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The Development of Rice Grains under Controlled Environment

IV. Properties of Protein of Rice Kernels Ripened under Different Temperatures Combined with Different Light Intensity and Air-Humidity

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Summary

The protein contents, protein properties and amino acid composition of the kernels obtained from the previous study (9), which matured under different day-night temperature regimes combined with light intensity or air-humidity, were determined.

IR-8 kernels had greater 1000-kernel weight and contained higher concentration of crude protein than Norin-17. In both varieties, crude protein concentration increased with increased ripening temperature, and with weaker light intensity and higher air-humidity. Crude protein contents per kernel tended to increase under weak light or high humidity. A strong negative correlation was found between protein concentration and 1000-kernel weight.

The kernels ripened at 20–15°C contained the least protein content, although their 1000-kernel weight was greatest.

Glutelin constituted about 60% of the total protein, followed by insoluble nitrogen, albumin, globulin and prolamine in that order. Kernels ripened at 35–30°C contained more albumin-globulin but less glutelin than those ripened at cooler temperatures.

The individual amino acid content increased in direct proportion to ripening temperature, air-humidity and in inverse proportion to light intensity, although its ratio to total amino acids changed little.

Many workers (1, 2, 4, 9) have recognized that nitrogen application, especially at the heading stage, increases the protein content of rice kernels. Taira et al (9) reported an increase of protein and some amino acids contents by nitrogen supply at heading stage. However, amino acid composition did not markedly change by transplanting dates (8) nor by nitrogen, phosphorus and potassium fertilization (7).

Work has not been conducted concerning the properties of rice kernel protein as influenced by climatic environments during ripening. This paper reports the change of protein property and amino acid composition of rice kernels ripened under different air-temperatures combined with different light intenisty and air-humidity.

Materials and Methods

I. Assay of Amino Acid Composition

Samples of Norin-17 and IR-8 rice grains were obtained from a previous study (9). Kernels were ground in a mortar and each 30 mg powder was hydrolyzed with 6 grams of 6N-HCl in a sealed airless tube, at 110°C for 24 hrs. After breaking the seal, HCl was removed under low air pressure at 60°C. The tube was stood overnight in a vacumed desicator and the liquid was filled up to 40 ml with pH 2.2 citrate buffer solution, and 1 ml aliquot of it was used for amino acid analysis with an autoanalyzer. Tryptophan was not estimated.

II. Fractionation of Protein

The kernels of cv. Norin-17 ripened for 5 weeks outdoors and in a high temperature chamber (day 35° night 30°C) under natural light were ground in a mortar after drying for 3 days at 45°C, then passed through a 120-mesh sieve. The flour was fractionated according to Ozaki (3) as shown in Fig. 1.

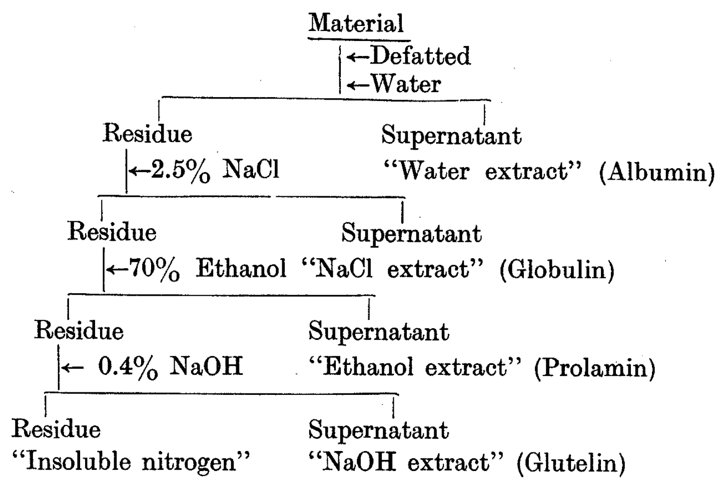

Fig. 1. Fractionation procedures of protein.

Two grams of flour were defatted with petroleum ether for one hour. The defatted material was shaken for one hour with 25 ml distilled water, then left standing overnight, and centrifuged at 4000 g (three times of repetition, each for 15 minutes). The supernatant "water extract" (Albumin) was obtained. The residue after water extract was shaken with 25 ml of 2.5% NaCl for 30 minutes, left standing overnight, centrifuged at 4000 g (three times of repetition, each for 15 minutes). The "NaCl extract" (Globulin) was obtained. After dewatered, the

TABLE 1. *Total Nitrogen Contents of Rice Kernels Matured under Different Ripening Environments*

