Studies on the Ripening of Rice-Grains

(Second Report)

By

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CHAPTER I

Introduction

The physical and chemical changes which occur during the growth of rice-plants have been thoroughly discussed by various scholars. The present authors have already introduced some of them in the previous report.[1] Here will be described briefly other researches on this point. Kelley and Thompson[2] have observed the change of each inorganic and organic chemical constituent and the effect of manures. Ash analyses of upland-rice were made by Gile and Carrero[3] at intervals from the early stage to complete maturity. Sen[4] has separated the whole life of rice-plants—from seedling-plants to dead ripeness—into six stages, and investigated the assimilation of each nutrient systematically. Suzuki[5] has also determined the development and chemical composition of the rice-plant at successive stages in its growth. He divided the whole life into five periods and investigated the effect of fertilizers during the growth of rice-plants and rice-grains. Van Rossem and Weber[6] determined the mineral matter extracted from the soil by young rice-plants in Dutch Indies, and afterward Van Rossem[7] determined the amounts of nutrient substances abstracted from the soil by rice-plants of Java at five different stages of growth from seedling to maturity.

As to the change of rice-kernels during the development, Uchida[8] and Yamazaki[9][10] have already studied the physical characters and

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some chemical composition of rice produced in Hokkaido and in Tottori Prefecture respectively. Recently, Matsuda[11] has investigated the rice of Kyoto on the relation between growth and actual weight, volume, water and ash contents, etc.

The authors[1][12][13][14] have also studied the change of physical and chemical characters of rice-grains during growth with a subvariety, Akashiho, produced in Hyogo Prefecture and with Toyokuni and Kamenoo produced in Akita Prefecture. Intending to study the physiology of ripening of rice-grains more precisely they carried out the following experiments.

CHAPTER II

Material and Some Physical Characters

The material used in this experiment is subvariety, Bôzu No. 5 (Hokunô No. 5), cultivated at the Kitami Branch of Hokkaido Agricultural Experiment Station in 1928, and the degree of ripening is shown by the date of harvest.

A. October 27th
B. November 3rd
C. November 10th
D. November 17th

After the actual weight and specific gravity of the unhulled rice were estimated, it was hulled, and the actual weight and specific gravity of the hulled rice were also determined. It was then powdered by a mill and used for the estimation of general constituents, distribution of nitrogen, etc. The defatted rice flour was used for the preparation of oryzenin and starch.

The physical properties and the contents of green rices were as follows:—

TABLE I

Physical Properties of Unhulled and Hulled Rices and the Contents of Green Rices

	A.	B.	C.	D.
Unhulled Rice				
Actual Weight (g)	29.0926	29.7366	30.1348	29.6828
Ratio	100.0	102.2	103.6	102.0
Specific Gravity	1.1242	1.1376	1.1380	1.1345
Ratio	100.0	101.2	101.2	100.9

TABLE I (Continued)

	A.	B.	C.	D.
Hulled Rice				
Actual Weight (g)	24.7202	25.6588	25.5470	25.3565
Ratio	100.0	103.8	103.3	102.6
Specific Gravity	1.4003	1.3928	1.3922	1.3950
Ratio	100.0	99.5	99.4	99.6
Green Rices				
Unripened (%)	8.0	4.4	2.5	1.2
Ratio	100.0	55.0	31.3	15.0
Half-ripened (%)	20.3	14.3	9.1	3.5
Ratio	100.0	70.4	44.8	17.2
Ripened Rice (%)	71.7	81.3	88.4	95.3
Ratio	100.0	113.4	123.3	132.9

As is seen in the above table, the actual weight of both unhulled and hulled rices first increased and afterward decreased, which resembles the results of previous reports[12][14] as well as those of some others. The specific gravity of unhulled rice, which may be mainly affected by the water content as is shown in the following chapter, first increased and then decreased, while that of hulled rice first decreased, afterward increased. The sharp drop in the number of unripened and half-ripened grains may perhaps be due to the dead ripeness.

CHAPTER III

General Analysis of Hulled Rice Flour

In a previous report,[12] the authors found that the content of crude fat of hulled rice decreased gradually with the ripening, while the total nitrogen showed no definite tendency. Afterward they[14] also found that the water content, ash and phosphoric acid contents in 1000 rice kernels decreased gradually and that of crude fat also showed a similar tendency. The total nitrogen seemed to increase gradually but the rate of the pure protein formation always increased more rapidly.

The hulled rice was powdered in a mill and was analyzed by the ordinary method. The numbers in the following table are the mean of two experiments.

TABLE II

General Analysis of Hulled Rice Flour

	A.	B.	C.	D.
Water (%)	11.1302	11.8545	11.9236	11.3415
Weight in 1000 Rice Grains (g)	2.7514	3.0413	3.0461	2.8758
Ratio	100.0	110.5	110.7	104.5
Solid Matters (%)	88.8698	88.1455	88.0764	88.6585
Weight in 1000 Rice Grains (g)	21.9688	22.6171	22.5009	22.4807
Ratio	100.0	103.0	102.4	102.3
Crude Ash (%)	2.3635	2.4388	2.3320	2.2467
Weight in 1000 Rice Grains (g)	0.5192	0.5516	0.5247	0.5051
Ratio	100.0	106.2	101.1	97.3
Phosphoric Acid (%)	1.1468	1.0350	1.0047	1.0284
Weight in 1000 Rice Grains (g)	0.2519	0.2341	0.2261	0.2312
Ratio	100.0	92.9	89.8	91.8
Phosphoric Acid/Crude Ash	48.5213	42.4389	43.0832	45.7738
Ratio	100.0	87.5	88.8	94.3
Crude Fat (%)	4.1611	3.8459	3.7536	3.7154
Weight in 1000 Rice Grains (g)	0.9142	0.8698	0.8446	0.8352
Ratio	100.0	95.1	92.4	91.4
Crude Protein (%)	9.8199	9.8621	9.2786	9.5752
Weight in 1000 Rice Grains (g)	2.1573	2.2305	2.0878	2.1526
Ratio	100.0	103.4	96.8	99.8
Pure Protein (%)	9.4505	9.6052	9.1373	9.4475
Weight in 1000 Rice Grains (g)	2.0762	2.1724	2.0560	2.1239
Ratio	100.0	104.6	99.0	102.3
Pure Protein/Crude Protein	96.2386	97.3958	98.4765	98.6667
Ratio	100.0	101.2	102.3	102.5

As is seen in the above table, the water content first increased and afterward decreased, suggesting the close relation with the specific gravity of hulled rice. The decrease of the weight of dry substances in rice grains is probably due to the dead ripeness, as Ivanov[15] already mentioned, showing the reversibility of physiological functions during the ripening of seeds. The absolute and the relative quantities of phosphoric acid in hulled rice decreased for certain periods and then increased, which resembles the results described in a previous report,[14] showing the tendency reported by Gile and Carrero.[3] Crude fat de-

creased gradually just like that of Akashiho and Kamenoo. The content of crude protein also showed no definite tendency in the present investigation. According to Kelley and Thompson,[2] the percentage of nitrogen is not affected by fertilizers when rice is attained to maturity. This statement also means the inconstancy of nitrogen content. The ratio of pure protein to crude protein, however, increased gradually during the whole period of ripening as already seen in the previous reports.

