STUDIES ON THE QUALITY OF PLANT FOOD I. INFLUENCE OF VARIETY AND ENVIRONMENT UPON THE RIBOFLAVIN CONTENT OF HULLED RICE (1). INVESTIGATIONS ON THE ESTIMATION OF RIBOFLAVIN AND THE SELECTION OF SAMPLE RICE

STUDIES ON THE QUALITY OF PLANT FOOD
I. INFLUENCE OF VARIETY AND ENVIRONMENT UPON THE RIBOFLAVIN CONTENT OF HULLED RICE (1). INVESTIGATIONS ON THE ESTIMATION OF RIBOFLAVIN AND THE SELECTION OF SAMPLE RICE

By

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I. Introduction

According to the results of the National Nutrition Survey of 1953 and 1954 (1), the greater part of the vitamins, which are daily taken to support our health, is supplied from the plant food, as shown in Table 1, and it is clear why plant foods are important as a source of vitamins. But the amount of vitamins really taken is got under by cooking and manufacturing than to the amount shown in Table 1, and vitamin A decreases to about 70 per cent, and the others to about 50 per cent of the standard amunt.

Table 1. Outline of vitamin taking.

Vitamins	Animal food		Plant food	
	Amount of taking*	%	Amount of taking*	%
A	150 I.U.	5	2673 I.U.	95
Thiamine	0.11 mg	17	0.94 mg	83
Riboflavin	0.18 mg	27	0.48 mg	73
Ascorbic acid	1.5 mg	2	74.5 mg	98

Note: * Yearly mean per capita per day in Japan.

This shows that the amount of the vitamins really taken is fairly deficient. Therefore the enrichment of vitamins is required for the daily foodstuffs. In the case of plant food, it is difficult to enrich the vitamins as in the general artificial enrichment process of other foodstuffs. But there are actually found considerable differences of vitamin content between those grown under different environments and by different varieties, so it may not be impossible to increase

their vitamin content, if we serect suitable varieties of food plants and culture them under favorable growth conditions.

Although scientific cares about food and nutrition have scarcely been taken in the field of agriculture, their importance in the field of food science, and dietetics is recognized. But, so far as plant food occupy an important position today as a source of nutrient supply, it is necessary to investigate them from many directions. Therefore it is necessary to investigate the riboflavin, an essential component of the flavin enzyme which takes an important part of biological oxidation in the living body, agronomically from the standpoint of riboflavin biosynthesis and its accumulation in the plant.

II. Materials and Methods

The four varieties of rice plant used in this experiment were raised in the fields of two experimental stations in Sendai City, Miyagi Prefecture, in accordance with the purpose of each experiment. In the investigation, other factors except the effective factors which I have for my object were controlled as carefully as possible. The details of the experiment materials and methods of their management are described under each item. As to the estimation of total riboflavin in hulled rice, the simplified fluorometric assay of lumiflavin, modified by the writer was used to obtain the results more rapidly and correctly. But, as to the ester type riboflavin (Flavinmononucleotide and Flavinadeninedinucleotide) Yagi's Method (2) was used. Details of the simplified fluorometric asay of lumiflavin are described below.

III. The Estimation of Riboflavin in Hulled Rice

When the total amount of riboflavin in rice kernels was estimated with the customary fluorometric assay of lumiflavin (3, 4, 5), the following facts were found: (*1*) When with two hydrolysis, the one which was hydrolyzed with takadiastase after pastualization of the sample and the other which was hydrolyzed with takadiastase without pastualization treatment, was compared, the formar gave a value higher than the latter. (*2*) Takadiastase hydrolysis was more promoted when the temperature of incubation reached 50°C than 38°C.

To make these facts clear and simplify the customary fluorometric assay of lumiflavin in hulled rice, the conditions of the treatment of the takadiastase hydrolysis and of other processes of the fluorometric assay of lumiflavin were investigated.

1. Materials and Methods

At the beginning of this experiment, the conditions of lumiflavin production and extraction were investigated using the pure riboflavin solution which dissolved the pure riboflavin crystal in distilled water. After the investigation

of the photolysis conditions of the riboflavin in alkalin solution using the water extractive of hulled rice, factors of hydrolysis treatment, temperature, time, pastualization were investigated basing on the results of the foregoing investigations to prepare the test solution. For this purpose the following apparatus was used for the photolysis of riboflavin shown in Fig. 1, and Beckmann Model DU Qualtz Spectrophotometer was used to measure the intensity of fluorescence of lumiflavin.

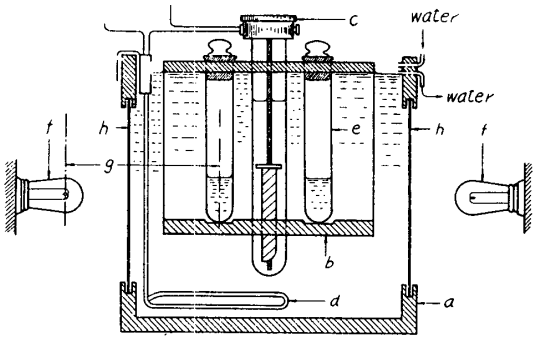

Fig. 1. Apparatus of the photolysis of riboflavin.

Note: a) Water tank—31.5 cm (width) × 24.0 cm (length) ×22.0 cm (height)
b) Test tube stand (wooden)
c) Temperature regulater
d) Electric heater
e) Photolysis glass tube—2.5 cm (diameter) × 11.0 cm (height)
f) Light source—White heat electric lamp (Matsuda), 200 W-100 V
g) Light source distance
h) Plate glass.

2. Results and Discussion

There are five treatments in the customary fluorometric assay of lumiflavin (3, 4, 5). The first treatment is the preparation of the test solution from the sample; the second, photolysis of riboflavin contained in the test solution; the third, decomposition or elimination of contaminated fluorescent substances; the fourth, extraction of lumiflavin from the above mentioned solution using organic solvent; and the fifth, measurement of fluorescence intensity. In this experiment, various conditions of the production of lumiflavin, extraction and measurement of fluorescence intensity, were first investigated, and then the preparation conditions of the test solution were undertaken.

(1) Influence of the temperature and the time of reaction and the total alkali concentration of reaction solution upon the production of lumiflavin

Relative values of the amount of lumiflavin which is obtained using pure riboflavin solution by the combination of three factors: temperature, time of reaction and total alkali concentration of reaction solution, are given in Table 2.