Treatment Day-Night, °C		Norin-17			IR-8		
		Tot-N (%)	1000-Kernel (g)	Protein* (g)	Tot-N (%)	1000-Kernel (G)	Protein (g)
SL	20-15	1.30	21.1	1.71	1.85	19.1	2.21
	25-20	1.59	18.3	1.78	2.06	21.4	2.76
	30-25	1.81	18.6	2.11	2.35	18.9	2.78
	35-30	2.00	15.6	1.95	2.59	18.0	2.91
	Mean	1.68	18.4	1.89	2.21	19.4	2.67
WL	20-15	1.64	17.7	1.81	2.38	15.4	2.29
	25-20	2.16	13.8	1.86	2.84	16.5	2.93
	30-25	2.61	14.4	2.35	3.10	14.6	2.83
	35-30	2.52	12.6	1.99	3.17	14.1	2.79
	Mean	2.23	14.6	2.00	2.87	15.2	2.71
HH	20-15	1.40	21.8	1.91	1.67	21.0	2.19
	25-20	1.60	20.2	2.02	2.02	22.1	2.79
	30-25	1.95	16.3	1.99	2.41	17.3	2.61
	35-30	2.29	15.4	2.21	2.93	18.1	3.31
	Mean	1.81	18.4	2.03	2.26	19.6	2.73
LH	20-15	1.32	21.6	1.78	1.75	22.1	2.42
	25-20	1.57	20.7	2.03	1.93	20.1	2.43
	30-25	1.83	18.0	2.06	2.43	18.6	2.83
	35-30	2.05	16.2	2.08	2.45	19.0	2.91
	Mean	1.69	19.1	1.99	2.14	20.0	2.65

* Crude Protein Content (Total-N Content × 6.25) per 1000 Kernels SL: strong light WL: weak light HH: high humidity LH: low humidity

residue was shaken for 30 minutes with 70% ethanol, left standing overnight, and centrifuged two times, each for 15 minutes at 4000 g. The "ethanol extract" (Prolamin) was obtained. The residue after ethanol extraction was shaken with 25 ml 0.4% NaOH for 30 minutes and left for 4 hrs, centrifuged two times at 4000 g, each for 15 minutes, and the supernatant was removed. Again 25 ml of 0.4% NaOH was poured and shaken for 30 minutes, left for 6 hrs, then was centrifuged two times, each for 15 minutes, and the supernatant was added to the previous one. The whole supernatant was then filtered by a Toyo-filter paper No. 5 B. The "NaOH extract" (Glutelin) was obtained. The residue after NaOH extraction was analyzed for insoluble nitrogen. All procedures were conducted during winter under low room temperatures.

Results and Discussion

I. *Protein Content*

As shown in Table 1, IR-8 kernels contained a higher concentration of total-N or crude protein and had a greater 1000-kernel weight than Norin-17, resulting in greater protein content per kernel in the former. Total-N or crude protein

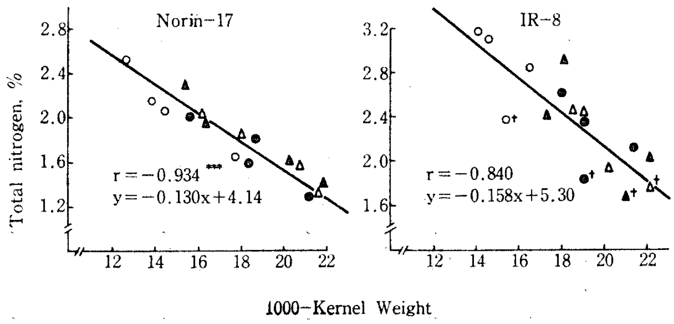

FIG 2. Correlations between 1000-kernel weight and total-nitrogen concentration in rice kernels ripened under different temperatures combined with different light intensity or air-humidity.

TABLE 2. *Fruiting Percentages of Rice Varieties as Affected by Ripening Environments*

Temp. °C Day-Night	Norin–17				IR–8			
	SL	WL	HH	LH	SL	WL	HH	LH
20–15	97.0	83.3	93.9	97.7	66.6	63.9	62.7	74.3
25–20	94.8	86.8	94.4	98.3	80.0	67.0	84.4	82.3
30–25	92.4	71.2	83.2	94.7	72.9	49.8	60.0	69.2
35–30	85.3	72.2	62.6	86.8	42.0	34.0	50.4	53.0
Mean	92.4	78.4	83.5	94.4	65.4	53.7	64.4	69.7

SL: strong light WL: weak light HH: high air-humidity LH: low air-humidity

concentration increased as the ripening temperature increased or 1000-kernel weight decreased, irrespective of light intensity or air humidity (Fig. 2). Weak light and high air humidity tended to increase total-nitrogen or crude protein concentration and to decrease 1000-kernel weight as compared to strong light and low air-humidity, respectively. However, crude protein content per 1000 kernels was generally greater under weak light or high humidity.