CHAPTER IV

Distribution of the Four Kinds of Rice Proteins

Preceding the study of transformation of nitrogenous substances, the authors investigated the distribution of the four kinds of rice proteins. Goto[16] has already studied on this problem and reported that the nitrogen of water-soluble protein decreased gradually with the ripening period while, on the contrary, that of alcohol-soluble protein increased. He also stated that those nitrogens of NaCl- and alkali-soluble proteins showed no definite tendencies. In the authors' previous report,[12] they found that the nitrogen of water-soluble protein first increased and afterward decreased, those of NaCl- and alcohol-soluble proteins increased constantly, while that of alkali-soluble varied variously. They also found, as in another report,[14] that the nitrogens of NaCl- and alcohol-soluble proteins increased while those of water-soluble decreased in one sample and increased in the other. The nitrogen of alkali-soluble seemed to increase perceptively. These phenomena are considered to manifest the physiological functions of the formation of reserve proteins during the ripening of seeds, showing a very interesting contrast when compared with those of transformations of various proteins during the germination. Takahashi and Shirahama,[17] observing the transformations of the four kinds of proteins during the germination and growth of malt, stated that the water-soluble protein in water-soaked barley grains increased at the rate of 100, 153, 234, 275 and 288 at 0, 24, 48, 72 and 120 hours after being put into the incubator; while the NaCl-soluble and alcohol-soluble decreased respectively at the rate of 100, 99, 102, 95 and 86, and 100, 49, 33, 29 and 10 during the same hours. The alkali-soluble protein decreased irregularly. All these tendencies seen in the

germination are quite opposite to those found in the present authors' investigation of ripening. There will be found a close relation between the germination and the ripening, the one shows the formation of soluble proteins by relaxing the condensation of the higher reserve proteins, and the other shows, on the contrary, the formation of reserve proteins by condensation of lower classes.

Determination of the four kinds of rice proteins was carried out by the method which had been described in a former report,[18] i. e., nitrogen of each protein was determined by the order of water-soluble, NaCl-soluble, alcohol-soluble and alkali-soluble. Total nitrogen was also estimated. Figures in the following table are the mean of two experiments.

TABLE III

Distribution of the Four Kinds of Rice Proteins During the Ripening

	A.	B.	C.	D.
Total N (%)	1.5712	1.5779	1.4846	1.5320
Weight in 1000 Rice Grains (g)	0.3452	0.3569	0.3340	0.3444
Ratio	100.0	103.4	96.8	99.8
Water-soluble N (%)	0.2700	0.2599	0.2262	0.2206
Weight in 1000 Rice Grains (g)	0.0593	0.0588	0.0509	0.0496
Ratio	100.0	99.2	85.8	83.6
NaCl-soluble N (%)	0.1946	0.2075	0.2118	0.2012
Weight in 1000 Rice Grains (g)	0.0428	0.0469	0.0477	0.0452
Ratio	100.0	109.6	111.4	105.6
Alcohol-soluble N (%)	0.0999	0.0986	0.1038	0.1103
Weight in 1000 Rice Grains (g)	0.0219	0.0223	0.0234	0.0248
Ratio	100.0	101.8	106.8	113.2
Alkali-soluble N I. (%)	0.4748	0.4582	0.4380	0.4382
Weight in 1000 Rice Grains (g)	0.1043	0.1036	0.0986	0.0985
Ratio	100.0	99.3	94.5	94.4
Alkali-soluble N II. (%)	0.1773	0.1787	0.1707	0.1736
Weight in 1000 Rice Grains (g)	0.0390	0.0404	0.0384	0.0390
Ratio	100.0	103.6	98.5	100.0

TABLE IV

Percentage of Each Nitrogen on the Basis of Total Nitrogen

	A.	B.	C.	D.
Total N	100.0000	100.0000	100.0000	100.0000
Water-soluble N	17.1845	16.4710	15.2366	14.3992
NaCl-soluble N	12.3856	13.1501	14.2667	13.1329
Alcohol-soluble N	6.3583	6.2487	6.9919	7.1996
Alkali-soluble N I.	30.2193	29.0381	29.5033	28.6026
Alkali-soluble N II.	11.2845	11.3250	11.4982	11.3314

As is seen in the above table, the quantity of nitrogen of water-soluble protein decreased gradually with the ripening period, showing the same tendency in both Akashiho and Toyokuni; and that of NaCl-soluble and alcohol-soluble always increased which is quite similar to the authors' previous results. The nitrogen of alkali-soluble resembles that of Kamenoo.

CHAPTER V

Transformation of Various Nitrogenous Substances During Ripening

Wassilieff[19] and Saliesskii[20][21] have stated, on the transformation of nitrogenous substances during growth, that the absolute and relative quantities of both the total and the protein nitrogen continuously increased with the ripening period, while the amount of non-proteid decreased gradually. That one part of their theory is not right is proved in the foregoing chapter. Nedokutschajeff,[22][23] Pfenninger[24] and Schulze and Winterstein[25] have obtained unsatisfactory results in early stages of ripening and the present authors also observed the non-conformity at complete maturity and at dead ripeness, i. e., the inconstancy of nitrogen content was repeatedly reported in the former reports[12][14] and in the foregoing chapters.

It is obvious that the change of free-amino nitrogen, on the other hand, plays an important rôle in the transformation of various nitrogenous substances; and therefore the determination of free-amino nitrogen is considered very significant to elucidate the relations

among these nitrogenous substances. On this point, Lüers,[26] determining the acid values of ripening grains of various plants, stated that both the acid and formol-titratable substances increased during ripening and decreased during storage, the reason therefor lying in the binding of acids in organic combination and the condensation of the nitrogenous compounds into more complex compounds.

The present authors also investigated the relations and transformations among these nitrogenous substances and described the results in their former reports. And now they intended to study more precisely and made the following experiments :—

One gram of rice flour was taken and the pure protein was estimated by Stutzer's method. The filtrates and washings were collected into a measuring flask of 250 cc capacity, 50 cc of which were used for the determination of non-proteid nitrogen and another 50 cc were used for the determination of free-amino nitrogen. Four grams of the sample, on the other hand, were soaked into 60 cc of 0.2% NaOH solution, and the whole was shaken vigorously for 30 minutes, allowed to stand overnight and then centrifuged. Ten cc of the supernatent liquid were used for the determination of soluble nitrogen and another 10 cc for the free-amino nitrogen. By these manipulations, the pure protein nitrogen, non-proteid nitrogen, free-amino nitrogen in non-proteid substances, soluble nitrogen and total free-amino nitrogen were obtained. Total nitrogen was also estimated. The results follow :—

TABLE V

Distribution of Protein Nitrogen, Non-proteid Nitrogen and Free-amino Nitrogen

	A.	B.	C.	D.
Total N (%)	1.5712	1.5779	1.4846	1.5320
Weight in 1000 Rice Grains (g)	0.3452	0.3569	0.3340	0.3444
Ratio	100.0	103.4	96.8	99.8
Pure Protein N (%)	1.5121	1.5368	1.4620	1.5116
Weight in 1000 Rice Grains (g)	0.3322	0.3476	0.3290	0.3398
Ratio	100.0	104.6	99.0	102.3
Pure Protein N/Total N	96.2386	97.3958	98.4765	98.6667
Ratio	100.0	101.2	102.3	102.5

TABLE V (Continued)