At 10°C, the amount of lumiflavin which is produced in the test solution in the cases that the total alkali concenstration of the solution is 0.5, 1.0, and 2.0 *N*, shows the tendency of increase up to 90 minutes, but over 20°C, it shows the tendency of decrease with the lapse of time, and over 40°C, it is found that the higher the alkali concentration is, the more remarkable this tendency is. At 20°C, the production of lumiflavin in the total alkali concentration 1.0*N* is 93 at eight minutes, and 99 at 15 minutes, so it is clearly known that the amount of production of alkali concentration 1.0*N* reaches the maximum within a very short time. And the amount of production of lumiflavin in the total

Table 2. Production of lumiflavin (I)
Temperature - Total alkali concentration - Time of photolysis.

Temperature (°C) / Total NaOH concentration (N) / Time (min)	10			20			30			40			50		
	0.5	1.0	2.0	0.5	1.0	2.0	0.5	1.0	2.0	0.5	1.0	2.0	0.5	1.0	2.0
30	84	89	92	96	100	99	100	99	94	91	90	79	85	75	54
60	85	90	94	96	100	97	97	97	85	86	78	64	68	56	32
90	85	91	95	95	98	93	95	93	78	81	70	53	63	43	24

Note: 1) Composition of the test solution
200 γ% pure Riboflavin solution (1 *ml*) + Distilled water (4 *ml*) + NaOH solution (5 *ml*)
2) Condition of photolysis
Light source—Electric lamp (200 W, 100 V)
Light source distance—20 cm
3) Value in Table 2 are given in the relative values obtained when the maximum of the average values of the readings—each of four samples was read three times in every case—was converted into 100, and values in other tables in this paper are given in the same way.

alkali concentration 1.0 N in 30 minutes gives the relative value 100, at both 20°C and 25°C, and at 30°C, 99.

From these results the condition of total alkali concentration 1.0 N at the temperature of riboflavin photolysis 20~25°C, or 0.5 N at 25~30°C is considered to be most suitable.

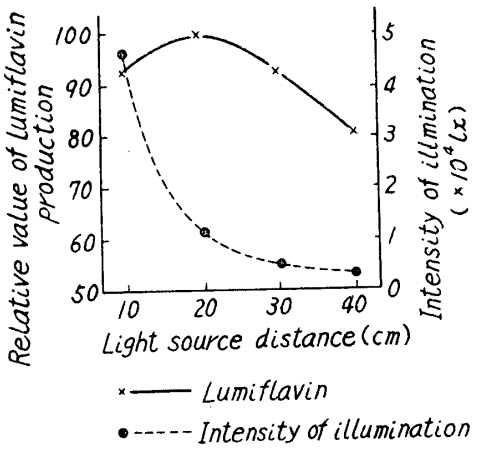

Fig. 2. Relation between the amount of production of lumiflavin and intensity of Illumination.

Note: 1) Composition of the test solution
200 γ% pure riboflavin solution (1 *ml*) + Distilled water (4 *ml*) + 2N-NaOH solution (5 *ml*)
2) Condition of photolysis
Light source—Electric lamp (200 W, 100 V)
Time of reaction—30 min
Temperature of reaction—30°C

(2) Effect of intensity of illumination.

The kind of lamp, the distance of light source and electric pressure are enumerated as factors influencing the intensity of illumination, but in this paper, the kind of lamp and the wave length of light are omitted because details of these factors were already reported by Yagi (6).

a. Distance of light source

The relation between the amount of production of lumiflavin and intensity of illumination under different conditions of distance of light source is shown in Fig. 2.

The maximum value of the amount of lumiflavin production is obtained when the distance of light source is

kept at 20 cm, and in this case the intensity of illumination is 11,600 lx, thus it is assumed that the suitable distance of light source is 20 cm which is usually applied to riboflavin photolysis of customary fluorometric assay of lumiflavin.

b. Electric pressure of light source

The results of the investigation upon the lumiflavin production related with the intensity of illumination which is varied by electric pressure is given in Fig. 3.

The maximum value of the amount of lumiflavin production is obtained when the electric pressure is 100 V, the intensity of illumination is 11,600 lx, and below 8,000 lx the amount of production decreases. From their results, the intensity of illumination, 11,600 lx is required to be kept during the riboflavin photolysis, and the pressure over 100 V is rather undesirable to keep the favorable intensity of illumination, 11,600 lx.

(3) Effect of the amount of the addition of glacial acetic acid to the extraction ratio of lumiflavin.

The results of investigation on the effect of addition of glacial acetic acid are given in Fig. 4.

Under these experimental conditions, the relative value of lumiflavin extraction is 100 when 1.0 *ml* to 1.25 *ml* of glacial acetic acid is added, but it is found that when the added amount increases over

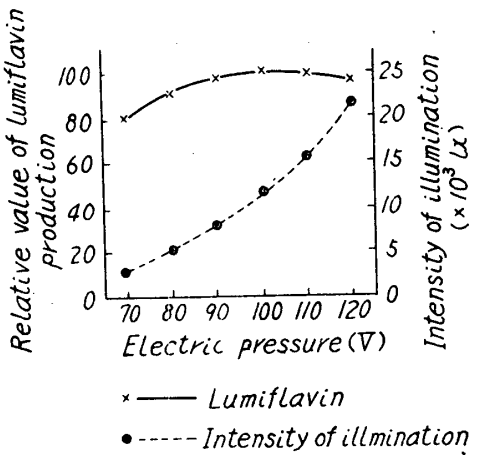

Fig. 3. Relation between the electric-pressure of light source and the amount of production of lumiflavin.

