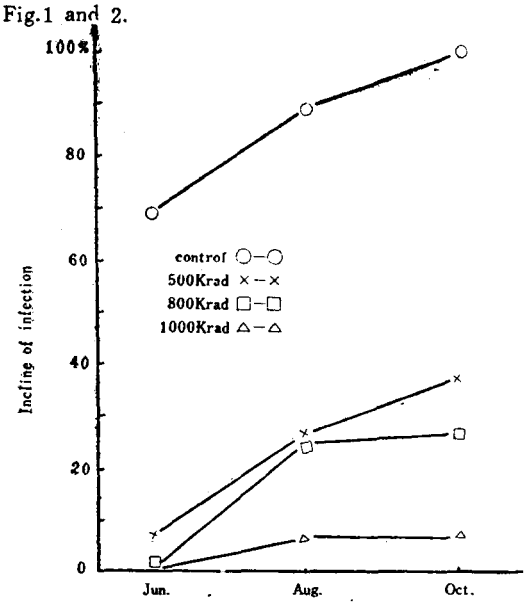

Fig. 1. Comparison with microbic infection in unpolished rice by irradiation with high dose during storage

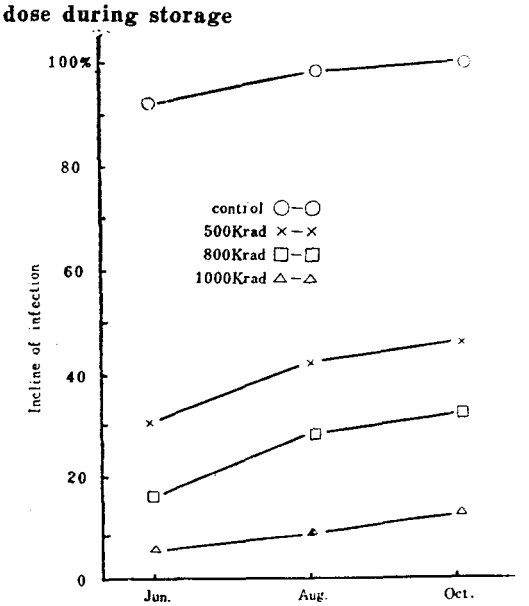

Fig. 2. Comparison with microbic infection in polished rice by irradiation with high dose during storage

As to unpolished rice, we couldn't find any mold in 1,000 krad lot until June, two months after irradiation, but slow increase in August; in October, there appeard more molds and 38% in 500 krad lot, 27% in 800krad lot, and 7% in 1,000 krad lot were their respective values and in case of polished rice, the infection was detected by 6% two months after irradiation and increased gradually. In October higher opportunities for mold growth were shown compared with the unpolished rice and the details were such as 46% in 500 krad lot, 32% in 800 krad lot, and 13% in 1,000 krad lot. Terui and Harada[9] found that most of molds in grains, such as *Penicillium* and etc., were almost sterilized with 800 krad dose at the rate of 400 krad/h and Ito *et al.*[10] reported that *Aspergillus* and *Penicillium* were major molds separated from rice and they were mostly perished with 300~400 krad doses. However, we couldn't sterilize the molds with 400 krad irradiation, as indicated in the previous report[1].

In this experiment we had no absolute sterilization even with 1,000 krad irradiation, so we think it is better to continue further research to determine whether the kraft paper packing caused a second contamination.

3. Changes in fat acidity

When the polished rice and the unpolished one were irradiated with relatively higher doses of 500~1,000 krad for sterilization during storage, the changes of fat acidity in them were shown as Fig.3 and 4.