	A.	B.	C.	D.
Free-amino N (%)	0.4064	0.4091	0.4018	0.3982
Weight in 1000 Rice Grains (g)	0.0893	0.0925	0.0904	0.0895
Ratio	100.0	103.6	101.2	100.2
Free-amino N/Soluble N	27.9697	28.8566	28.5268	29.9917
Ratio	100.0	103.2	102.0	107.2
Free-amino N/Total N	25.8656	25.9269	27.0645	25.9922
Ratio	100.0	100.2	104.6	100.5
Non-proteid N (%)	0.0326	0.0267	0.0226	0.0102
Weight in 1000 Rice Grains (g)	0.0072	0.0060	0.0051	0.0023
Ratio	100.0	83.3	70.8	31.9
Non-proteid N/Total N	2.0749	1.6921	1.5223	0.6658
Ratio	100.0	81.6	73.4	32.1
Free-amino N in Non-proleid (%)	0.0147	0.0076	0.0064	0.0022
Weight in 1000 Rice Grains (g)	0.0032	0.0017	0.0014	0.0005
Ratio	100.0	53.1	43.8	15.6
Free-amino N in Non-proteid/ Non-proteid N	45.0920	28.4644	28.3186	21.5686
Ratio	100.0	63.1	62.8	47.8
Free-amino N in Non-proteid/ Total N	0.9356	0.4817	0.4311	0.1436
Ratio	100.0	51.5	46.1	15.3
Soluble N (%)	1.4530	1.4177	1.4085	1.3277
Ratio	100.0	97.6	96.9	91.4
Weight in 1000 Rice Grains (g)	0.3192	0.3206	0.3169	0.2985

The transformation of various nitrogens during ripening is observable in the above table. That the quantity of total nitrogen and that of protein nitrogen showed no definite tendency is already mentioned in the foregoing chapter, but the ratio of protein nitrogen to total nitrogen increased gradually during ripening and non-proteid nitrogen, on the contrary, rapidly decreased. The total nitrogen percentage also decreased almost at the same rate. Lüers' so-called formol-titratable substance, i. e., the free-amino nitrogen, is seen, as he pointed out, to increase for a while and afterward slightly to decrease. The absolute and relative quantities of non-proteid nitrogen decreased rapidly, which is the same tendency as seen in the previous report. These facts show not only protein formation from non-proteid substances, but also the condensation of higher proteins from lower classes in rice kernels,

and it is almost decisive that the quantity of water-soluble protein decreased and that of NaCl-soluble and alcohol-soluble proteins increased with the advance of ripening period, as is seen in the foregoing chapter. It suggests, moreover, from the decreasing velocity of both free-amino nitrogens, that the rate of the formation of protein from the non-proteid substances exceeds that of the polymerization of higher proteins from the lower. The amount of 0.2% NaOH-soluble nitrogen gradually decreased, which shows that the protein decreased its solubility as a whole.

The free-amino nitrogen, from the other point of view, showed a moderate decreasing tendency, which may be due to the interference caused by the reverse function, namely, disintegration of higher proteins into lower classes, as reported by Spitzer, Carr and Epple[27] and Ivanov.[15] Further investigations are wanted to solve these problems.

CHAPTER VI

Transformations of Carbohydrates in Rice Grains

Many studies have been carried out by various authors on the transformation of carbohydrates. On the change of carbohydrates in the ripening seed of *Zea mays*, Portele[28] has stated that the content of starch increased while that of glucose and cane sugar decreased. Blagowestschenski[29] obtained similar results with *Vicia faba minor* as shown in the following table:—

Harvest of 1923

Ripening Stage	Glucose		Sucrose		Starch	
	%	In 100 Grains (g)	%	In 100 Grains (g)	%	In 100 Grains (g)
I.	3.07	0.10	2.23	0.07	8.53	0.2864
II.	1.94	0.15	2.69	0.29	21.24	2.3146
III.	0.57	0.09	2.21	0.32	48.08	4.7532
IV.	0.21	0.08	1.76	0.69	47.87	19.2875
V.	0.23	0.11	1.52	0.70	47.91	22.0195

Harvest of 1924

Ripening Stage	Glucose		Sucrose		Starch	
	%	In 100 Grains (g)	%	In 100 Grains (g)	%	In 100 Grains (g)
II.	5.80	0.23	5.74	0.23	19.41	0.7699
III.	2.55	0.11	1.99	0.09	11.92	0.5198
IV.	2.28	0.22	2.78	0.27	19.79	1.9090
V.	0.91	0.11	3.10	0.38	30.62	3.6422
VI.	0.96	0.14	3.18	0.47	33.03	4.8871
VII.	0.48	0.09	2.62	0.48	33.62	6.1093
VIII.	0.37	0.09	2.01	0.44	29.96	7.0630
IX.	0.39	0.14	2.02	0.71	39.87	13.9765

Colin and Belval[30] also found the same tendency in wheat, and Colin and Franquet[31] investigated the phenomena in potato. Kelley and Thompson[2] have reported that the reducing sugars were found in notable quantities in all stages of growth, especially in the first period. Sucrose, while present as a trace at the first period, occurred in the stem at the second period to the extent of about 10%, and at maturity, this had been largely converted to starch which had gradually increased throughout the growth of the plant. Pentose-forming compounds constitute a large portion of the carbohydrates at every stage.

Recently, Belval[32] studied the sugars of leaves and stems of rice and reported that the content varied with the ripening period and these variations appeared to be largely dependent on the water content of the vegetable tissues. As the water content of rice grains is far less than that of the leaves and stems, it must have an important physiological rôle in grains.

As to the existence of dextrin in grains, Belval[33][34][35] stated that the soluble carbohydrate reserve which accompanies the starch in all stages of development of grains is composed either of sucrose and reducing sugars or of these together with some levulosans. He confirmed the views of Dehérain and Meyer[36] and Müntz[37] and opposed those of Péligot,[38] Payen and Persoz[39] and Hébert,[40] insisting upon the absence of dextrin, and declared that it was entirely illusory to formulate the processes of synthesis on the basis of the hydrolytic phenomena, but that it was advisable to imagine that when glucose is

condensed to starch, in the plant, there must occur the various steps through maltose and dextrins.

According to Colin and Belval,[30] the wheat grains have no dextrin, and the soluble carbohydrates are composed of sucrose, reducing sugars and levosin which had been isolated and described by Tanret.[41][42] These latter decreased with the advance of the ripening period and the starch increased. At maturity the soluble carbohydrate reserve was mainly a levulosan the same as exists in the stems. Moreover, Estienne,[43] concluding from Belval's thesis on the origin of starch in cereals, stated that the starch of cereal grains is formed from carbohydrates which are elaborated by the leaves and which pass into the stalk, mainly in the form of hexoses. The carbohydrates then pass into the grains, being transformed on the way into simple sugars which enter into the grains and build up the reserve of starch. The carbohydrates stored temporarily in the stem consist either of a mixture of sucrose, glucose and fructose, or of these together with levulosans. Very young wheat grains may contain up to 35% levulosans, while the starch content does not exceed 15%. The major portion of these levulosans consists of levosin, isolated and described by Tanret as already mentioned, to the exclusion of what has been called levulin or synanthrose. The levulosans must be considered as a temporary reserve, which is of importance only in the young grains. He finally concluded that whichever class of cereals was used, no trace of dextrins was ever found in any part of the plant. What has been taken for dextrins is only sucrose or a mixture of sucrose and levulosans.

Five grams of rice flour were used for the determination of reducing sugars and dextrin, and another 3 grams were used for the determination of starch by Bertrand's method. The results are as follows. Figures are the mean of two experiments.

TABLE VI

Reducing Sugar, Dextrin and Starch of Hulled Rice Flour

	A.	B.	C.	D.
Reducing Sugar (%)	1.8741	1.6962	1.4482	1.6198
Weight in 1000 Rice Grains (g)	0.4117	0.3836	0.3259	0.3641
Ratio	100.0	93.2	79.2	88.4
Dextrin (%)	2.1564	2.1572	2.2637	2.5589

TABLE VI (Continued)

	A.	P.	C.	D.
Weight in 1000 Rice Grains (g)	0.4737	0.4879	0.5094	0.5753
Ratio	100.0	103.0	107.5	121.4
Starch (%)	65.3327	65.0886	66.0730	65.8941
Weight in 1000 Rice Grains (g)	14.3528	14.7211	14.8670	14.8090
Ratio	100.0	102.6	103.6	103.2

As is seen in the above table, the quantity of reducing sugar decreased with the ripening time and then afterward slightly increased, reversely proportional to the water content. On the contrary, dextrin, which may be a group of sucrose and the levulosans of Belval and Estienne, and starch showed an increasing tendency.