Note: 1) Composition of the test solution
200 γ% pure Riboflavin solution (1 *ml*) + Distilled water (4 *ml*) +2*N*-NaOH solution (5 *ml*)
2) Condition of photolysis
Light source—Electric lamp (200 W, ? V)
Time of reaction—30 min
Temperature—25°C
Light source distance—20 cm

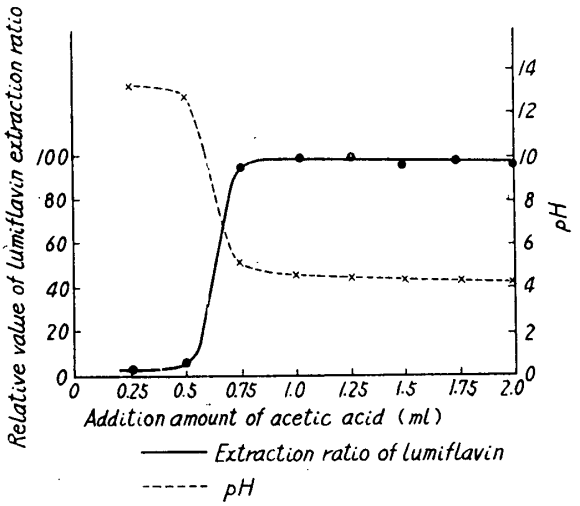

Fig. 4. Relation between the extraction ratio of lumiflavin and the amount of addition of glacial acetic acid.

Note: 1) Composition of the test solution
200 γ% pure Riboflavin solution (1 *ml*) + Distilled water (4 *ml*) +2*N*-NaOH (5 *ml*)
2) Condition of photolysis
Light source—Electric lamp (200 W, 100 V)
Time of reaction—30 min
Temperature of reaction—25°C
Light source distance—20 cm

1.25 *ml*, the amount of extraction of lumiflavin is decreased. Therefore, to extract the lumiflavin satisfactorily into chloroform, the added amount of glacial acetic acid calculated by the following formula is necessary:

[Amount of addition of glacial acetic acid (*ml*)]
= [Total alkali concentration (N)] × [Volume of test solution (*ml*)]/10

The relation between the extraction ratio of lumiflavin and the pH value of test solution is also shown in Fig. 4. From this result it is found that the extraction of lumiflavin is carried out satisfactorily between 4 and 5 of pH value. Therefore, it is possible to understand that the amount of addition of glacial acetic acid obtained by the above formula is the amount which is necessary to bring the pH between 4 and 5.

(4). *Relation between the extraction ratio of lumiflavin and the volume of used chloroform*

The results of actual reading of lumiflavin contained in 1.0 *ml* of chloroform which has been obtained, altering the volume of used chloroform to the same volume of photolyzed solution is shown in Table 3.

Table 3. Relation between the extraction ratio of lumiflavin and the volume of used chloroform.

Used chloroform (*ml*)		5.0	7.5	10.0	12.5	15.0
Reading value	Theoritical	200	133	100	80	67
	actual	177	127	100	83	69
Extraction ratio (%)		89	96	100	104	103

Note: 1) Composition of the test solution
200 γ% pure Riboflavin solution (1 *ml*) + Distilled water (4 *ml*)
+2*N*-NaOH solution (5 *ml*)
2) Condition of photolysis
Light source—Electric lamp (200 W, 100 V)
Time of reaction—30 min
Temperature of reaction—25°C
Light source distance—20 cm

In the case when 10 cm of chloroform was added to 10 *ml* of photolyzed solution is set as a standard, the extraction ratio in Table 3 becomeg higher than the theoritical ratio in proportion as the increase of the amount of added chloroform because the actual reading values increase more than the theoritical values, and the extraction ratio becomes lower in proportion as the decrease of the amount of the added chloroform. Therefore, it is desirable that the volume of chloroform and that of photolyzed solution are equal at any time.

(5) Relation of the riboflavin concentration of test solution to the amount of production of lumiflavin.

From the results of the investigation on the relation of the amount of production of lumiflavin to the amount of the used riboflavin, which is varied within the range of 0.02 μg to 3.0 μg per milli liter, under the above mentioned conditions of riboflavin photolysis, it is found that the amount of lumiflavin dissolved in chloroform shows the linear relation with the corresponding theoretical amount within the range of 0.02 μg to 2.4 μg per milli liter of the used riboflavin.

(6) Investigation of photolysis time by using the extractive of hulled rice

The relation of the amount of production of lumiflavin to the total alkali concentration is shown in Table 4, which is obtained by using the extractive of hulled rice prepared by the customary takadiastase treatment (5).

Table 4. Production of lumiflavin (II)
Temperature - Total alkali concentration - Time of photolysis.

Total NaOH concentration (N) \ Time (min)		15	30	60	90	120	180	240
0.5	A	—	98	100	100	99	93	—
	B	—	98	100	100	100	100	—
1.0	A	92	98	100	99	98	95	93
	B	96	99	100	99	99	96	92

Note: 1) Preparation of the extractive of hulled rice
Hulled rice flour (10g) + Distilled water (40 *ml*) + 2N-Hydrochloric acid (→ pH 4.5) → + Distilled water (→ 50 *ml*) + Toluol (5 drops) + Takadiastase powder (0.1 g) → Incubation (24 hrs at 38°C) → + Distilled water (→ 50 *ml*) → Filtration (Filtrate = Extractive)

2) Composition of the test solution
A : Filtrate (4 *ml*) + Distilled water(0.5 *ml*) + NaOH solution (4.5 *ml*)
B : Filtrate (4 *ml*) + 200 γ% pure Riboflavin solution (0.5 *ml*) + NaOH solution (4.5 *ml*)

3) Condition of photolysis
Light source—Electric lamp (200 W, 100 V)
Temperature of reaction—25°C
Light source distance—20 cm

From the results shown in Table 4, the production velocity of lumiflavin is dilatory compared with that of pure riboflavin solution in the case of the water extractive of hulled rice, and it reaches the maximum value in 60 minutes since the beginning of photolysis, and maintains the highest value up to 120 minutes. At 25°C, the amount of production of lumiflavin is the same either in 0.5 N or 1.0 N of total alkali concentration, and it is found that the addition test (B) indicates the same tendency as the empty test (A). From the above

mentioned result it is assumed as a favorable condition to adopt the 0.5 N of the total alkali concentration for 60 minutes at 25°C when riboflavin photolysis is performed to the extractive of hulled rice according to the aforementioned conditions.

(7) *Investigation of extraction method of riboflavin contained in hulled rice*

From previous, reasons, takadiastase treatment was investigated in connection with the two cases: the one was not-paste and the other paste, and the results are shown in Table 5.

Table 5. Extraction of riboflavin contained in hulled rice.

