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# Chemical Studies on the Ripening of Rice-seed and Chemical Properties of Rice of the Early Ripening Subvarieties

By

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## INTRODUCTION

Physical properties of the ripening seed were studied by Schjerning<sup>(1)</sup> who stated that during the ripening of barley, the weight of 1000 grains reached its maximum then decreased gradually in the later period, while the weight of dry matter still increased. On the contrary, Blagowestschenski<sup>(2)</sup> reported that no decrease in the weight of 1000 grains could be observed in the later ripening period of *Vicia fava minor*. The chemical composition of the ripening seed was investigated by Storer and Lewis.<sup>(3)</sup> They stated that in the ripening of sorghum seed, the content of nitrogen free extracts increased while the cellulose and the mineral substance decreased, and that the content of crude protein came up to its maximum then decreased in the later ripening period. Further, Schulze, Wassiliff and Emmerling<sup>(4)</sup> investigated more critically the chemical composition of ripening seed and reported that the content of crude protein increased during the ripening period while the content of non-proteid and amide nitrogen decreased gradually. Blagowestschenski<sup>(2)</sup> observed the same facts with other materials. Coordinate with the change of carbohydrate in the ripening seed of *Zea mays*, Portells<sup>(5)</sup> stated that the content of starch increased while that of glucose and cane sugar decreased. Blagowestschenski obtained the same results and added that the content of hemicellulose reached the maximum then decreased gradually. Other authors isolated some organic bases and enzymes from the ripening seed but there is no

report concerning the content and chemical properties of the four kinds of seed proteins. Spitzer<sup>(6)</sup> is the sole investigator in this field. He found that in the case of *Zea mays*, the formation of glutelin occurs very early in the ripening period followed by globulin and albumin while the formation of zein is late.

In rice seed, the change of such physical properties as actual weight, volumetric weight and specific weight has been reported repeatedly by Kato, Ando, Yanagihara, Kondo, Oikawa and the Agricultural Experiment Station of the Government of Japan, but the studies on chemical composition of the ripening rice seed are very scarce. Goto<sup>(7)</sup> is the only one who reported the change of rice during the ripening period. He stated that the ash content of seed increased in the later part of the period while the content of crude fibre and crude fat decreased but that there was no marked difference in the proportion of protein and nitrogen free extracts during the ripening period. Yet the content of sugar and water soluble protein decreased and that of alcohol soluble protein increased. These reports of previous investigators are not sufficient to explain the protein formation in the ripening rice seed therefore the author undertook the following investigation.

Commonly the early ripening subvarieties of rice are distinguishable from those ripening later in that they always produce small and bad seeds and do not show strong physiological functions. These characteristics of the early ripening subvarieties have been studied by many authors from the standpoint of heredity but there is no chemical investigation on the difference between the early ripening and the late ripening subvarieties. Of course the two kinds always differ in their subvarieties and it is very difficult to determine the absolute differences between them though they showed some chemical differences. The author obtained good samples from Aomori Prefecture where are cultivated two kinds of early and later ripening rice derived from the same subvarieties called "*Kamenuo*." These two kinds showed a difference of 4 or 5 days in their ripening time. With these materials, the authors began the following experiment in order to give a chemical explanation to the characteristics of the early ripening subvarieties. Therefore the following experiments are divided into two parts.

This experimental work was done in our laboratory by the author and the following collaborators, viz. Makoto Abe, Shukichi Watanabe, Sugano Eguchi and Yoshi Araki.

## PART I.

## Chemical studies on the ripening of rice.

## 1. Materials and some physical properties.

The materials of the present investigation were collected from a kind of common rice called "*Akashiho*" produced during the year 1925 in Kako, a Province of Hyogo Prefecture, and two kinds called "*Toyokuni*" and "*Kamenoö*" produced during the year 1926 in Akita Prefecture. The ripening time is stated according to the date upon which each sample was collected: i. e. 12th, 17th, 22nd, 27th of October and 6th of November; 23th and 30th of September and 7th of October; and 27th of September, 4th and 11th of October respectively. The actual weight of 1000 grains and the specific weight of the rice seed were first determined.

TABLE I.

Weight of 1000 grains and specific weight of hulled and unhulled rice.

Ripening date	Akashiho				
	12th Oct.	17th Oct.	22nd Oct.	27th Oct.	6th Nov.
Unhulled rice Wt. 1000 grains.	30.7125	31.0952	31.1828	30.1352	30.3462
Specif. weight	1.2366	1.2103	1.2368	1.2243	1.0238
Hulled rice Wt. 1000 grains.	25.6474	25.6532	25.6270	25.6952	25.6894
Specif. weight	1.4779	1.4800	1.4519	1.4282	1.4468

  

Ripening date	Toyokuni			Kamenoö		
	23rd Sept.	30th Sept.	7th Oct.	27th Sept.	4th Oct.	11th Oct.
Unhulled rice Wt. 1000 grains.	26.136	26.379	26.276	23.356	23.414	22.186
Specif. weight	1.1476	1.1640	1.1610	1.1652	1.1627	1.1596
Hulled rice Wt. 1000 grains	22.013	21.796	21.796	20.064	19.815	18.927
Specif. weight.	1.4147	1.4199	1.4251	1.4252	1.4248	1.4316

In the above results the author observed that the two physical properties of rice seed show no definite relation to the ripening period.