CHAPTER VII

Physico-chemical Properties of Oryzenin

In the authors' previous reports,[12][13] they separated the principal protein, oryzenin, from each rice different in ripening degree, and investigated the physico-chemical characters of it. Proteins which had been separated heretofore as oryzenin, etc. may be different in their physico-chemical properties according to the condensation degree, and if the changes of their properties caused by the ripening degree become clear, the physiological function of the formation of proteins in cereal grains will also be explained without difficulty. The authors already reported that the ash and phosphorus content decreased and the iso-electric point moved to the alkaline side at a definite period in the ripening time, while the specific rotatory power, at the same time, increased, and nitrogen, sulphur and free-amino nitrogen content also increased.

These phenomena are considered to show that the oryzenin is most condensed at that period, and that afterward the oryzenin again somewhat disintegrated. The authors also observed the distribution of various amino acids in protein molecule and found that the mono-amino acids decreased and diamino acids increased, which coincides

with the tendency of decreasing crude fat and non-proteid and increasing pure protein during ripening. They also found that the lysin increased and assumed a relation between that tendency and decrease of ash, elevation of iso-electric point and specific rotatory power and increase of free-amino nitrogen, concluding that the oryzenin has no definite properties but always manifests the denaturation phenomena during ripening and that whoever utilizes the principal protein, oryzenin, must pay attention to the harvest time of rice.

The authors desiring to know the changes more accurately attempted the following experiments.

§ 1. Preparation of Oryzenin, Water and Ash Contents

Oryzenin was separated from each rice crop by the ordinary method repeatedly described in former reports and was preserved in a H_2SO_4 desiccator. Water and ash contents are as follows:—

TABLE VII

Water and Ash Contents of Oryzenin

	A.	B.	C.	D.
Water (%)	5.6903	4.8421	4.5766	4.2291
Ash (%)	0.6830	0.5889	0.5437	0.5970
Ratio	100.0	86.2	79.6	87.4

The ash content decreased with the advance of the ripening period and afterward increased slightly, which shows the change of composition of protein as stated in the former report.

§ 2. Changes of Oryzenin Alkaline Solution in Titration with HCl

As the chemical composition is considered to be somewhat varied

according to the ash content of each oryzenin, the authors determined the change of protein alkaline solution in titration with HCl, by which the change of isò-electric point is supposed.

One-tenth gram of water-freed oryzenin was dissolved in 10 cc of 1/50-n-NaOH solution. After complete dissolution it was made up to 100 cc with redistilled water, to each 10 cc of which were added various quantities of 1/200-n-HCl solution for the purpose of finding the maximum point of turbidity and of surface tension. The turbidity was measured by a Duboscq's colorimetric nephelometer and the surface tension by a de Noüy's torsion balance. The degree of turbidity is shown by the "×" sign in the following table.

TABLE VIII

Change of Turbidity of Oryzenin Alkaline Solution in Titration with HCl

HCl (cc)	A.	B.	C.	D.
3.70				×
3.80	×××	××	××	××
3.90	×××	×××	×××	×××
4.00	×××	××××	××××	×××
4.10	××××	××××	××××	××××
4.20	××××	××××	×××××	×××××
4.30	××××	×××××	××××	××××
4.40	×××××	×××××	××××	×××
4.50	××××	××××	××××	×
4.60		×	×	

As is seen in the above table, the quantity of HCl solution wanted to bring about the maximum point of turbidity gradually decreased with the ripening period just like in the former report. The iso-electric point seems to move somewhat to the alkaline side. Moreover, it seems to decrease the iso-electric range.

The changes of surface tension were as follows:—

Figures are dyne per square centimeter on the basis of water as 75.

TABLE IX

Change of Surface Tension of Oryzenin Alkaline Solution in Titration with HCl

HCl (cc)	A.	B.	C.	D.
3.70				63.3
3.80	62.6	64.3	61.9	63.6
3.90	63.0	64.0	60.6	64.0
4.00	60.6	63.0	64.0	63.3
4.10	62.6	63.3	64.3	64.3
4.20	63.0	64.3	64.7	64.7
4.30	59.9	64.7	63.0	64.0
4.40	64.0	63.3	61.2	63.0
4.50	63.3	63.6	63.3	63.3
4.60		64.0	62.6	

The result of the experiment coincides well with that of turbidity showing the tendency described also in the first report.[1]

§ 3. Specific Rotatory Power of Oryzenin Alkaline Solution

In a former report, the authors found that the specific rotatory power reached its maximum at a definite time in the ripening period, and afterward decreased again. The same experiment was carried out with Bôzu No. 5.

One-tenth gram of oryzenin was dissolved in 15 cc of 1/10-n-NaOH solution and after 8 hours, 24 hours, 48 hours and 1 week the specific rotatory power was estimated by a Haensch-Schmidt's half-shadow polariscope, calculating the $[\alpha]_D$ from the equation

$$[\alpha]_D^t = \frac{\pi \times 0.346 \times 15}{1 \times 0.1},$$

where π is the reading of the polariscope at t°C. 0.346 is the constant of the apparatus and 1 is the length of the observation tube expressed in decimeters. The experiment was carried out at 18°C.

TABLE X

Change of Specific Rotatory Power of Oryzenin Solution

	A.	B.	C.	D.
8 Hours Reading	−1.50	−1.40	−1.65	−1.65
$[\alpha]_D^{18}$	−82.5472	−76.3574	−89.7421	−89.4852
Ratio	100.0	92.5	108.7	108.4
24 House Reading	−1.55	−1.50	−1.65	−1.70
$[\alpha]_D$	−85.2987	−81.8115	−89.7421	−92.1969
Ratio	100.0	95.9	105.2	108.1
48 House Reading	−1.25	−1.20	−1.05	−1.25
$[\alpha]_D^{18}$	−68.7893	−65.4492	−57.1086	−67.7918
Ratio	100.0	95.1	83.0	98.5
1 Week Reading	−1.00	−0.95	−1.20	−1.25
$[\alpha]_D^{18}$	−55.0314	−51.8139	−65.2670	−67.7918
Ratio	100.0	94.2	118.6	123.2

As is seen in the above table, the specific rotatory power at first decreased and then increased, which differs from the result of the former report. But it reached its maximum at the third period early in the time of dissolution. It decreased gradually with the lapse of time though the changes became somewhat irregular.

§ 4. Nitrogen, Sulphur and Phosphorus Contents of Oryzenin

The authors have described, in a former report, the difference of chemical construction of oryzenins obtained from rice, different in ripening degree, by the foregoing experiments. They also determined and reported the quantities of nitrogen, sulphur and phosphorus.

The nitrogen content reached its maximum at a definite period of ripening and then decreased while the phosphorus content decreased gradually. The sulphur content showed a like tendency. The authors intended to certify those results and carried out the following experiments.