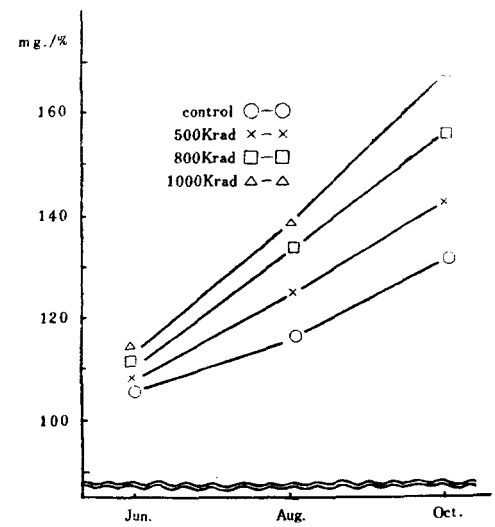

Fig. 3. Changes in fat acidity of unpolished rice by irradiation with high dose during storage

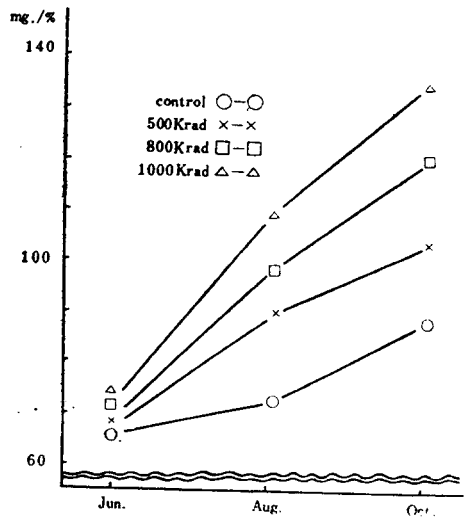

Fig. 4. Changes in fat acidity of polished rice by irradiation with high dose during storage

Two months after irradiation, that is, in June, fat acidity of stored rice levelled at 100~110mg% for the unpolished rice and 65mg % for the polished one. In reviewing control lot, continuous increase culminated to gain about 20% in October, but, since the fat content of the unpolished rice was twice as much as that of the polished one, higher fat acidity was found in the unpolished rice. The acidities were also increased with irradiation doses, and, in the unpolished lot, the acidity reached up to 170mg% with about 60% of increase when 1,000 krad dose was applied, on the other hand, in the polished lot it was increased to 130mg% with 1,000 krad dose. We reported that fat acidity was increased a little with increased dose when stored rice was irradiated with 10~50 krad γ-ray disinfestation, and, in this occasion of higher doses of disinfection, the fat acidity in rice increased considerably.

In the previous report[1], thiobarbituric acid test referred more rancidity to the increased irradiation dose. Therefore, it was easy to predict a deteriorated, unusual odor and taste in rice, which were derived from accelerated rancidity with increased fat acidity.

4. Changes in riboflavin

The effects of the range of 500~1,000 krad doses, used for disinfecting stored rice, upon the riboflavin contents of the unpolished rice and the polished one were shown in the Table 1.

Table 1. Changes in riboflavin contents of irradiated rice in kraft paper bag

(mg%)

Samples		Jun.	Aug.	Oct.
Control	unpolished	0.126	0.120	0.104
	polished	0.051	0.046	0.034
500 krad	unpolished	0.078	0.074	0.065
	polished	0.029	0.022	0,018
800 krad	unpolished	0.062	0.057	0.051
	polished	0.022	0.019	0.014
1,000krad	unpolished	0.060	0.057	0.050
	polished	0.019	0.016	0.013

There appeared in the table that the riboflavin content of the unpolished rice was two and a half times as that of polished one, and also, in the content changes during storage, obvious damages were induced with 500~1,000 krad irradiation though we reported that no effects of 10~50 krad irradiation on the changes of riboflavin content could be seen. The destruction of riboflavin by higher dose irradiations was supposed to occur immediately after irradiation and the decreasing ratio during storage was relatively slow.