## 2. General analysis of rice flour.

The air-dried hulled rice was powdered and its water and fat

content determined by the ordinary methods. Then the defatted rice power was used for the determination of the nitrogenous substances.

TABLE II.  
General analysis of rice flour.

Ripening date	Akashiho				
	12th Oct.	17th Oct.	22nd Oct.	27th Oct.	6th Nov.
Water %	13.511	13.099	13.291	13.318	13.488
Crude fat %	2.043	2.024	1.984	1.944	1.946
Water % of defatted rice	10.385	11.090	12.201	13.644	11.752
Total nitrogen %	1.433	1.373	1.450	1.504	1.282

  

Ripening date	Toyokuni			Kamenoo		
	23rd Sept.	30th Sept.	7th Oct.	27th Sept.	4th Oct.	11th Oct.
Water %	12.851	12.550	12.785	12.407	12.908	11.203
Crude ash %	2.157	2.163	2.104	2.149	1.895	1.828
P <sub>2</sub> O <sub>5</sub> %	0.8140	0.7267	0.7911	0.6530	0.5810	0.5210
Crude fat %	3.4714	3.7242	3.5299	3.5165	3.1313	3.4884
Crude protein %	7.4413	7.3563	7.6794	8.4113	8.7981	9.0488
Proteid %	6.8706	8.8256	7.4850	7.6213	8.3138	8.7125
Proteid N% in Total nitrogen	92.33	92.78	97.46	90.60	94.49	96.28

According to the above results, the fat and phosphoric acid content of the rice seed decrease gradually in the later portion of the ripening period while the nitrogen content is irregular. The formation of the proteid increases gradually in the later part of the ripening period.

### 3. Content of the four kinds of proteins.

Goto<sup>(7)</sup> stated that among the four kinds of rice proteins, the alcohol soluble increases in the later period of the ripening, the water soluble decreases gradually while the NaCl and the NaOH soluble proteins show no marked change during the ripening time. The author determined the content of the four kinds of seed proteins by the method which was described in a former report.<sup>(8)</sup> The following figures are the percentage of nitrogen in the dry matter.

TABLE III.  
Content of the four kinds of proteins.

Ripening date	Akashiho				
	12th Oct.	17th Oct.	22nd Oct.	27th Oct.	6th Nov.
Nitrogen of Water sol. protein	0.0894	0.1353	0.1463	0.1353	0.0894
NaCl sol. protein	0.1353	0.1372	0.1485	0.1485	0.1583
Alcohol sol. ,,	0.0495	0.0569	0.0603	0.0550	0.0895
NaOH sol. Protein	0.3781	0.3763	0.3818	0.3419	0.3763

  

Kinds of rice	Ripening Date	N. of water sol. protein	NaCl sol. protein	Alcohol sol. protein	NaOH sol. protein	
					I	II
Toyokuni	23rd Sept.	0.2255	0.2164	0.1095	0.3898	0.1955
	30th Sept.	0.2184	0.2145	0.1131	0.4015	0.1924
Kamenoo	7th Sept.	0.2231	0.2270	0.1245	0.4436	0.1790
	27th Sept.	0.2091	0.1963	0.1342	0.4724	0.2349
	4th Oct.	0.2299	0.2092	0.1446	0.4724	0.2349
	11th Oct.	0.2448	0.2292	0.1588	5.5026	0.2587
		Total nitrogen %				
Toyokuni	23rd Sept.	18.9400	18.1757	9.1970	32.7398	16.4203
	30th Sept.	18.5556	18.2243	9.6092	34.1121	16.3466
Kamenoo	7th Oct.	18.1574	18.4748	10.1327	36.1032	14.5682
	27th Sept.	15.5372	14.5787	9.9718	35.1018	17.4543
	4th Oct.	16.3316	14.8611	10.2721	35.0785	17.5250
	11th Oct.	16.9084	15.8309	10.9684	34.7147	17.8685

The above results show that the content of water soluble protein first reaches its maximum at a certain time in the ripening period, then decreases later. The content of NaCl soluble continues to increase until the last ripening date. The content of alcohol soluble protein increases a very little, while that of NaOH soluble protein shows no marked difference during the whole ripening period.

**4. Content of proteid and non-proteid nitrogen.**

Many authors have reported that with the later ripening time of seed, the proteid nitrogen always increases while the non-proteid nitrogen and amide nitrogen decrease gradually during the whole period.

Goto<sup>(7)</sup> obtained the same results with respect to the ripening time of rice. The authors intending to get more exact information on the point, determined, first the free amino nitrogen content of the defatted rice powder, next the proteid nitrogen by Stutzer's reagent, then the non-proteid nitrogen, the free amino nitrogen and the free amino nitrogen in the same filtrate by Sørensen's method.

The free amino nitrogen content of the defatted rice powder is the sum of the free amino nitrogen of non-proteid and proteid. The author has already stated in his former report<sup>(8)</sup> that the free amino nitrogen content of proteid decreases in the order of protein in its water soluble, NaCl soluble, NaOH soluble and alcohol soluble<sup>(9)</sup> form. Also the content of the free amino nitrogen of the defatted rice powder decreases according to the degree of polish of hulled rice which decrease seems to be one of the scales for measuring the degree of polish. (See footnote).