Nitrogen was determined by Kjeldahl's method, sulphur by

Benedict-Denis method and phosphorus by volumetric method after the sample was incinerated in a platinum crucible. The results are as follows:

TABLE XI

Nitrogen, Sulphur and Phosphorus Contents of Oryzenin

	A.	B.	C.	D.
Nitrogen (%)	17.3403	17.3951	17.4606	17.2649
Ratio	100.0	100.3	100.7	99.6
Sulphur (%)	0.8445	0.9236	1.1081	1.0610
Ratio	100.0	109.4	131.2	125.6
Phosphorus (%)	0.1936	0.1652	0.1230	0.1326
Ratio	100.0	85.3	63.5	68.5

As seen in the above table, the nitrogen content showed its maximum at a definite period of ripening but phosphorus, on the contrary, showed its minimum value at the same period. The sulphur content showed a tendency similar to that of nitrogen as in the former report. All these phenomena are considered to show that the oryzenin is most condensed at a definite period, which is the same as in the former report.

§ 5. Free-amino Nitrogen Content of Oryzenin

The authors found, in a former report, that the free-amino nitrogen of oryzenin determined by Sörensen's method of formol titration showed its maximum quantity at a definite period of ripening and then decreased gradually, which coincides with the other results showing the increase of optical density, maximum quantities of nitrogen and sulphur, minimum quantity of phosphorous and the elevation of the isoelectric point at the same period. These showed that the oryzenin increased the density of its structure at that time and then afterward disintegrated again gradually.

On the other hand Spitzer, Carr and Epple,[27] studying the order of protein formation in Indian corn, had already suggested that the glutelin is made at the first place and then the globulin, and the

albumin, the lowest class of reserve protein in cereals. Ivanov[15] also recognized the reversibility of physiological functions during the ripening of seeds. These are very interesting problems with regard to the determination of free-amino nitrogen as already mentioned above.

The authors next determined various properties of oryzenin obtained from Bôzu No. 5 and found that the oryzenin decreased its ash and phosphorus quantities, increased the optical density, increased the nitrogen and sulphur contents and elevated the iso-electric point. From these results the authors also assumed the change of free-amino nitrogen playing the most important rôle in protein molecule, and carried out the following experiment.

One-tenth gram of the water-freed oryzenin was completely dissolved in 25 cc of 1/5-n-NaOH solution. Neutralized formaldehyde was added to 20 cc of the solution as usual and titrated back with 1/20-n-HCl solution. The authors also observed the change of free-amino nitrogen content to understand the decomposition of oryzenin alkaline solution when exposed to the ultraviolet rays under the quartz lamp for 30, 60 and 90 minutes at the distance of one foot. The free-amino nitrogen was estimated as usual. The results are as follows:—

TABLE XII

Content of Free-amino Nitrogen and the Change in It When Exposed to Ultraviolet Rays

	A.	B.	C.	D.
Without Exposure (%)	1.0206	1.1034	1.3388	1.0781
Ratio	100.0	108.1	131.2	105.6
Exposed for 30 Minutes (%)	1.1134	1.2873	1.4671	1.3522
Ratio	100.0	115.6	131.8	121.4
Exposed for 60 Minutes (%)	1.4845	1.5264	1.5405	1.4984
Ratio	100.0	102.8	103.8	100.9
Exposed for 90 Minutes (%)	1.7071	1.7655	1.8339	1.7542
Ratio	100.0	103.4	107.4	102.8
Total Nitrogen (%)	17.3403	17.3951	17.4606	17.2649

The above results were calculated on the basis of total nitrogen as 100. The rate of free-amino nitrogen formation by exposing to ultraviolet rays was measured as follows:

TABLE XIII

Total Nitrogen Percentage of Each Free-amino Nitrogen and the Rate of Formation of the Latter when Exposed to Ultraviolet Rays

	A.	B.	C.	D.
Without Exposure	5.8857	6.3432	7.6675	6.2445
	100.0	100.0	100.0	100.0
Exposed for 30 Minutes	6.4209	7 4004	8.4023	7.8321
	109.1	116.7	109.6	124.5
Exposed for 60 Minutes	8.5610	8.7749	8.8227	8.6789
	145.5	138.2	115.1	139.0
Exposed for 90 Minutes	9.8447	10.1494	10.5031	10.1605
	167.3	160.0	137.0	162.7

As is seen in the above tables, the free-amino nitrogen also showed a definite tendency during ripening, coinciding well with the other results. That is, the free-amino nitrogen increased its quantity with the advance of ripening, showing its maximum at a definite period, and afterward decreased markedly. The formation of free-amino nitrogen by ultraviolet rays was also most prominent at that period, but the rate of formation was less compared with the other ones, which also explains the construction and other properties of protein.

§ 6. Amino Acids Content of Oryzenin

A great many authors have investigated the distribution of amino acids in the protein molecule of rice. Suzuki, Yoshimura and Fuji[44] have investigated the quantity of each amino acid together with the distribution of the four kinds of rice protein. Osborne, Van Slyke, Leavenworth and Vinograd[45] have reported that the amount of various diamino acids of rice proteins is comparatively greater than that of the other cereals, and Kurosawa[46][47] has also determined the diamino acids in rice proteins. Hamada[48] has thoroughly investigated on this point. One of the present authors,[49] with Nakamura and Watanabe, has studied the distribution of amino acids of oryzenin obtained from

common and glutinous rices. There are many other investigations with regard to the distribution of amino acids, for example, those of Hoffmann,[50] Jones and Gersdorff,[51] Jones and Csonka,[52] Tadokoro, Ito and Watanabe,[53] Tadokoro, Nakamura and Watanabe,[54] Tadokoro, Tsuji and Watanabe,[55] etc.

Recently, one of the authors and Araki[13] have studied the change of amino acids in oryzenin during ripening of Akashiho and found that the monoamino acids decreased while the diamino increased, showing a relation between that tendency and the decrease of crude fat and non-proteid and increase of pure protein during the ripening of rice grains. They also found a tendency to the decrease of arginin and increase of lysin.

The authors repeated the same experiments already described in the former report, except the "Insoluble N." The latter was determined as follows:—

After the oryzenin was digested with HCl, it was filtered before distillation of the bulk of HCl. The black powdery precipitate on the filter paper was thoroughly washed with water until no chlorine reaction was observed. The nitrogen in this insoluble precipitate was estimated as "Insoluble N" by Kjeldahl's method. The results are as follows:—

Figures are the percentages of dry matter.

TABLE XIV

Distribution of Amino Acids in Oryzenin

	A.	B.	C.	D.
Total N	17.3403	17.3951	17.4606	17.2649
Insoluble N	0.1306	0.1066	0.0930	0.1059
Amide N	1.6611	1.6196	1.5715	1.5884
Melanin N	0.1402	0.1332	0.1310	0.1248
Monoamino N	9.9739	9.8261	9.3831	9.7539
Diamino N	5.4345	5.7096	6.2820	5.6919
Arginin N	3.0878	3.1517	3.7350	2.9046
Histidin N	1.2931	1.5030	1.9476	2.2204
Cystin N	0.2481	0.1829	0.0566	0.1378
Lysin N	0.8055	0.8720	0.5428	0.4291

Each Nitrogen was calculated on the base of total nitrogen as 100.

TABLE XV

Amino Acids Content, Percentages of Total Nitrogen

	A.	B.	C.	D.
Total N	100.0000	100.0000	100.0000	100.0000
Insoluble N	0.7532	0.6128	0.5326	0.6134
Amide N	9.5794	9.3107	9.0003	9.2002
Melanin N	0.8085	0.7657	0.7503	0.7229
Monoamino N	57.5186	56.4877	53.7387	56.4955
Diamino N	31.3403	32.8230	35.9781	32.9680
Arginin N	17.8071	18.1183	21.3910	16.8237
Histidin N	7.4572	8.6404	11.1543	12.8608
Cystin N	1.4308	1.0514	0.3242	0.7982
Lysin N	4.6452	5.0129	3.1087	2.4854

As is seen in the above two tables, the distribution of amino acids, *Bausteine* of Oryzenin, differs according to ripening degree. The insoluble nitrogen and amide nitrogen gradually decreased with the advance of ripening up to a definite period and then slightly increased. The melanin nitrogen seemed to decrease gradually. The monoamino nitrogen, just like insoluble and amide nitrogens, decreased and afterward increased, while the diamino acids, on the contrary, increased till that period and afterward decreased markedly. The last two tendencies affect the iso-electric point of protein as a whole.