Temperature (°C) / Time of hydrolysis (hr) / Treatment	38±0.5				50±1.0					
	12	24	36	48	1	3	5	10	15	25
Hot water	46	50	58	59	—	—	—	—	—	—
Not-Paste	49	58	60	59	—	49	52	52	—	58
Paste	51	60	—	—	58	60	60	59	58	58

Note: 1) Materials

Hulled rice which contains moisture of 15.2 per cent, the name of its variety is unkown.

2) Treatment

Hot water: Hulled rice flour (10 g) + Distilled water (50 *ml*) + 2N-Hydro-chloric acid (pH→4.5) + Toluol (5 drops)→Incubation (38±0.5°C)

Not-Paste: After sampling of hulled rice (10 g), Takadiastase treatment was performed by Wada's method (5) omitting the treatment of pastualization.

Paste: After addition of Distilled water (50 *ml*) to hulled rice flour (10 g), and making the pH to 4.5 with 2N-Hydrochloric acid, the sample was pastualized in the water bath at 85°C, and 5 drops of Toluol and 0.1 g of Takadiastase powder were added after cooling, then the sample was incubated. After the incubation for 1 to 24 hrs., its weight was corrected adding Distilled water and then filtrated.

At 38°C, 24 hours is required to hydrolyze the sample solution satisfactorily in both treatment of paste and not-paste to extract the riboflavin from the hulled rice. But a longer time is required to extract the riboflavin than that of the other treatment. Considerable difference of time required for the hydrolysis is found between the case of paste and not-paste at 50°C. In the case of not-paste, the required time for the hydrolysis of the sample solution at 50°C hardly differs from the case of 38°C incubation, but, in the case of paste, hydrolysis is performed rapidly three hours and the value of the real measurement is maintained during 24 hours after it. But, in the case of hydrolysis at 50°C, saccharification of starch paste is not yet sufficient in three hours and not fit to be put in practice because the viscosity of the solution is still high

and it is difficult to filtrate it, So, it is desirable in practice to incubate the pastualized sample solution at 50°C for five hours.

Results of the investigation about photolysis conditions nearly coincide with the results which were previously reported by Hotta (7) and Sasaki (8), but, some differences were found partially about total alkali concentration and temperature. Judging from the inspection of the results of the experiment, the amount of production of lumiflavin, as total alkali concentration becomes higher, becomes rapidly higher and reaches a value which is high but unstable. In the case of relatively high alkali concentration, the ratio of lumiflavin decomposion becomes higher and higher with the rise of reaction temperature, compared with the the case of the low alkali concentration. So, in practice, it is recommended to apply the 1.0 of total alkali concentration in the range of reaction temperature 20~25°C, and 0.5 *N* in 25~30°C.

Table 6. Shama of the simplified fluorometric assay of the total riboflavin in hulled Rice.

Extraction of lumiflavin	Hulled rice flour (10 g) + Distilled water (50 *ml*) → + 2*N*-Hydrochloric acid (→ pH 4.5) → Pastualization (in the water bath at 80°C) → Cooling down → + Toluol (5 drops) + Takadiastase powder (0.1 g) → Incubation (5 hrs at 50°C) → Cooling down → + Distilled water (correct the total weight) → Filtration (Filtrate = Prepared sample solution)		
	A	B	Standard solution
Photolysis	Filtrate (4.0 *ml*) + Distilled water (0.5 *ml*)	Filtrate (4.0 *ml*) + 200 γ% pure Riboflavin solution (0.5 *ml*)	200 γ% pure Riboflavin solution (0.1 *ml*) + Distilled water (3.5 *ml*)
	+ *N*-Sodium hydroxide solution (4.5 *ml*)		
	λ (200 W, 100 V, 20 cm, 25°C, 60 min)		
Oxidation	+ 4 % Sodium permanganate solution + 3 % Hydrogen peroxide solution		+ Distilled water (0.5 *ml*)
Extraction of riboflavin	+ Chloroform (10.0 *ml*)		
	Shaking (severely for about 30 sec)		
	Centrifugalization		
Measurement	Collect the lower layer (chloroform containing the lumiflavin) and measure the intensity of fluorescence.		
	F	F′	100

Calculation Formula :

$$\text{Total riboflavin } (\gamma\%) = 10\{(12.5 \times F)/(F' - F) - D\}$$

Note : F Reading value of A
F′ Reading value of B
D Quantity of total riboflavin (γ) contained in 0.1 g of Takadiastase powder

As to the takadiastase extraction at high temperature, we have already the reports of McLaren (9) and Fujita (10), but from the results of this investigation, the takadiastase treatment for 15 hours at 45~50°C which was determined by McLaren is too long to extract the riboflavin, and one hour at 45°C which was determined by Fujita, is too short. Of course, Fujita's extraction conditions are partially different from McLaren's, that is, in Fujita's method the acid hydrolysis is performed for 15 minutes at 80°C using 0.25 *N*-sulfuric acid after the treatment of takadiastase. But in this case, there is danger of possible contamination by other fluorescent substances when sulfuric acid is applied to the phdrolysis of cereals as previously reported by Wada and her coworker (5) and still more the saccharification of starch paste cannot be sufficiently proceeded under the conditions of this treatment, and so the filtration of saccharified solution falls into a difficulty because the viscosity of the solution is still high. Therefore, in the extraction of riboflavin it is required to perform at least five hours incubation with takadiastase at 50°C after pastualization.

The estimation procedure which was obtained by the above mentioned investigation is shown in Table 6. The same kinds of instruments and chemicals are safely used if they are selected and used in the same way as that was described in the previous reports (3, 4, 5). And the total riboflavin contained in hulled rice is estimated within ± 3.1 per cent of estimation error at five per cent level of significance by this simplified lumiflavin fluorometric assay. Its recovery is about 100 per cent, and so the addition test (B) may be omitted.

IV. Investigation on the Selection of Sample

In the case of the rice plant, thiamine is synthesized at leaves during the early stage of maturity and the greater part of free thiamine is transported to the ear (11), and it is distributed in beard, ovary wall and embryo. Especially, in the embryo and seed skin, a large quantity of thiamine accumulation is found in the case of ripened rice (12), and 14.3 per cent of total thiamine is contained in the endpermis (13). As to the riboflavin, it was also reported that about 48~55 per cent of total riboflavin is distributed in the endpermis (13), but, details of other facts are not yet clear today. But to certify the nutritive value of rice and quality correctly, it is necessary to make clear how the transportation of riboflavin is performed, when it is concluded and in what type of riboflavin exists in a rice kernel. From these viewpoints, the change of riboflavin content was first investigated upon the hulled rice harvested at various stages and of different post harvest handling.