The following numbers are the nitrogen percentage of dry matter, an increase being shown by the sign “+” and decrease by “-”.

TABLE IV.

Content of free amino, proteid and non-proteid nitrogen.

Ripening date	12th Oct.	17th Oct.	22nd Oct.	27th Oct.	6th Nov.
Free amino nitrogen	0.3429	0.3426	0.3420	0.3403	0.3314
Variation	(-) 0.0003	(-) 0.0006		(-) 0.0017	(-) 0.0089
Proteid nitrogen	1.2343	1.2698	1.3277	1.3605	1.3209
Variation	(+) 0.0355	(+) 0.0579		(+) 0.0328	(+) 0.0396
Non. proteid nitrogen	0.2278	0.1837	0.1395	0.0945	0.0925
Variation	(-) 0.0441	(-) 0.0442		(-) 0.0450	(-) 0.0020
Free amino nitrogen in nonri- tend (filtrate of Pr.)		0.0945	0.0858	0.0781	0.754 0.0420
Variation	(-) 0.0089	(-) 0.0077		(-) 0.0027	(-) 0.0334

The above results show that the free amino nitrogen content of the defatted rice powder and that of the non-proteid decrease gradually with the advance of ripening date accelerating in the later period. This shows not only proteid formation from non-proteid but also the polymerization of higher class protein from lower class. The amount of proteid

increases and inversely, that of non-proteid nitrogen decreases gradually at the same rate. The velocity of the decrease of the free amino nitrogen of non-proteid in the later ripening period shows that the formation of proteid from the non-proteid occurs more rapidly than the condensation of the higher class of proteid from the lower.

##### 5. The difference of physicochemical properties of oryzenin of different ripening dates.

The principal proteid of rice, oryzenin, having been prepared by the following method from each kind of rice, and compared for the difference of physico-chemical properties of the different oryzenins, their condensation degree could be determined. The residual rice powder from which were separated the water soluble and the NaCl soluble protein, was washed several times until no chlorine reaction was observed and 2 litres of 0.2% NaOH solution were added to it. The upper liquid was decanted and the same treatment repeated three times. The upper liquid was filtered several times until no alkaline reaction to starch was observed by a Buchner's funnel whereupon the alkali soluble protein was precipitated by the same treatment as above described. The precipitate was dialysed for one week to separate it from the liquid. The precipitate was mixed stirring with 70% alcohol, placed over night in an ice chamber and the upper alcohol solution was separated by a centrifugal machine. The treatment was repeated three times until the

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The change of the free amino nitrogen of the defatted rice powder with the degree of polishing.

Five g. of defatted hulled rice powder were mixed with 50 cc of 0.2% NaOH solution, shaken for half an hour and settled over night in an ice chamber. The mixture was centrifuged for 15 minutes and 20 cc of the upper liquid was titrated with HCl by Sørensen's method for the determination of free amino nitrogen. The following results were obtained. The albumin and globulin nitrogen were determined by the same method which was used to estimate the four kinds of proteins.

Polishing degree	Free amino-N	Globulin-N	Albumin-N
10.0%	0.3778	0.2838	—
12.5%	0.3654	0.2660	—
15.0%	0.3556	0.2595	—
17.5%	0.3480	0.2446	0.1018
20.0%	0.3429	0.2405	0.0949
22.5%	0.3337	0.2123	0.0944



upper alcohol solution had no protein reaction. The residual protein which was separated from 70% alcohol soluble protein was washed with absolute alcohol and ether and dried in a sulphuric acid desiccator. The ash content was determined by the ordinary method.

TABLE V.

Water and ash contents of the protein samples.

Ripening date	12th Oct.	17th Oct.	22nd Oct.	27th Oct.	6th Nov.
Water %	7.7111	8.6075	8.9093	9.4689	9.9745
Ash	0.6630	0.3932	0.2503	0.4500	0.4468
Variation	(-) 0.2693	(-) 0.1429	(-) 0.1997	(-) 0.0032	

From the above table, it will be seen that there is a variation of ash content of the different oryzenins with the ripening date of the rice.

(a) As stated in the former report,<sup>(8)</sup> the iso-electric point of oryzenin is to be inferred from the change of surface tension of its alkaline solution by an acid titration. One tenth g. of water free oryzenin was dissolved in 10 cc of 1/50 n-NaOH solution. The solution was stood still over night for the completion of solution. One cc of the solution was diluted with 9 cc of redistilled water, to which mixture was then added 1/100 n-HCl solution. The surface tension of the mixture was measured by Nouy's torsionmeter. The figures of Table VI have been calculated on the basis of water whose surface tension is 75 dyn/sq. cm. Another factor that is suitable for the determination of the iso-electric point of oryzenin is the change of turbidity of its alkaline solution by an acid titration. As in the former experiment, the turbidity of the alkaline solution was determined by means of a Dubosque's colorimetric nephelometer. The table shows the degree of turbidity by the use of the "+" sign.

TABLE VI.