The tendencies of individual diamino acids are seen to differ somewhat from the results of the former report. According to these tables, the arginin increased with the diamino nitrogen and then decreased markedly, while the histidin kept a constant increase during ripening. Cystin showed a sharp drop at a definite time and lysin decreased in the later part of the ripening period.

For convenience in understanding the change of each nitrogen during the ripening, ratio is taken on the basis of the nitrogen of maximum quantity as 100.

TABLE XVI

Ratios of Each Nitrogen During the Ripening

	A.	B.	C.	D.
Insoluble N	100.0	81.4	70.7	81.4
Amide N	100.0	97.2	94.0	96.0
Melanin N	100.0	94.7	92.8	89.4
Monoamino N	100.0	98.2	93.4	98.2
Diamino N	87.1	91.2	100.0	91.6
Arginin N	83.2	84.7	100.0	78.6
Histidin N	58.0	67.2	86.7	100.0
Cystin N	100.0	73.5	22.7	55.8
Lysin N	92.7	100.0	62.0	49.6

§ 7. Determination of the Iso-electric Point of Oryzenin

Heretofore, there have been reported by many authors the iso-electric point of oryzenin and various other plant proteins. But it is the authors' opinion that the physical and chemical properties of protein are always changed during ripening as is obvious in the foregoing experiments and that the iso-electric point, accordingly, is also always affected by many factors, as already mentioned in the preliminary test of the iso-electric point. It is undoubtedly reasonable to think so from the findings on the distribution of amino acids in protein molecule.

The determination of the iso-electric point was carried out by Michaelis and Nakajima's method[56] based on the principle of maximum mutual precipitation which occurred between two proteins different from each other in iso-electric point. If a greater quantity of one colloid was used than the other, the optimum precipitation point approached the iso-electric point of the former. In this experiment, the authors used the following mastix sol and protein solution:—

Mastix sol:—Five grams of mastix were dissolved in 100 cc of 96% alcohol solution and filtered. Ten cc of the filtrate were poured into 200 cc of distilled water. The milky solution was filtered and preserved for the experiment.

Protein solution:—One-twentieth gram of each oryzenin was completely dissolved in 50 cc of 1/200-n-NaOH solution.

Mixtures were made in different proportions of the two solutions with the ratios of mastix to protein, 5 : 2, 5 : 5, 5 : 15 and 5 : 20. The buffer solutions were prepared according to the following formulae.

TABLE XVII

Number	1/10-n-$NaCH_3COO$ Solution cc	1/10-n-CH_3COOH Solution cc	n-CH_3COOH Solution cc	H_2O cc	Colloid Mixture cc
1.	2.0	0.10	—	3.90	2 0
2.	2.0	0.25	—	3.75	2.0
3.	2.0	0.50	—	3.50	2.0
4.	2.0	1.00	—	3.00	2.0
5.	2.0	2.00	—	2.00	2.0
6.	2.0	4.00	—	—	2.0
7.	2.0	—	0.80	3.20	2.0
8.	2.0	—	1.60	2.40	2.0
9.	2.0	—	3.20	0.80	2.0

The pH value of the maximum precipitation was estimated by a potentiometer of Cambridge type using the quinhydron electrode and calculated by the following equation,

$$pH = \frac{0.4541 - \pi(18°)}{0.0577},$$

where π denotes the electromotive force between two electrodes.

In the following table, the signs denote respectively as follows:—

(—) No precipitation took place or a slight turbidity was observed.

(×) Precipitation formed.

(× ×) Precipitaton formed but the upper solution remained turbid.

(× × ×) Maximum precipitation formed and the upper solution became clear.

The turbidity was observed at the time indicated in the table after the whole had been mixed.

TABLE XVIII (a)

A

Colloid Ratio	Time	1.	2.	3.	4.	5.	6.	7.	8.	9.
5:2										
	10 m	—	×	×	×	×	×	×	×	×
	30 m	—	×	××	××	××	××	××	××	×
	1 h	—	××	×××	×××	×××	×××	×××	×××	×
	24 h	—	×××	×××	×××	×××	×××	×××	×××	××
5:5										
	10 m	—	—	×	×	×	×	×	—	—
	30 m	—	—	××	××	××	××	×	—	—
	1 h	—	×	×××	×××	×××	×××	××	—	—
	24 h	—	×××	×××	×××	×××	×××	×××	×	—
5:15										
	10 m	—	—	×	×	×	×	×	—	—
	30 m	—	—	×	××	××	××	×	—	—
	1 h	—	—	××	×××	×××	×××	×	—	—
	24 h	—	—	×××	×××	×××	×××	××	—	—
5:20										
	10 m	—	—	×	×	×	×	—	—	—
	30 m	—	—	×	×	××	×	—	—	—
	1 h	—	—	××	×××	×××	××	×	—	—
	24 h	—	—	×××	×××	×××	×××	×	—	—
	EMF					179.9 mv				
	pH					4.752				

TABLE XVIII (b)

B

Colloid Ratio	Time	1.	2.	3.	4.	5.	6.	7.	8.	9.
5:2										
	10 m	—	×	×	×	×	×	×	×	×
	30 m	—	×	××	××	××	××	××	××	×
	1 h	—	××	×××	×××	×××	×××	×××	×××	××
	24 h	—	×××	×××	×××	×××	×××	×××	×××	××

TABLE (b) (Continued)

Colliod Ratio	Time	1.	2.	3.	4.	5.	6.	7.	8.	9.
5:5										
	10 m	—	—	×	×	×	×	×	—	—
	30 m	—	—	×	××	××	××	×	—	—
	1 h	—	×	××	×××	×××	×××	××	—	—
	24 h	—	×××	×××	×××	×××	×××	×××	×	—
5:15										
	10 m	—	—	×	×	×	×	×	—	—
	30 m	—	—	×	××	××	××	×	—	—
	1 h	—	—	××	×××	×××	×××	×	—	—
	24 h	—	—	×××	×××	×××	×××	×	—	—
5:20										
	10 m	—	—	—	×	×	×	—	—	—
	30 m	—	—	×	××	××	××	—	—	—
	1 h	—	—	××	×××	×××	×××	—	—	—
	24 h	—	—	×××	×××	×××	×××	×	—	—
	EMF					179.2 mv				
	pH					4.764				

TABLE XVIII (c)

C

Colloid Ratio	Time	1.	2.	3.	4.	5.	6.	7.	8.	9.
5:2										
	10 m	—	×	×	×	×	×	×	×	×
	30 m	—	×	××	××	××	××	××	××	××
	1 h	—	××	×××	×××	×××	×××	×××	×××	××
	24 h	—	×××	×××	×××	×××	×××	×××	×××	××
5:5										
	10 m	—	—	×	×	×	×	×	×	—
	30 m	—	—	×	××	××	××	××	×	—
	1 h	—	—	××	×××	×××	×××	×××	××	—
	24 h	—	×	×××	×××	×××	×××	×××	××	—

TABLE XVIII (c) (Continued)