5. Results of organoleptic test

In April, the sample rice was hulled into the unpolished rice and the polished one for storage. They were packed hermitically in kraft paper bags and stored at the room temperature after 500~1,000krad irradiation. The packing was opened in October and the panel test was performed with boiled rice cooked by usual method. The results of the difference test[6] about taste, odor, color and viscosity were as follows.

a) Taste

Analysis of variance

Source of variance	df	SS	MS	F
Sample	4	1.51	0.38	0.48 <2.52
Panelist	18	43.63	2.42	
Error	72	56.63	0.79	
Total	94			

b) Flavor

Analysis of variance

Source of variance	df	SS	MS	F	
Sample	4	14.8	3.7	5.44	>2.52
Panelist	18	47.94	2.66		
Error	72	48.8	0.68		
Total	94	111.54			

Of flavor, in 5% level and 1% level there were significant differences, but no distinction of significance was found between doses because Duncan's multiple range test manifested all the resemblance in the variance ratios of 500 krad, 800 krad and 1,000 krad.

c) Color

Analysis of variance

Source of variance	df	SS	MS	F	
Sample	4	126.59	31.65	56.5	>2.52
Panelist	18	23.43	1.30		
Error	72	40.41	0.56		
Total	94	190.43			

Of color, also obvious significant differences were found in 5% level and 1% level. Although irradiated rice samples with 500 krad and 1,000 krad changed yellow in October, no significance differed between doses when Duncan's multiple range test was applied.

d) Viscosity

Analysis of variance

Source of variance	df	SS	MS	F	
Sample	4	3.32	0.83	0.63	>2.52
Panelist	18	42.95	2.38		
Error	72	93.68	1.30		
Total	94	140.95			

The polished rice, which was irradiated with higher doses and stored until October, didn't change taste and viscosity in the light of difference test, but evident significances shown in flavor and color led to the conclusion that such irradiated rice couldn't be accepted by consumers.

6. The effects of γ-ray on the hydrolysis of starch by amylase

In Fig.5 and 6, there appeared the degree of hydrolysis caused by the action of purified bacterial amylase (α-amylase) on the purified rice starch irradiated with 50, 500, 1,000 and 3,000 krad each. In comparison of blue colors created by the reaction between the remaining starch unhydrolyzed and iodine the lowest O.D. value was given to control lot and conspicious increase of the value paralled with increased dose.

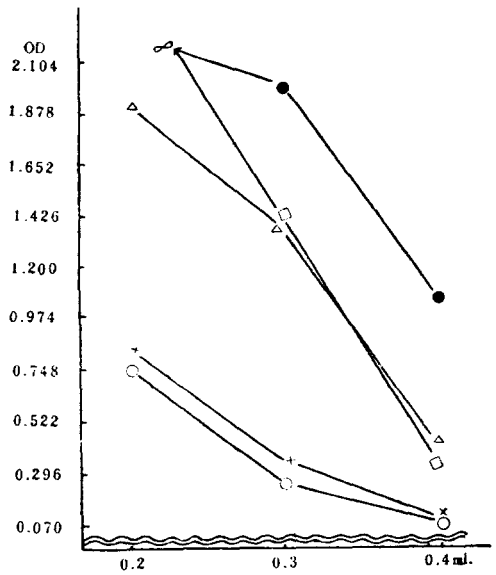

Fig. 5. Changes in optical density of the remained starch, by irradiation, after reaction with purified α-amylase (control-o, 50 krad-(×), 500 krad-□, 1,000 krad-△, 3,000 krad-•)

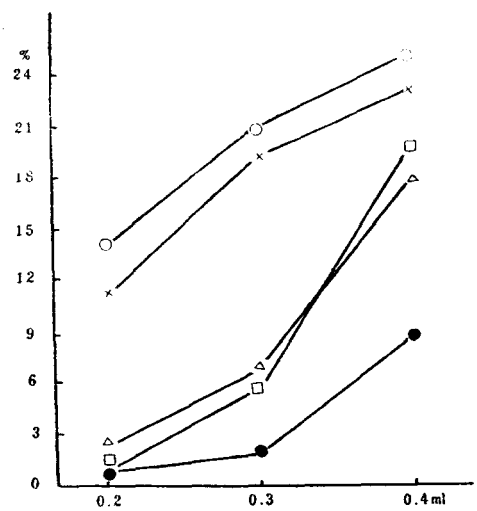

Fig. 6. Changes in reducing sugar content produced from starch, by irradiation, reacted with α-amylase(control-o, 50 krad-(×),500 krad-□,1000 krad-△, 3000 krad-•)

The higher O.D. values shown in the iodine reaction seemed to be due to the fact that the change in molecular structure was caused by irradiation which was not agreeable to the α-amylase action on the starch. The less effective action of α-amylase on the irradiated starch was also proved by the amount of reducing sugar produced by the action of α-amylase. The quantity of glucose and maltose produced decreased with the raised level of irradiation dose in the digestion by α-amylase.