Surface tension and turbidity of the protein solutions.

cc of HCl	3.00	3.10	3.20	3.30	3.40	3.45	3.50	3.55	3.60	3.65	3.70
Surface tension (20°C)											
12th Oct.	61.0	61.0	60.0	60.3	60.3	60.0	60.0	59.5	59.5	59.5	60.5
17th Oct.	61.0	61.0	60.5	60.3	60.3	60.0	59.5	59.5	60.5	58.0	59.5
22nd Oct.	61.0	61.0	60.5	60.3	60.3	59.5	59.0	60.5	59.5	59.5	—
27th Oct.	61.0	61.0	60.5	60.3	60.0	60.0	59.5	59.5	59.5	60.5	—
6th Nov.	61.0	61.0	60.5	60.3	60.3	60.0	60.0	59.5	59.5	60.5	—
Turbidity											
12th Oct.	—	—	—	—	—	+	+	+	++	++	+++
17th Oct.	—	—	—	—	—	+	++	+++	+++	++	++
22nd Oct.	—	—	—	—	+	++	++	+++	++	++	+
27th Oct.	—	—	—	—	+	+	+	++	++	+++	++
6th Nov.	—	—	—	—	—	—	+	+	++	+++	++

The above results show that the iso-electric point of the five kinds of oryzenin varies according to their degree of ripening; that the iso-electric point of the oryzenin whose ash content was the smallest shows the highest pH value and that of the oryzenin obtained in the later part of the ripening period shows low pH value.

(b) The specific rotatory power of a compound is generally said to be caused by its asymmetric carbon and to be enlarged by the increase of the complication and the saturation of the molecular structure. One tenth g. of water freed oryzenin was dissolved in 15 cc of 1/10 n-NaOH solution and the specific rotatory power of the solution was observed by means of a Haensch-Schmidt half shadow polariscope.

TABLE VII.

Specific rotatory power of the various oryzenin solutions.

Ripening date	12th Oct.	17th Oct.	22nd Oct.	27th Oct.	6th Nov.
Specific rotatory power	(-) 63.577	(-) 66.172	(-) 66.172	(-) 63.577	(-) 60.982
„	(-) 68.889	(-) 72.404	(-) 72.644	(-) 70.227	(-) 67.739

The oryzenin whose iso-electric point is situated near the alkaline side and ash content is the smallest, shows the maximum specific rotatory power. The oryzenin of the later ripening rice decreases in its specific rotatory power.

(c) The sulphur was estimated by Denis-Benedict's method, the phosphorus by the volumetric method and the nitrogen by Kjeldahl's method. The results are indicated in Table VIII.

TABLE VIII.

Nitrogen, sulphur and phosphorus contents of various oryzenins.

Ripening date	12th Oct.	17th Oct.	22nd Oct.	27th Oct.	6th Nov.
Nitrogen %	16.7732	17.2689	17.2153	17.0988	16.9034
Sulphur %	1.0166	1.0939	1.2856	1.1531	0.9789
Phosphorus %	0.2152	0.1501	0.1402	0.1332	0.1445

From the table, the nitrogen and sulphur content of oryzenin reach their maximum at a definite point of the ripening period and decrease later. But the phosphorus content decreases gradually during the ripening time.

(d) The free amino nitrogen of different oryzenins was determined by Sørensen's method which has been described in the former report.<sup>(8)</sup>

TABLE IX.

Free amino nitrogen contents of the various oryzenins.

Ripening date	12th Oct.	17th Oct.	22nd Oct.	27th Oct.	6th Nov.
Dry matter %	0.7757	1.0330	1.0161	0.9810	0.9474
Nitrogen %	4.6246	5.9819	5.9023	5.7372	5.6048

From the above table the free amino nitrogen content of oryzenin is seen to come to the maximum point at a certain time in the ripening period, coinciding with the results of the former experiments.

(e) The kinds and quantities of amino acids of the different

oryzenins were determined employing the same treatment as described in the former report.

TABLE X.

Kinds of nitrogen found in various oryzenin samples.

Ripening date	12th Oct	17th Oct.	22nd Oct.	27th Oct.	6th Nov.
The following figures are the percentage of dry matter.					
Total N.	16.3634	16.3503	16.5445	16.5587	16.3239
Ammonia N.	0.9895	0.9899	1.0315	1.0051	1.0192
Melanin N.	0.2998	0.3010	0.3011	0.3007	0.3000
Mono-amino N.	9.4620	9.4620	9.3998	9.4116	9.2648
Diamono N.	5.6121	5.5974	5.8121	5.8413	5.7999
Arginin N.	3.8980	3.8510	3.9013	3.9024	3.9208
Lysin N.	1.1206	1.1281	1.3019	1.3443	1.2889
Histidin N.	0.3225	0.3307	0.3255	0.3162	0.3240
Cystin N.	0.2710	0.2875	0.2835	0.2784	0.2790
The following figures are total nitrogen percentage.					
Ammonia N.	6.0468	6.0542	6.2339	6.0700	6.2690
Melanin N.	1.8320	1.8409	1.8197	1.8160	1.8452
Mono-amino N.	57.8255	57.8711	56.8202	56.8371	56.2111
Di-amino N.	34.2955	34.2337	35.1260	25.2761	35.6746
Arginin N.	23.8206	23.5527	23.5773	23.5670	23.9934
Lysin N.	7.0498	7.9036	7.8682	8.1283	7.9721
Histidin N.	1.9708	2.0207	1.9672	1.9095	1.9938
Cystin N.	1.6560	1.7566	1.7133	1.6812	1.7161

Judging from the data of the above table, the monoamino nitrogen content of oryzenin decreases and the diamino nitrogen increases during the ripening period. The arginin content is low and the lysin content is high at a definite ripening date.