IV-1. Riboflavin content of hulled rice harvested at different stages

A. Preliminary experiment

Preliminary investigations were performed to know the outline of the suitable time for measurement in the post harvest stage of the rice plant.

1. Materials

Fukubozu No. 1, one of the mediate varieties of rice plant, was harvested from the experiment field. After hanging it was airdried in a room, and successively threshed and hasked. And then its total riboflavin was estimated. The conditions of the preliminary experiment are shown in Table 7.

Table 7. Conditions of the Preliminary Experiment.

Variety of rice plant	Fukubozu No. 1, mediate variety
Condition of Experiment-field	Clayey loam, Drainage unsatisfactory
Seeding date	April 15, 1952
Harvest date	October 30, 1952

2. Method of Estimation

Moisture: According to the general method, the amount of moisture was culculated with decrease in weight by drying at 100~105°C. Total riboflavin: Wada's method (5) was applied to prepare the riboflavin extractive, but, as to the other treatments, customary lumiflavin fluorometric assay (3) was applied.

3. Results and Discussion

Samples, which were obtained at the harvest date, were successively threshed and husked, and then, the amount of moisture and total riboflavin in the hulled rice were estimated. The results are shown in Fig. 5.

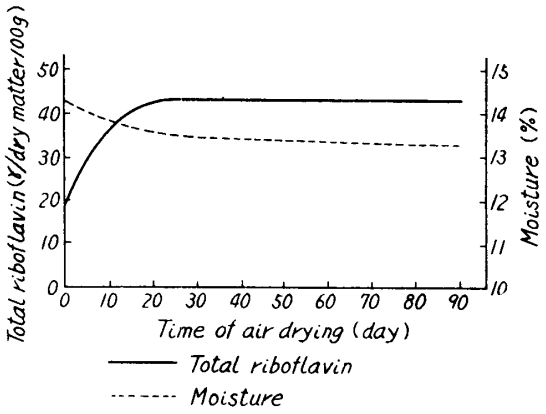

Fig. 5. Change of total riboflavin amount contained in hulled rice during the time of Air-drying.

From Fig. 5 it is found that the moisture of hulled rice decreases in proportion to the prolongation of the time of drying, besides, total riboflavin increases up to the 20th day after the harvest date and then it becomes constant. From these results, it is found that the content of total riboflavin is required, at least, to be compared together after threshing and husking 20 days after the harvest date.

B. Regular experiment

From the preliminary experiment it was roughly found that the total riboflavin in hulled rice increases up to the 20th day after the harvest date and it becomes constant in proportion as the fixation of moisture, but, it is not yet ascertained how the total riboflavin contents of hulled rices, harvested at different stages of ripening, differ from one another. The following experiments were performed to give a solution of these questions and to ascertain the suitable time for selection of samples.

1. Materials

Two varieties of rice plants were applied for this experiment and they were planted in the field. The conditions of the experimental fields and culture, and several dates of their growing are shown in Table 8. The experimental conditions of the treatment on the days after different harvest dates are shown in Table 9.

Table 8. Condition of field, culture and growing date of rice plant.

Two seedlings per one rice stubble		
Variety / Conditions	Aikoku No. 1 (Late variety)	Futaketori (Late variety)
Soil	Clayey loam	Clayey loam
Drainage	satisfactory	satisfactory
Fertilizer (Kan/Tan)		
Ammonium sulfate	6.57	(N : 1.38)
Calcium superphosphate	10.30	(P_2O_5 : 1.70)
Potassium chloride	3.54	(K_2O : 1.70)
Farmyard-manure	100	450
Damage by disease	none	none
Seeding date	April 20, 1955	April 20, 1955
Transplanting date	June 16, 1955	June 16, 1955
Heading date	August 18, 1955	August 10, 1955
Ripening date*	October 15, 1955	October 3, 1955

Note : * The ripening date means the time that the ear of the rice plant turned to yellow over 90 per cent.
1 Kan = 8.2672 lb, 1 Tan = 0.2451 acre

2. Method of Estimation

As to the estimation of moisture and total riboflavin, the same method, as were applied in the preliminary experiment, was also applied in this experiment, but, to measure the fluorescence intensity of the produced lumiflavin,

Table 9. Conditions of experiment after the harvest date.

Date of harvest	September 30, October 5, October 15, October 31 (1955)
Air-Drying	I. After hung with stems and leaves, rice plant were airdried in a dark room. II. After threshing, unhulled rice was airdried in a dark room. III. After threshing and husking, hulled rice was airdried in a dark room.
Preparation of the samples	Each samples, which were obtained by the above mentioned treatment I-III, were served for the determination of moisture and total riboflavin at the 5th, 10th, 15th, 20th and 30th day after the harvest date.

Beckmann Model DU Qualtz Spectrophotometer was applied.

3. Results and Discussion

The content of total riboflavin in hulled rice and hull of a rice plant, which were cultured accordingly to the conditions given in Table 8 and harvested at the time in Table 9, are shown in Fig. 6.

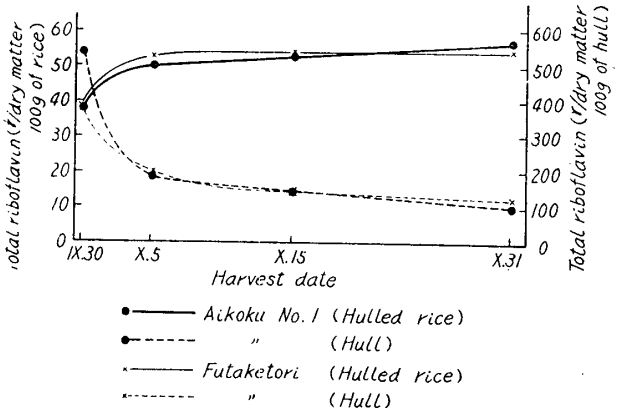

Fig. 6. Change of total riboflavin content in hulled rice and hull harvested at differented dates.