Fig. 2 shows a strong negative correlation between 1000-kernel weight and nitrogen concentration, suggesting that kernels well ripened with abundant starch accumulation contained less protein, and the kernels ripened at 20–15°C contained the least protein of all in spite of their greatest 1000-kernel weight in both varieties. The kernels ripened at higher temperatures (35–30°, 30–25°C), on the contrary, tended to contain more protein due to a higher concentration of nitrogen in spite of their smaller 1000-kernel weight (Table 1). These facts were already reported in the previous paper (9); the rate of ripening increased but starch deposition ceased earlier as the ripening temperature increased, thus resulting in less starch and higher nitrogen concentrations. High air-humidity and weak light also depressed starch accumulatuon and caused a higher nitrogen concentration.

Higher temperatures, weaker light intensity and higher air-humidity all caused smaller fruiting percentages as shown in Table 2, thus decreasing the amount

TABLE 3. *Properties of Protein of Mature Kernels as Influenced by High Ripening Temperature (Day 35° Night 30°C)*

Protein fraction	DW %		% of total nitrogen	
	Outdoors	35/30°	Outdoors	35/30°
Albumin	0.13	0.18	8.1	10.0
Globulin	0.09	0.17	5.6	9.4
Prolamin	0.06	0.03	3.7	1.7
Glutelin	1.06	1.04	65.8	57.8
Insoluble-N	0.28	0.36	17.4	20.0
Total-N	1.61	1.80	100	100

of ripened grain. This is one reason why the nitrogen concentration increased under these ripening environments: translocated nitrogen supply from straw for each kernel increased in amount.

II. Protein Properties

As shown in Table 3, glutelin contents occupied around 60% of the total nitrogen, followed in order by insoluble nitrogen, albumin, globulin and prolamin in kernels of both varieties. The proteins of 35/30°C kernels contained more albumin and globulin, but less glutelin as compared to those ripened under outdoor cooler temperatures. The albumin-globulin/prolamin/glutelin ratios of outdoor and 35/30°C kernels were 16.4/4.5/79.1 and 24.6/2.1/73.2, and albumin/globulin ratios were 1.44 and 1.06, respectively. These facts may show higher contents of smaller molecule, water or NaCl soluble proteins and relatively higher protein decomposition in the kernels during ripening at high temperatures.

III. Amino Acid Composition

As shown in Fig. 3 and 4, each amino acid in Norin-17 and IR-8 kernels increased its content as the ripening temperature increased. The only exception is an low glutamic acid content at 25–20°C with low air-humidity in IR–8 probably due to an unknown experimental error. These increments coincided with the increments of total nitrogen, indicating that nitrogen of kernels increased along with protein increase. In both varieties, glutamic acid content was always highest, followed by aspartic acid, arginine, and leucine, in that order. Many works also reported the highest content of glutamic acid in rice kernels, but the second or third order amino acid was not similar (5, 7–9). At each temperature regime, amino acid content was higher with weaker light-intensity and higher air-humidity, except at 20–15°C in IR–8, where it was less with high-humidity than with low-humidity (Table 4).

Although each amino acid content increased markedly with increasing temperature and with weak light and high air-humidity, its ratio to total amino acid