**6. The difference of chemical properties of starches in different ripening periods.**

From the above results the physico-chemical properties of oryzenins are seen to vary with the different ripening dates. The same differences were supposed in the case of starches. The starch was prepared as

described in the former report and the following chemical properties were examined.

(a) The content of water, ash and phosphoric acid was determined by the ordinary method giving the following results :

TABLE XI.

Water, ash and phosphoric acid content of starches.

Ripening date	12th Oct.	17th Oct.	22nd Oct.	27th Oct.	6th Nov.
Water %	5.7213	5.2294	8.0585	8.1953	8.2402
Ash (dry matter %)	0.1241	0.1285	0.1046	0.1236	0.2044
Phosphorus (dry matter %)	0.0130	0.0103	0.0080	0.0101	0.0134

From the table the ash and phosphorus content of starch reach their minimum at a definite point of the ripening time and thereafter increase.

(b) Acetylation of starch was carried out using acetic acid anhydride and  $ZnCl_2$  as follows. Five g. of starch were mixed with 40 g. of acetic acid anhydride and 0.2 g.  $ZnCl_2$  and kept at a constant temperature of  $160^\circ C$ . for four hours in an oil bath under a reflux condenser. When the mixture became a transparent, dark brown solution, it was poured into water and acetylated starch was precipitated. The precipitate was then washed many times with water until the washed water had no acetic acid reaction, next washed with alcohol and ether and then dried in a sulphuric acid desiccator.

The content of the acetyl group of acetyl starch was determined by Wenzel's method.<sup>(10)</sup> One-tenth g. of each sample was saponified with 1 : 1 sulphuric acid and after the addition of metaphosphoric acid and Na-phosphate solution, the distilled acetic acid was collected in standard NaOH solution and titrated with standard sulphuric acid, using phenolphthalein as indicator. Results are shown in the following table.

TABLE XII.

Acetyl radical of the acetyl starch.

Ripening date	Average ccm. of $H_2SO_4$ sol. not titrate	Acetyl radical	%
12th Oct.	6.9	0.0473618	48.1501
17th ,,	7.3	0.0501074	50.7597
22nd ,,	7.9	0.0542258	55.5347
27th ,,	7.2	0.0494210	50.0459
6th Nov.	7.0	0.0480482	48.7820

The content of acetyl radical of the acetyl starch varies with the ripening of rice. In the middle of the ripening period it reaches the maximum, then decreases gradually. This shows that the hydroxyl group content of starch which can be replaced by acetyl group, is at its richest midway in the ripening period.

The specific rotatory power of acetyl starch which was dissolved in pyridin solution was determined by a Haensch-Schmidt half shadow polariscope at 25°C. and the following results were obtained.

Table XIII.

Specific rotatory power of acetyl starch.

Reading		Specific rotatory power at 25°C.
12th Oct.	5.00	$\alpha_D^{25} = \frac{5.00 \times 0.346 \times 15}{2. \times 0.1} = 131.909$
17th Oct.	4.80	$\alpha_D^{25} = \frac{4.80 \times 0.346 \times 15}{2. \times 0.1} = 126.181$
22nd Oct.	4.40	$\alpha_D^{25} = \frac{4.40 \times 0.346 \times 15}{2. \times 0.1} = 116.936$
27th Oct.	4.75	$\alpha_D^{25} = \frac{4.75 \times 0.346 \times 15}{2. \times 0.1} = 124.821$
6th Nov.	4.90	$\alpha_D^{25} = \frac{4.90 \times 0.346 \times 15}{2. \times 0.1} = 129.097$

The specific rotatory power of the acetyl starches varies also with the same correlation to the content of acetyl group. Its value shows the minimum midway in the ripening period.

These differences of the chemical properties of starches during the ripening period showed that at the middle of the ripening time the polymerization degree of starch decreases followed by an increase during the later part of the ripening period.

### Résumé.

(1) During the ripening of rice seed the non-proteid decreases and the protein increases. The composition of the principal protein, oryzenin, changes by a decrease of monoamino and increase of diamino nitrogen during the ripening period.

(2) Among the four kinds of rice proteins, the contents of the NaCl-soluble and alcohol-soluble proteins increase gradually during the whole ripening time. The composition of oryzenin increases in its specific rotatory power, in the content of free amino nitrogen and lysin but decreases in the content of arginin and of ash up to a definite point in the ripening period. At this date the iso-electric point decreased in its acidic nature and then these physico-chemical properties under consideration became reversed in the later period.

(3) During the ripening period, the chemical properties of starches vary and in the middle part of the ripening period, the ash and phosphorus contents decrease, the acetyl group content of acetyl starch increases and its specific rotatory power decreases.