When the rice starch was reacted with the purified diastase under the same condition as above, the changes were noted as in Fig.7 and 8. The O.D. value of the control lot, that is, the blue color presented through the reaction between the remaining starch and iodine, had the highest, and in irradiated lot the values decreased with increased doses. Diastase is an enzyme of strong β-activity, so it has weak liquefying power but strong saccharifying power. In the present experiment, if the water in enzyme solution increased in amount, O.D. value by iodine reaction would be almost same irrelevant to the doses. Same reaction was observed from another point of view in Fig.8, and the reducing sugars, namely, maltose and glucose were increased in accordance with doses. This fact suggested that irradiation rendered the rice starch more soluble and helped diastase produce more maltose and glucose from rice starch. As authors reported[1] previously on the increase of amylose content in the irradiated rice starch, the easiest and natural reasoning may be that diastase of strong saccharifying power acted on the rice starch with increased amylose content to produce more maltose and glucose.

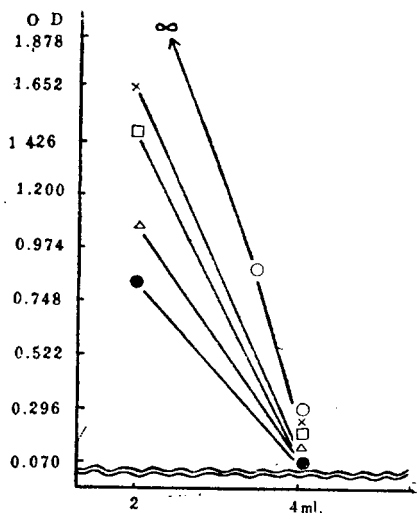

Fig. 7 Change in optical density of the remained starch by irradiation, after reaction with purified diastase (control-o, 50 krad-×, 500 krad-□, 1,000 krad-△, 3,000 krad-・)

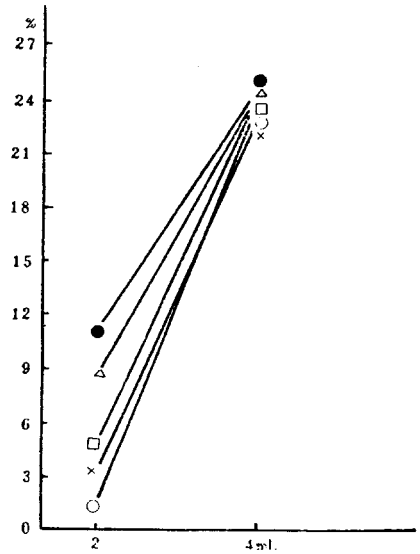

Fig. 8. Changes in reducing sugar content produced from starch, by irradiation reacted with diastase (control-o, 50 krad-×, 500 krad-□,1,000 krad-△, 3,000 krad-・)

Summary

For the purpose of disinfection and efficient storage, Korean Paldal variety rice was hulled into the unpolished and polished ones and packed in the kraft paper bags, irradiated with relatively high doses, 500, 800, and 1,000 krad from the CO-60 source and stored at room temperature, a variety of changes were observed as follows.

1) With 1,000 krad irradiation, mold was almost sterilized.
2) Fat acidity increased during storage and continued to increase more with increased doses.
3) The content of riboflavin was severely reduced with higher doses.
4) The results of organoleptic test were featured by a yellow color and a keen irradiation odor appearing in the boiled rice.

5) In the irradiated rice starch with 3,000 krad, hydrolysis with α-amylase was not effective while accelerated with diastase.

Acknowledgements

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