As to the Aikoku No. 1, its total riboflavin in unpolished state increases rapidly up to the 10th day before ripening date and still more it increases slowly after then. However, the greater part of Aikoku No. 1, harvested on October 31, was lodged down by the rain fall and an indication of germination was found in a part of the rice plants and perfect samples were hardly obtained. Considering the fact that the amount of total riboflavin increases at the time of germination (14), (15), the effect of germination on the increase of total riboflavin must be taken into consideration, and so the increase in this case becomes a subject for consideration. As to the Futaketori, its total riboflavin increases up to the second day after ripening date in unpolished state, but after then, the total riboflavin content becomes constant, and it is found that the ripening date, which is determined by observation with the naked eye, nearly coincides with the time that the content of total riboflavin becomes constant. Total riboflavin, contained in the hull, decreases in both varieties nearly symmetrically with the increase of total riboflavin in hulled rice. From these results

it is presumed that the increase of total riboflavin in hulled rice is derived from the movement of riboflavin from the hull. The change of total riboflavin content in hulled rice and in hull, which were obtained in both varieties under different conditions of air-drying, is shown in Fig. 7 and 8. The mean temperature and humidity taken every ten days during the time of this experiment are shown in Table 10.

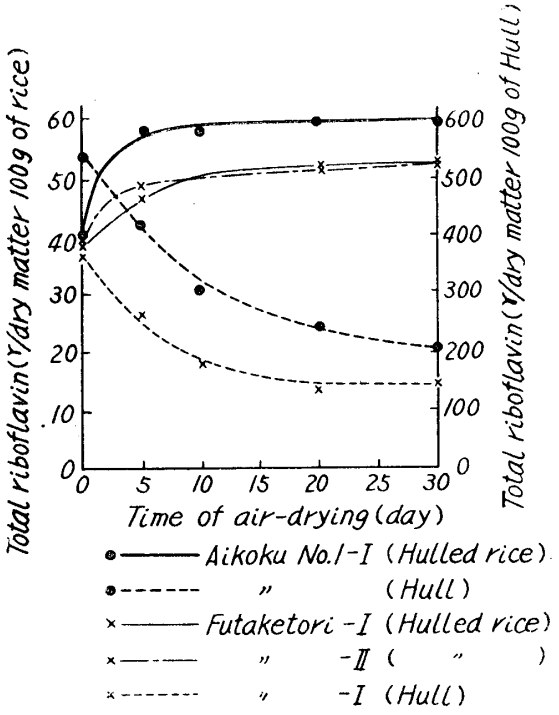

Fig. 7. Change of total riboflavin content in hulled rice and in hull under different conditions of air-drying.
(harvested on September 30)
Note : I Air-drying with stem and leaves
II Air-drying of unhulled rice

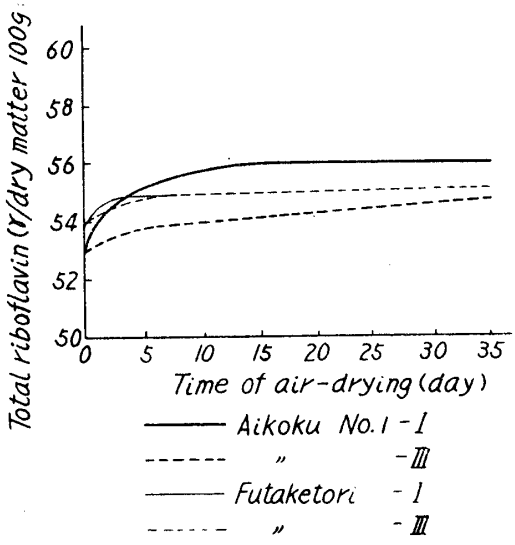

Fig. 8. Change of total riboflavin content in hulled rice and in hull under different conditions of air-drying.
(harvested on October 15)
Note : I Air-drying with stem and leaves
III Air-drying of hulled rice

Table 10. Average of the room temperature and humidity of each 10 days during the time of air-drying test.

Temperature and humidity \ Period	October 1～10	October 11～20	October 21～31	November 1～10	November 11～20	November 21～30
Temperature (°C)	19.5	17.5	16.1	13.8	11.9	12.2
Relative humidity (%)	80	80	76	70	70	71

As to the samples, which were harvested on Sept. 30, it was very difficult to husk them, and a large quantity of broken rice was found in it, thus, the tests of II and III in the case of Aikoku No. 1 and the test of III in the case of Futaketori could not be done.

From Figure 7, the change of total riboflavin in hulled rice nearly shows the same tendency as in the case of Fig. 5, but in the hull, the amount of decrease is comparatively slow. That is to say, in both the test I of Aikoku No. 1 and the test II of Futaketori, it is found that the content of riboflavin increases rapidly up to the fifth day and increases gradually up to the 15th day after the beginning of air-drying. But in the test I of Futaketori it increases gradually up to the 15th day and it becomes constant after then. These facts are conjectured to coincide considerably with the results of the preliminary experiment. As to the total riboflavin amount in the hulled rice, which was harvested on Oct. 15, it is found in Fig. 8 that it is difficult to find the difference between I and II in the case of Futaketori, and the amount of total riboflavin in both tests gives a constant value. This is, it is conjectured, because the after ripening already has been finished, 10 days having passed after the ripening date. In the case of Aikoku No. 1, the content of total riboflavin increases till the 15th day and then it becomes constant, but, the content in test III gives comparatively a lower value than that in test I.

This fact is, it is conjectured, because the movement of riboflavin or riboflavin precursor from the hull, which is seen in Fig. 7, could not be found in this case, and the increase of total riboflavin in hulled rice resulted from the after ripening of riboflavin precursor which was contained in the hulled rice at the harvest date.

That is to say, when the content of total riboflavin reached a constant value there was no difference in the value between the two varieties both in Test I and II except a case of Futaketori, which was harvested on Sept. 30, and the final value of them considerably coincided with each other. And the same results were also obtained in samples which were harvested on Oct. 5 and 31. Furthermore, the following fact was found in this experiment: the amount of total riboflavin increases till about the fifth day after the day that the moisture of rice became about 15 per cent at the time of air drying, and, turned constant. Thus, it is conjectured that the time that the content of total riboflavin becomes constant is affected by the temperature and humidity of the outside world, and there may be some differences of the time which was described above.