## PART II.

### Chemical properties of early ripening subvarieties.

#### 1. Materials and some physical properties of early ripening subvarieties.

Some physical properties of early and late ripening subvarieties which originated from "*Kamenö*" were determined. The following results were obtained.

TABLE XIV.

Weight of 1000 grains, specific weight and number of various grains.

Subvarieties	No.	Weight of 1000 grains	Specific weight	Numbers of transparent grains	Numbers of white spotted grains	Numbers of greenish grains
Late ripening subvarieties	1	22.4788	1.4434	730	248	13
	2	22.3100	1.4303	863	71	62
	3	22.7734	—	836	144	20
	4	23.3070	1.4491	543	430	27
	5	22.4913	1.4425	499	449	52
	6	22.2403	1.4446	648	311	41
Early ripening subvarieties A	1	22.9642	1.4437	613	342	45
	2	22.7744	1.4385	462	514	24
	3	22.9904	1.4224	834	—	119
	4	23.2119	1.4304	857	99	44
Early ripening subvarieties B	1	24.1185	1.4370	613	255	132
	2	22.8989	1.4325	650	185	165
	3	22.4461	1.4519	670	312	18
	4	22.8904	1.4275	—	—	—
	5	23.9894	1.4328	674	293	33
	6	23.3955	1.4242	666	319	12
Average of late subvarieties		22.6001	1.4419	686	275	36
Early subvarieties A		22.9852	1.4337	691	277	58
Early subvarieties B		23.2898	1.4336	674	355	71

From the above table, the early ripening subvarieties show the greater absolute weight of 1000 grains, the lower specific weight and a larger percentage of rich greenish grains compared with the late ripening subvarieties.

**2. General analysis of the early ripening subvarieties.**

The water, ash, crude protein, crude fat, proteid and non-proteid of the hulled rice were determined by the ordinary methods.



TABLE XV.

Water, ash, crude fat, crude protein, proteid and non-proteid contents.

Subvarieties	No.	Water	Ash %	Crude fat	Crude protein	Proteid	Non-proteid
Late ripening subvarieties	1	13.1680	1.4375	2.5796	6.2244	6.0640	0.1740
	2	13.9147	1.4071	2.6456	8.0450	7.3926	0.6504
	3	12.9771	1.3444	2.3562	6.8263	6.6776	0.1296
	4	12.0641	1.4854	2.6465	6.8544	6.7537	0.1007
	5	11.6675	1.3925	2.8087	7.0831	6.7236	0.3595
	6	13.0889	1.4728	2.6314	6.9256	6.6738	0.2518
Early ripening subvarieties A	1	12.2218	1.5037	2.4721	7.9574	7.3833	0.5711
	2	11.6912	1.4712	2.4516	8.1525	7.3629	0.6896
	3	13.6656	1.5290	2.8367	7.3338	7.0102	0.3236
	4	13.5260	1.4767	2.5854	7.3456	7.0213	0.3243
Early ripening subvarieties B	1	12.7374	1.4898	2.4341	8.5888	8.1591	0.4297
	2	13.9113	1.3823	2.5662	7.9588	7.7435	0.2153
	3	12.5631	1.4868	2.5710	8.5206	7.7566	0.7640
	4	8.1242	1.6217	2.3444	7.6813	7.5501	0.1312
	5	13.1464	1.4968	2.5423	8.7163	7.8449	0.8714
	6	12.0293	1.3188	2.3188	9.5669	9.0883	0.4786
Average of late ripening subvarieties		12.8134	1.4333	2.6113	6.9931	6.7174	0.2770
Average of early ripening subvarieties A		12.7761	1.4951	2.5864	7.6973	7.2589	0.4771
Average of early ripening subvarieties B		12.0849	1.4660	2.4873	8.5054	8.0237	0.4871

From the above table, the early ripening subvarieties are seen to be rich in ash and crude protein content but to have a rather lower content of proteid. They are rich in non-proteid and fat content in comparison with the later ripening ones. When the authors compare this result with that of the above experiments of Part I, they understand that the seed of the early ripening subvarieties has been taken in the early part of the ripening period.

### 3. Content of the four kinds of proteins.

The content of the four kinds of proteins, water-soluble, NaCl-soluble, NaOH-soluble and alcohol-soluble were determined by the method which the authors described in their former report. The following numbers represent the nitrogen percentage of dry matter.

TABLE XVI.

Content of the four kinds of proteins.