Colloid Ratio	Time	1.	2.	3.	4.	5.	6.	7.	8.	9.
5:15										
	10 m	—	—	×	×	×	×	—	—	—
	30 m	—	—	×	××	××	×	—	—	—
	1 h	—	—	××	×××	×××	××	×	—	—
	24 h	—	—	×××	×××	×××	×××	××	—	—
5:20										
	10 m	—	—	×	×	×	×	—	—	—
	30 m	—	—	×	××	×	×	—	—	—
	1 h	—	—	×	×××	×××	×××	×	—	—
	24 h	—	—	×××	×××	×××	×××	××	—	—
	EMF				160.8 mv					
	pH				5.083					

TABLE XVIII (d)

D

Colloid Ratio	Time	1.	2.	3.	4.	5.	6.	7.	8.	9.
5:2										
	10 m	—	×	×	×	×	×	×	×	×
	30 m	—	×	××	××	××	××	××	××	×
	1 h	—	××	×××	×××	×××	×××	×××	×××	××
	24 h	—	×××	×××	×××	×××	×××	×××	×××	×××
5:5										
	10 m	—	—	×	×	×	×	×	×	—
	30 m	—	—	××	××	××	××	××	××	—
	1 h	—	—	×××	×××	×××	×××	×××	×××	—
	24 h	—	××	×××	×××	×××	×××	×××	×××	—
5:15										
	10 m	—	—	×	×	×	×	×	—	—
	30 m	—	—	×	××	××	×	×	—	—
	1 h	—	—	××	×××	×××	×××	×	—	—
	24 h	—	—	×××	×××	×××	×××	×	—	—

TABLE XVIII (d) (Continued)

Colloid Ratio	Time	1.	2.	3.	4.	5.	6.	7.	8.	9.
5:20										
	10 m	—	—	×	×	×	×	—	—	—
	30 m	—	—	×	××	××	×	—	—	—
	1 h	—	—	××	×××	×××	×	×	—	—
	24 h	—	—	×××	×××	×××	×××	××	—	—
	EMF	175.0 mv								
	pH	4.837								
	Compare									
			A.		B.		C.		D.	
	pH		4.752		4.764		5.083		4.837	

As shown in the above tables, the iso-electric point of oryzenin gradually moved toward the alkaline side with the advance of the ripening period and afterward returned to acidic side, as was assumed in the preliminary test and distribution of amino acids. When the iso-electric point of oryzenin came to the alkaline side, the protein decreased in ash and phosphorus contents; but it increased in nitrogen, sulphur and free-amino nitrogen; it was optically most condensed; in distribution of amino acids, the monoamino nitrogen decreased and diamino nitrogen increased. The oryzenin, from these results, seems to be very complicated indeed during the period of ripening.

CHAPTER VIII

Chemical Properties of Starch

Chemical characters of rice starch have been thoroughly discussed by many authors, but unfortunately no one has attempted the investigation of the change of constitution of rice starch during ripening. In the authors' previous report[12] on the study of ripening they investigated the change of starch, the most important reserve carbohydrate of rice, as well as the protein, oryzenin, and found that the ash and phosphoric acid contents reached their minimum at a definite time in the ripening

period. They concluded that the starch also changes its condensation degree like oryzenin during the ripening period. Later, they[14] undertook the acetylation of the same starch to find the chemical construction, and reported that the specific rotatory power decreased until that certain time and afterward increased, while the content of acetyl radical reached the maximum at the same time and then decaeased again. The increase of acetyl radical content means the richness of hydroxyl group which can be replaced by acetyl radical in starch. All this is caused by degradation of carbohydrate as a whole. The decrease of specific rotatory power also indicates the disintegration of starch. The authors concluded from these results that the starch is least condensed at that time of ripening, and that one who wants to utilize the strach of rice must choose the proper time of harvest.

The authors undertook the following experiments with the rice starch obtained from Bôzu No. 5.

§ 1. Preparation of Starch, Its Ash and Phosphoric Acid Contents

Starch was prepared from the residue freed from oryzenin as usual. It was thoroughly washed, dried and preserved in a H_2SO_4 desiccator.

Water, ash and phosphoric acid were determined by the ordinary method.

Results are as follows:—

TABLE XIX

Water, Ash and Phosphoric Acid Contents of Starch

	A.	B.	C.	D.
Water (%)	5.3553	6.0131	5.7483	5.7169
Ash (%)	0.2190	0.2044	0.1571	0.1114
Ratio	100.0	93.3	71.7	50.9
Phosphoric Acid (%)	0.0928	0.0779	0.0763	0.0700
Ratio	100.0	83.9	82.2	75.4

As is seen in the above table, the ash and phosphoric acid content gradually decreased with the advance of ripening period, which had been observed in the former report, showing the change of chemical construction of starch during ripening.

§ 2. Saponification Value of Starch

According to Rosenthaler,[57] the saponification value of starch is due to the amylopectin in starch molecule, and is expressed by the number of mg of KOH to neutralize the phosphoric acid liberated from amylopectin by saponification. The figures are considered to be affected by the quantity or construction of amylopectin. Rosenthaler reported that the saponification value of rice starch was 4.9.

One gram of rice starch was boiled with 20 cc of absolute alcohol and equal volume of 1/5-n-KOH solution in a water-bath under a reflux condenser for one hour. After cooling it was titrated with 1/20-n-HCl solution using phenolphthalein as an indicator. A blank control experiment was run and the necessary correction was made. The results are as follows :—

TABLE XX

Saponification Value of Rice Starch

	A.	B.	C.	D.
Equivalent cc of 1/20-n-HCl.	0.710	0.730	1.035	1.360
Saponification Value	2.106	2.179	3.081	4.047
Ratio	100.0	103.5	146.3	192.2

As is seen in the above table, the saponification value of rice starch gradually increased with the ripening, showing the increase of phosphoric acid liberated from amylopectin. That the quantity of phosphoric acid thus freed increased with the ripening, notwithstanding the decrease of phosphoric acid in starch molecule as shown in Table XIX, showed the change of construction of amylopectin but not only the quantity of the latter. According to the authors' investigation[58]

moreover, amylose isolated and purified by Ling and Nanji's method[59] contained phosphoric acid exclusively as its ash component. These problems will be studied later.

§ 3. Hydrolysis of Starch by Hydrochloric Acid

Physical and chemical investigations on the decomposition of starch by acids or enzymes have been heretofore attempted by many authors, but such studies on rice starches different in degree of ripeness were entirely neglected. The authors, finding by the above experiments that these starches are different from each other in their condensation degree, wished to ascertain the velocity of their decomposition by hydrochloric acid and carried out the following experiment.

One-half gram of each rice starch was weighed in an Erlenmeyer flask of about 200 cc capacity. One hundred cc of 2% HCl were added and the whole was warmed in a boiling water-bath for the purpose of inversion. Ten cc of the solution were pipetted out at intervals of 10, 20, 40, 80 and 160 minutes and the amount of glucose was estimated by Bertrand's method.

The figures in the following table are the quantity of glucose in number of mg produced from one gram of rice starch.

TABLE XXI

Amount of Glucose Formed from Starch

	A.	B.	C.	D.
10 Minutes	20.0677	26.7148	28.5706	24.6987
Ratio	100.0	133.1	142.4	123.1
20 Minutes	48.9485	53.4625	56.8911	55.6299
Ratio	100.0	109.2	116.2	113.6
40 Minutes	80.9038	79.9837	88.8030	87.3420
Ratio	100.0	98.9	109.8	108.0
80 Minutes	106.4624	105.3111	107.2668	106.0456
Ratio	100.0	98.9	100.8	99.6
160 Minutes	107.0755	107.5661	109.4057	106.8776
Ratio	100.0	100.5	102.2	99.8

According to the above table, the amount of glucose formed from starch by inversion for a definite time increased with the ripening period and afterward decreased. The starch of the maximum formation of glucose is considered as nearly completely condensed as possible. The rate of formation, on account of its condensation, is prominent during the earlier part of decomposition, decreasing the ratio by longer manipulation.