From the above described results, the following facts are conjectured: the substances of riboflavin source move into rice kernels from a stem, leaves and hulls, in the form of free riboflavin or riboflavin precursor, till the time that the moisture reaches to about 15 per cent, and the increase of total riboflavin in rice kernels results from its after ripening. Then, in the case of perfect rice kernels, if treatment of air-drying and after ripening are satisfactorily employed after the harvest of rice plants, no difference of total riboflavin content is found between the early harvested rice and the rice at the best

harvest time, and then, both samples of hulled rice are satisfactorily able to serve for the experiment of varietal and environmental differences.

IV-2. Change of riboflavin type in unhulled rice during maturity

As to the possibility of the increase of total riboflavin content in rice kernels, which resulted from the movement of free riboflavin and riboflavin presursor from the stem, leaves and hulls during the time of maturity of a rice plant, is previously described. This result considerably coincides with the result of thiamine which was already described by Kondo and his coworkers (11). But it is not yet solved, whether riboflavin, in the case of a rice plant, is removed from the leaves and stems to the ears in the form of free fiboflavin as in the case of thiamine. Considering the enzymatic action of riboflavin in the plant, it is supposed that the quality of plant food is influenced by any type of riboflavin. Then, to find the outline at first, the change of free and esterified riboflavin in unhulled rice was investigated in connection with the change of saccharides during the maturity of a rice plant.

1. Materials

Two varieties of rice plants were applied in this experiment. The conditions of culture and growth of them are shown in Table 11.

Table 11. Conditions of culture and growth of rice plant.

Condition \ Variety		Futaketori		Aikoku No. 1	
Field	Component of fertilizer	Quantity of fertilizer			
Nursery-bed (clayey loam)	N	9.9[a]	Monme/Tsubo		
	P_2O_5	10.7[b]	,,		
	K_2O	12.0[d]	,,		
Paddy-field (clayey loam)	N	1.600[a]	Kan/Tan	0.244[a]	Kan/Tan
	P_2O_5	1.700[b]	,,	1.700[c]	,,
	K_2O	1.700[d]	,,	1.700[d]	,,
	Farmyard-manure	300.000	,,	300.000	,,
Damage by insect and disease		none		none	
Seeding	date	April	19, 1956	April	25, 1956
Transplanting	date	June	8, 1956	June	12, 1956
Heading	date	August	27, 1956	August	30, 1956
Ripening	date	October	15, 1956	October	28, 1956

Note: a) Ammonium sulfate b) Calcium superphosphate
c) Fused phosphate d) Potassium chloride
1 Kan = 1,000 Monme = 8.2672 lb
1 Tan = 300 Tsubo = 0.2451 acre

Gathering of the sample begun from the 10th day after the heading date and 25 ears of each variety of the same heading date were selected voluntarily and gathered almost every seven days, and then unhulled rices were separated from the ears. The quantity of moisture, riboflavin and succharides of these unhulled rices were estimated.

2, Method of Estimation

Moisture: The moistere of unhulled rice was estimated at 100~105°C by the customary method.

Total riboflavin: The content of total riboflavin was estimated by the fluorometric assay of lumiflavin which was described before in this paper.

Free riboflavin and esterified riboflavin: Esterified riboflavin is given by the addition of Flavinadeninedinucleotide and Flavin-mononucleotide in this paper. Content of free riboflavin and esterified riboflavin was estimated by Yagi's method (2).

Total sugar, reducing sugar and non-reducing sugar: After the sample solution by the custamary method was prepared, each saccharides was estimated by Henmi's method (16).

3. Results and Discussion

The quantitative change of total riboflavin and free riboflavin in unhulled rice during the maturity of rice plants is shown in Fig. 9 and 10.

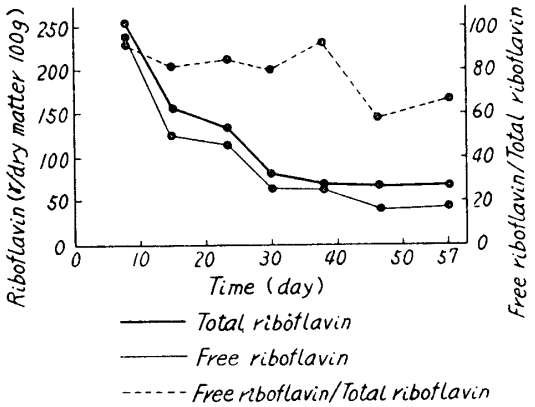

Fig. 9. Change of riboflavin content in unhulled rice.
(Futaketori)

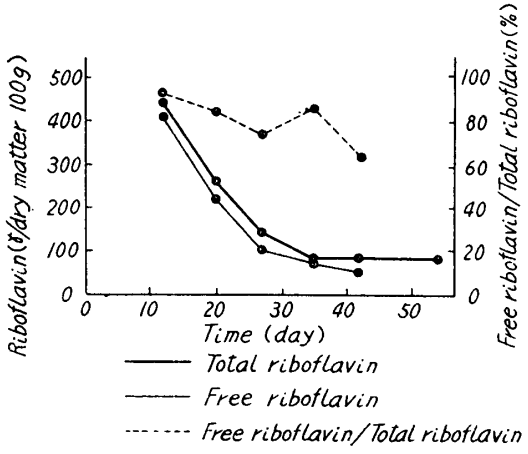

Fig. 10. Change of riboflavin content in unhulled rice.
(Aikoku No. 1)

On the 10th day after the heading date, a large quantity of total riboflavin was distributed in the hulled rice of each variety, and 90 per cent of it was occupied by free riboflavin, still more, the quantity of total riboflavin was fixed by the 35th day. The time necessary to fix the quantity of total riboflavin was shortened by about three weeks in the case of Futaketori and by about

four weeks in the case of Aikoku No. 1 than that of the preliminary experiment. This is presumably because it was influenced by the conditions of weather. Free riboflavin decreased abnormally for a while in the case of Futaketori, but, decreased by degrees in both varieties, and the ratio of free riboflavin to total riboflavin apparently decreased after the 40th day. According to the last measurement—April 10, 1957, the ratio of free riboflavin to total riboflavin was 25 per cent in the case of Futaketori. From this fact, it was found that the esterified riboflavin increased in unhulled rice after the ripening date. Increase and decrease of the quantity of saccharides in unhulled rice are shown in Figs. 11 and 12.