Subvarieties	No.	Total-N	Water-sol. N	Alcohol-sol. N	NaCl-sol. N	NaOH-sol. N
Late ripening subvarieties	1	0.9959	0.1374	0.0341	0.1334	0.2910
	2	1.2872	0.1305	0.0593	0.1395	0.2783
	3	1.0922	0.1417	0.0604	0.0604	0.3111
	4	1.0967	0.1167	0.0590	0.1434	0.2379
	5	1.1333	0.1395	0.0498	0.1713	0.3387
	6	1.1081	0.1610	0.0528	0.1407	0.3518
Early ripening subvarieties A	1	1.2727	0.1492	0.0645	0.1476	0.3461
	2	1.3044	0.1326	0.0470	0.1530	0.3771
	3	1.1734	0.1121	0.0656	0.1553	0.3024
	4	1.1753	0.1035	0.0318	0.1434	0.2752
Early ripening subvarieties B	1	1.3742	0.1549	0.0811	0.2020	0.4066
	2	1.2732	0.1534	0.0721	0.1625	0.3869
	3	1.3632	0.1430	0.0679	0.1879	0.3847
	4	1.2290	0.1536	0.0614	0.1700	0.1490
	5	1.3946	0.1395	0.0697	0.1482	0.4149
	6	1.5307	0.1305	0.0627	0.1653	0.3914
The following figures are the percentage of total nitrogen.						
Late ripening subvarieties	1	100.00	13.7966	3.4441	13.3949	29.3102
	2	100.00	10.1382	4.5991	10.8142	21.6206
	3	100.00	12.9738	5.5301	13.4316	28.4838
	4	100.00	11.1516	5.3798	13.0756	21.6923
	5	100.00	13.7966	5.4410	13.3949	21.6206
	6	100.00	15.8740	4.7649	12.6975	31.7480
Early ripening subvarieties A	1	100.00	11.7231	5.0680	11.5974	28.6635
	2	100.00	10.1656	3.6032	11.7295	28.9098
	3	100.00	9.5534	5.5906	13.3350	25.7713
	4	100.00	8.8078	4.4074	12.2011	23.4153
Early ripening subvarieties B	1	100.00	11.2720	5.9016	14.6995	29.5881
	2	100.00	12.0465	5.6620	12.7611	30.3832
	3	100.00	10.4893	4.9806	13.7827	28.2183
	4	100.00	12.4980	4.9559	13.8342	24.0928
	5	100.00	10.0029	4.9978	11.8529	29.7505
	6	100.00	8.5255	4.0962	10.7990	25.5700
Average of the late ripening subvarieties B			12.9551	4.5270	12.8014	25.7459
Early ripening subvarieties A			10.0625	4.6673	12.1907	26.6899
Early ripening subvarieties B			10.8057	5.0990	12.9546	29.6005

From the above table, it will be seen that the early ripening sub-varieties are more rich in NaOH-soluble protein than in water soluble compared with the late ripening subvarieties.

#### 4. Some physico-chemical properties of oryzenin.

The pure oryzenin of different rices was prepared by the method used before and the following physico-chemical properties were examined.

(a) As stated above, (Part I, 5, a) the iso-electric point of oryzenin is to be inferred from the change of the surface tension and the turbidity of its alkaline solution by an acid titration. One tenth g. of water freed oryzenin was dissolved in 10 cc of 1/50 n-NaOH solution; 1 cc of the solution was diluted to 10 cc and then 1/200 n-HCl solution was added.

TABLE XVII.

Surface tension and turbidity of the protein solutions.

ccm of HCl	3.00	3.05	3.10	3.15	3.20	3.25	3.30	3.35	3.40	3.45	3.50	3.55
Surface tension Late ripening var.	—	60.1	60.1	60.1	60.1	59.5	<u>61.2</u>	60.1	60.1	60.1	—	—
Early ripening tar. A	—	60.1	60.1	60.1	60.1	59.5	59.5	60.1	<u>61.2</u>	60.1	60.1	—
Early ripening tar. B	—	60.1	60.1	60.1	60.1	60.1	60.1	60.1	60.1	60.1	59.5	<u>61.2</u>
Turbidity Late ripening var. M	—	+	+	+	++	++	+++	++	++	+	+	—
Early ripening tar. A	—	+	+	+	++	++	++	++	+++	++	+	—
Early ripening tar. B	—	+	+	+	+	+	++	++	++	++	++	+++

As shown in the above table, the iso-electric point of the oryzenin of early ripening subvarieties is more acidic than that of the later ripening ones.

(b) The specific rotatory power of the oryzenin alkaline solution was determined by the method described above (Part I, 5, 6) and a comparison made of the specific rotatory power of the different kinds of oryzenins.

TABLE XVIII.

Specific rotatory power of the various oryzenin solutions.

Late ripening var. No.	1	2	3	4	5
Specific rotatory power					
(5 hrs)	-57.09	-62.28	-64.88	-62.28	-62.28
(24 hrs)	-50.67	-62.28	-62.28	-62.28	-62.28
(48 hrs)	-62.28	-64.88	-62.28	-64.88	-64.88
(72 hrs)	-58.39	-59.97	-55.80	-59.67	-60.98
Early ripening subvarieties A					
(5 hrs)	-64.88	-62.28	-59.67	-59.67	
(24 hrs)	-62.28	-62.28	-59.67	-59.67	
(48 hrs)	-62.28	-64.88	-57.09	-62.28	
(72 hrs)	-58.39	-59.67	-54.50	-59.67	
Early ripening subvarieties B					
(5 hrs)	-59.67	-57.09	-55.80	-54.50	-54.50
(24 hrs)	-59.67	-59.67	-59.67	-57.09	-57.09
(48 hrs)	-60.98	-59.67	-59.67	-59.67	-54.50
(72 hrs)	-59.09	-57.09	-57.09	-55.80	-57.09

From the above table, the specific rotatory power of oryzenin of the early ripening subvarieties is seen to be always lower than that of the later ripening ones.