§ 4. Preparation of Soluble Starch and Acetylated Starch

The authors found, in their first report, that the hydroxyl group which can be replaced by acetyl radical reached its maximum at a definite time of ripening, and the specific rotatory power of acetylated starch reached its minimum at that period. They concluded, from these facts, that the starch is disintegrated to the greatest degree at that period and that afterward it was again somewhat condensed. The authors, on the other hand, recognized in the above experiments that the properties are quite opposite, that is, the starch seemed to be extremely condensed at a definite period of ripening. They had to study on this point further and prepared the soluble and acetylated starches.

A. Soluble Starch

The soluble starch was prepared as follows from rice starch different in degree of ripeness:—

Fifty grams of starch were soaked in 200 cc of 7.5% hydrochloric acid and the whole was shaken vigorously several times a day and then allowed to stand for ten days at room temperature. It was washed with distilled water by decantation method until no chlorine reaction was observed. It was again washed with 70% alcohol, filtered with a silk sieve, and at last washed with absolute alcohol and ether. It was preserved in a H_2SO_4 desiccator. Water, ash and phosphoric acid contents were estimated as usual. The results are as follows:—

TABLE XXII

Water, Ash and Phosphoric Acid Contents of the Soluble Starch

	A.	B.	C.	D.
Water (%)	5.9243	9.0059	5.5854	8.8213
Ash (%)	0.1437	0.0982	0.0805	0.0711
Ratio	100.0	68.3	56.0	49.5
Phosphoric Acid (%)	0.0133	0.0115	0.0075	0.0095
Ratio	100.0	86.5	56.4	71.4

According to the table, the ash and phosphoric acid contents of soluble starch are markedly less than those of the original starches due to the demineralization process of hydrochloric acid. The ash content decreased continuously but the phosphoric acid slightly increased after it reached the minimum quantity at a certain period.

B. Acetylated Starch

The acetylated starch was prepared by the method already described in the former report. The content of acetyl radical was determined by Wenzel's method[69] and the specific rotatory power in pyridin solution was estimated by a Haensch-Schmidt's half-shadow polariscope.

The results are as follows:—

TABLE XXIII

Specific Rotatory Power and Acetyl Radical Content of Acetylated Starch

	A.	B.	C.	D.
Water (%)	9.8433	7.9114	8.7609	9.1811
Specific RotatoryPower Reading	5.02	5.12	5.42	5.22
$[\alpha]_D$	130.2690	132.8640	140.6490	135.4590
Ratio	100.0	102.0	108.0	104.0
Acetyl Radical Equivalent cc of Sulphuric Acid	6.16	6.10	5.93	6.01
C_2H_3O (%)	52.5974	52.0851	50.6344	51.3166
Ratio	100.0	99.0	96.3	97.6

As is seen in the above table, the specific rotatory power of acetylated starch gradually increased with the ripening, reaching the maximum at a definite period and afterward decreasing. The content of acetyl radical showed its minimum value at that period, on the contrary, and then increased slightly. Elevation of specific rotatory power implies the advance of condensation of starch and the depression of acetyl radical content shows the decrease of hydroxyl group as already mentioned, which is nothing but the advance of the condensation. The starch is therefore, from these points of view, most nearly altogether polymerized at that period when the specific rotatory power increased and the acetyl radical content decreased. This will also be obvious from the results of decomposition of starch by hydrochloric acid and from phosphoric acid content of soluble starch.

All these present results seem to be quite opposite to those of the previous report, but it is the authors' opinion that these differences are mainly due after all, not only in the rice starch but also in all other plant constituents, to the harvest period of the sample; and that each individual constituent is concerned with the other, sometimes condensed and sometimes depolymerized, to perform the vital phenomena.

CHAPTER IX

Summary

The results of the above experiments may be briefly summarized as follows:—

1. The actual weight of unhulled and hulled rices was increased till a certain period but always afterward decreased. The specific gravity of unhulled rice increased at first and afterward decreased while that of hulled rice at first decreased and then increased. The specific gravity seemed likely to be most affected by the water content. The sharp drop of unripened and half-ripened grains may perhaps be due to the dead ripeness.

2. The water content of hulled rice increased at first and then decreased, showing a close relation with the specific gravity. The decrease of dry substances in 1000 grains is probably due to the reversibility of physiological functions during ripening. The ash and phosphoric acid contents decreased for a while and crude fat also showed a tendency to decrease. As to the total nitrogen, it showed no de-

finite tendency during the whole period of ripening, but the ratio of pure protein to crude protein, constantly increased.

3. In determination of the distribution of the four kinds of rice proteins, the nitrogen of water-soluble form gradually decreased with the ripening time while the nitrogens of NaCl-soluble and alcohol-soluble forms always increased during that period. That of the alkali-soluble was inconstant showing a slight decreasing tendency. All these changes are quite opposite to those seen in germination.

4. Quite opposite to the increase of the ratio of pure protein to crude protein, there appeared a sharp decrease of non-proteid substances. The free-amino nitrogen in the non-proteid substances sharply decreased with the free amino-nitrogen in protein substances. From this fact, it is obvious that the protein substances are formed from non-proteid substances with the accompaniment of the formation of higher proteins from the lower. The decreasing velocities of both free-amino nitrogens, moreover, suggest that the formation of protein substances from the non-proteid substances occurs more rapidly than the polymerization of higher proteins from the lower. The protein decreases its solubility with the advance of ripening as a whole.

5. The reducing sugar of the hulled rice gradually decreased with the ripening period, showing also a relation with water content. On the contrary, the quantity of so-called dextrin and that of starch increased.

6. Oryzenin, the principal protein of hulled rice, decreased its ash and phosphorus contents at a definite time in the ripening period, while the content of nitrogen, sulphur and free-amino nitrogen increased at that period. The quantity of hydrochloric acid required to cause the maximum turbidity and surface tension of the protein solution also decreased at the same period, thus placing the iso-electric point of oryzenin on the alkaline side, and the specific rotatory power increased. The amount of free-amino nitrogen liberated from protein molecules by the action of ultraviolet rays gradually increased till that certain period while the rate of liberation decreased on account of its high condensation. As to the distribution of amino acids, the monoamino nitrogen decreased and the diamino nitrogen increased till that time. Arginin also increased with the diamino nitrogen and afterward decreased while histidin increased continuously. The lysin nitrogen decreased at the later part of ripening and the cystin nitrogen at first decreased and then increased. The insoluble and the amide nitrogen gradually de-

creased with the advance of the ripening period, slightly increasing afterward, and the melanin nitrogen constantly decreased. From these results, it seems that oryzenin is probably at its highest degree of condensation at the above mentioned period of ripening, and then disintegrates again.

7. The ash and phosphoric acid contents of rice starch decreased gradually with the ripening period while the saponification value increased showing the change of quantity or construction of starch molecule. The amount of glucose formed from starch by hydrolysis with hydrochloric acid reached its maximum at a definite time of the ripening period. The ash content of soluble starch decreased gradually like that of the original starch while the phosphoric acid slightly increased in the last period. The specific rotatory power of acetylated starch reached its maximum at the definite time and the content of acetyl radical its minimum, showing the least quantity of hydroxyl group replaced by the acetyl radical, by condensation. These results suggest that the starch is also in its most condensed form at that period of ripening.

After all, all these reserve materials in cereal grains are subjected to vital phenomena, sometimes condensed and sometimes disintegrated according to the urgency of the case.

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