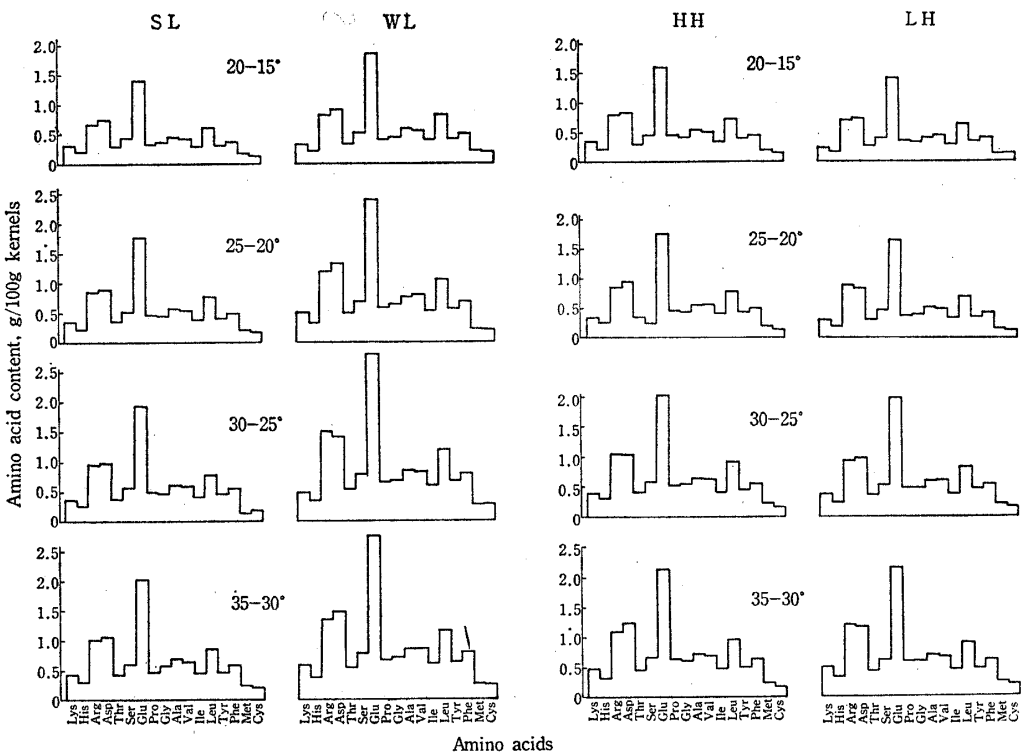

FIG 3. Amino acid composition of Norin-17 rice kernels ripened under different air-temperatures combined with light-intensity and air-humidity
SL: strong light WL: weak light HH: high humidity LH: low humidity

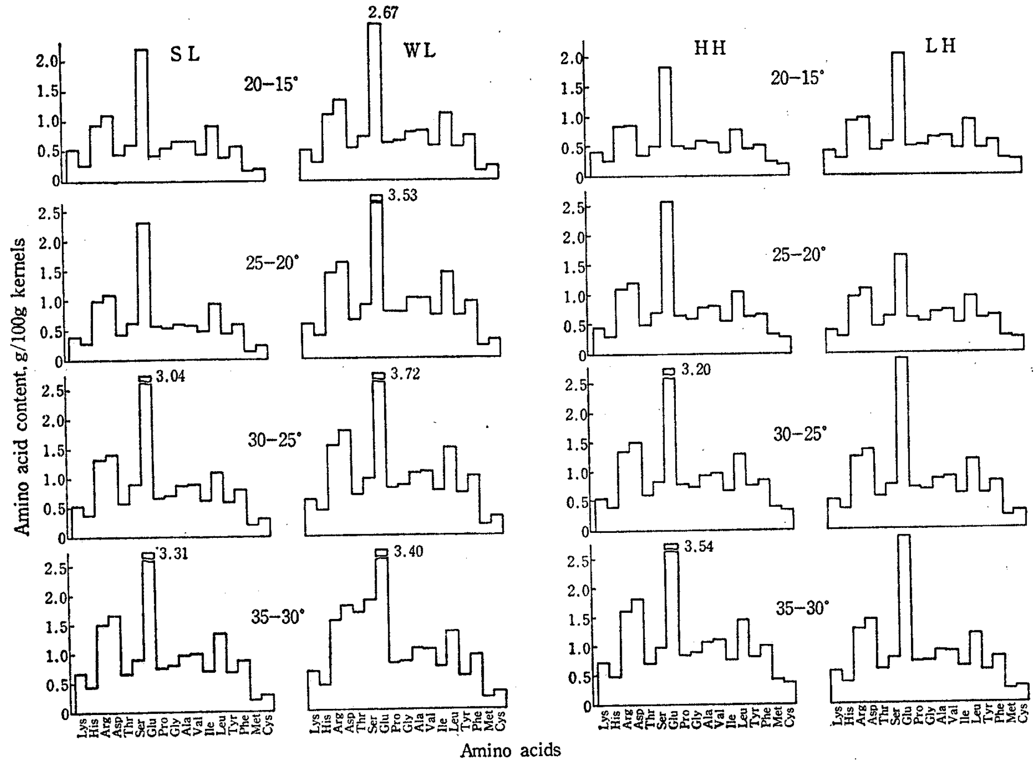

FIG. 4. Amino acid composition of IR–8 rice kernels ripened under different air-temperatures combined with light-intensity and air-humidity

TABLE 4. *Total Amino Acid Contents (g/100 g Kernels) as Influenced by Ripening Environments*

Temp. °C Day-Night	Light Humidity	Norin-17		IR-8	
		g	Ratio	g	Ratio
20–15	SL	7.28	100	11.28	100
	WL	9.52	130	13.55	120
25–20	SL	9.27	127	11.67	103
	WL	12.97	178	17.24	152
30–25	SL	9.92	136	14.94	132
	WL	14.58	200	18.30	162
35–30	SL	10.84	149	16.80	148
	WL	14.68	201	17.52	155
20–15	LH	7.42	100	10.65	100
	HH	8.71	117	9.59	90
25–20	LH	8.73	117	11.12	104
	HH	9.19	123	13.05	122
30–25	LH	10.47	141	14.45	135
	HH	10.91	147	16.17	151
35–30	LH	11.70	157	14.61	137
	HH	12.18	164	18.47	173

content changed little. Taira and Taira (8) showed a similar change with difference in transplanting date.

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