5. Some chemical properties of oryzenin.

(a) The water and ash content of oryzenin was determined by the ordinary method, the phosphorus content by volumetric method and the sulphur by Denis-Benedict's method.

TABLE XIX.

Water, ash, phosphorus and sulphur contents of protein samples.

	No.	Water %	Ash	Dry matter % phosphorus	Sulphur
Late ripening var.					
	1	3.9755	0.3600	0.1944	0.7473
	2	6.4053	0.3856	0.2105	0.8583
	3	4.1406	0.4851	0.2025	0.7209
	4	10.7656	0.4011	—	0.6797

TABLE XIX. (Continued).

	No.	Water %	Ash	Dry matter % phosphorus	Sulphur
Average	5	7.9972	0.3998	—	0.8541
Early ripening var. A	1	—	0.4063	0.2025	0.7715
	2	7.7510	0.4082	0.2150	0.7473
	3	5.6034	0.3116	0.2703	0.7896
	4	4.0692	0.3250	0.2025	0.7322
	5	5.0932	0.3420	0.2103	0.8239
Average	—	—	0.3467	0.2245	0.7732
Early ripening var. B	1	2.3616	0.5378	0.2824	0.8239
	2	4.0900	0.4748	0.2415	0.7072
	3	3.0047	0.4640	0.2911	0.6710
	4	3.7037	0.2749	0.1673	0.8308
	5	3.0237	0.3709	0.2213	0.7209
	6	6.3138	0.3681	0.2087	0.8926
Average	—	—	0.4150	0.2353	0.7744

(b) The elementary composition of oryzenin was determined by the ordinary method: i. e. carbon and hydrogen were determined by an electric furnace, nitrogen by Kjeldahl's method and sulphur by Denis-Benedict's method. The following figures representing the dry matter percentage were calculated.

TABLE XX.

Elementary composition of various oryzenins.

	Carbon %	Nitrogen %	Hydrogen %	Sulphur %
Late ripening var.	52.0702	17.1429	7.5247	0.7473
Early ripening var.	51.4695	17.3541	7.4399	0.7322

(c) The free amino nitrogen of different oryzenins was determined by Sørensen's method as described in the former report.<sup>(8)</sup>

TABLE XXI.

Free amino nitrogen of the various oryzenins.

		No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
Late ripening var.	Protein %	1.330	0.840	1.330	1.715	1.505	
	Total N. %	7.4803	4.9517	7.9385	9.6387	8.8984	
Early ripening var. A	Protein %	1.330	1.645	0.875	0.980		
	Total N. %	7.5993	9.7261	5.1965	5.7577		
Early ripening var. B	Protein %	0.875	1.330	1.225	1.225	0.875	0.980
	Total N. %	4.9449	7.2384	7.0373	7.5795	4.9177	5.4115

  

Average	Late ripening var.	Early ripening var. A	Early ripening var. B
Protein %	1.330	1.225	(1) 1.155 (2) 1.015
Total N %	7.7583	7.0734	6.4795 5.8486

From the above table, it may be seen that the free amino nitrogen of oryzenin of the early ripening subvarieties is always less than that of the latter ones.

**Résumé.**

(1) In general analysis of rice, the early ripening subvarieties are rich in fat and non-proteid content while the content of ash and proteid is less in comparison with the later ripening subvarieties.

(2) The early ripening rice is rich in water soluble protein, the iso-electric point of its oryzenin is acidic and its specific rotatory power is low in comparison with the later ripening subvarieties. In the early ripening subvarieties, the free amino nitrogen content of oryzenin is less than in the later. These characteristics show that the seed of the early ripening subvarieties is not completely ripened.

SUMMARY.

(A) Chemical studies on the ripening of rice seed.

(1) During the ripening of rice seed, the content of non-proteid

decreases and proteid content increases. The composition of the principal protein, oryzenin, changes by a decrease of mono-amino and an increase of diamino nitrogen during the ripening period.

(2) Among the four rice proteins, the content of water soluble and alcohol-soluble proteins increases gradually during the whole ripening time. The composition of oryzenin increases in specific rotatory power, in content of free amino nitrogen and of lysin nitrogen but decreases in content of arginin and of ash to a definite ripening date. At this date the iso-electric point moved toward the alkaline side. Then that chemical character reversed itself in the later part of the ripening period.

(3) Therefore the chemical structure of oryzenin seems to be changed during the ripening time of rice.

(4) The chemical properties of rice starch also vary with the ripening period and show that its polymerization degree decreases in the middle of the ripening period and then increases later.

(B) Chemical properties of the early ripening subvarieties.

(5) In general analysis of rice, the early ripening subvarieties are rich in fat and non-proteid content while the content of ash and proteid is less in comparison with the later ripening kinds.

(6) The early ripening rice is rich in water soluble protein, the iso-electric point of its oryzenin is acidic and its specific rotatory power is low as compared with the oryzenin of the later ripening subvarieties. In the former, the free amino nitrogen content of oryzenin is less than in the later. These characteristics show that the seed of the early ripening subvarieties is not usually completely ripened.

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