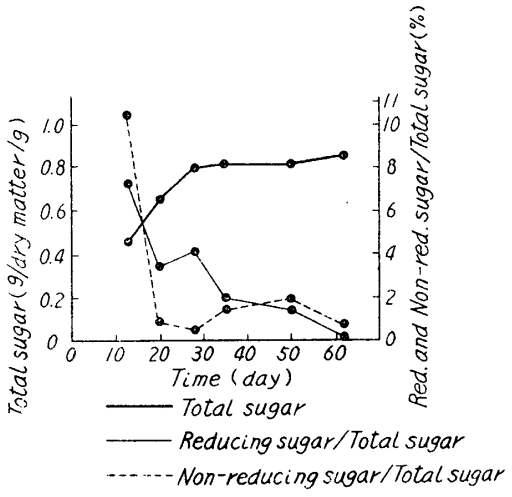

Fig. 11. Change of saccharides in unhulled rice.
(Futaketori)

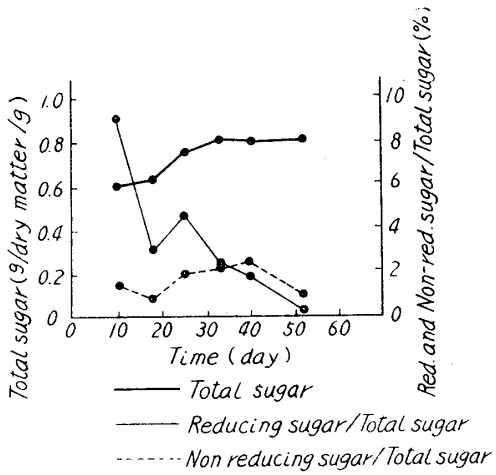

Fig. 12. Change of saccharides in unhulled rice.
(Aikoku No. 1)

In the case of Futaketori. total sugar decreased till the 27th day and this tendency considerably coincided with the quantitative change of rice kernels during the ripening period, but, both reducing sugar and non-reducing sugar increased rapidly till the 20th day after the heading and showed a tendency different from the total sugar. Still more, total sugar showed a little quantitative change in unhulled rice after the 27th day of the heading date, but, from this time reducing sugar increased up to the 50th day. Both reducing sugar and non-reducing sugar decreased gradually from the 50th day in two varieties, and they were nearly fixed at the 62nd day, and then they showed a little change till the 10th day in April of the next year (1957) from this 62nd day of heading date. These tendencies, which were found in the case of Futaketori, were also observed in the case of Aikoku No. 1, but in this variety little more time was neccessary to reach the constant value compared with than that of Futaketori. These differences are conjuctured to have resulted

from the quantity of this rice variety. The relation of starch production and total riboflavin amount, which was calculated per dry matter 1 g, is given in Figs. 13 and 14.

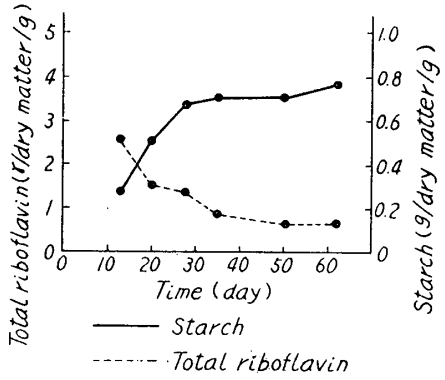

Fig. 13. Relation of starch production and total riboflavin amount in unhulled rice.
(Futaketori)

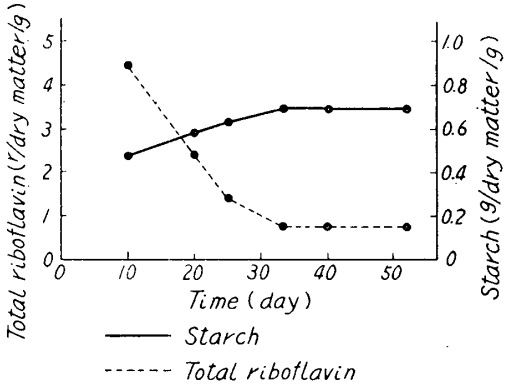

Fig. 14. Relation of starch production and total riboflavin amount in unhulled Rice.
(Aikoku No. 1)

An inverse relationship seems to exist, in general, between the production of starch and the total riboflavin amount in both varieties, but, of the other saccharides no special relationship to the riboflavin in this experiment was found.

In the above experiment the following facts were found. Riboflavin which was synthesized in the leaves of a rice plant moves the leaves to the ear through the stem in a form of free riboflavin as in the case of thimine (11) at the early time of ripening, and in accordance with the increase of total sugar, the content of riboflavin decreases relatively in unhulled rice. But the quantity of total riboflavin in hulled rice increases till the ripening date of a rice plant by the translocation of free riboflavin and its precursor which are contained in leaf, stem and hull. After the repening date, the quantity of total riboflavin is fixed in a short time in hulled rice, and by and by free riboflavin turns into esterified riboflavin and then esterified riboflavin comes up gradually to about 75 per cent of total riboflavin.

Summary

(1) Fluorometric assay of lumiflavin was investigated about hulled rice and a simplified method was obtained as shown in Table 6. The total riboflavin contained in hulled rice is estimated within ± 3.1 per cent error at five per cent level of significance by this method. Its recovery is about 100 per cent.

(2) After heading, synthesized riboflavin which was produced at a leaf of a rice plant moves from the leaf to an ear and the greater part of riboflavin moves again from the hull to the rice kernel in a form of free riboflavin as

in the case of thiamine. And it is roughly known that the total riboflavin in hulled rice is fixed at the time of the ripening date, and after this date free riboflavin turns gradually to esterified riboflavin. As to the riboflavin content of early harvest rice, it comes to coincide with the riboflavin content of rice harvested at the best time, because synthesized riboflavin and its precursor move into rice kernels and increase the content of riboflavin by after ripening when a rice plant is airdried with stem and leaf sufficiently after harvest. And then both rice of early harvest and rice harvested at the best time are satisfactorily served to the sample of this experiment if they are sufficiently airdried.

Acknowledgememt

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