Physico-chemical Studies on the Specificity of Proteins of Different Rice Varieties and Subvarieties.

By

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Introduction.

Almost every study concerning the specificity of protein of different kinds of plant and animals or the specificity of their expressed juice has been carried out from the standpoint of physiological reactions. Previous studies will be briefly reviewed in order to connect the authors' study with them.

The precipitin, anaphylaxis, agglutinins and opsonins were physiological reactions. They were adopted for the determination of animals[1] or plants[2]. However, when these experiments were carried out with serum, blood corpuscle, plant cell sap or plant extract they were found to contain other constituents or to be mixtures of many kinds of protein. Wells and Osborne who were the only ones to experiment with pure globulins of seeds of hemp, flax, squash and other plants, found a marked difference among them and suggested the name "Artspezifität" for this condition. This difference has been verified recently from the study of physico-chemical reactions by many investigators. Amongst them Radsmann and Hamburger[3] described the different phenomena of discharge of blood corpuscle by different species of animals. Abderhalden[4] observed a difference of sinking velocities of blood corpuscle in serum among different species of animals and stated that the velocity of cow's corpuscle was the greatest and that of rabbit's situated between those of horse's and man's. Brown differentiated the "Artspezifität" by the system of crystalization of haemoglobin. Straub and Meier[5] stated that the red blood corpuscles of different species of mammalia possessed different isoelectric points respectively.

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Though there are no investigations about the "Artspezifität" of pure protein from the physico-chemical standpoint, the authors are able to abstract from previous experiments the following information related to this point. The results of Kossel, Kutscher and Dakin(6) on protamin and histone, of Osborne, Abderhalden and Hopkins on albumin and globulin, and of Schultze, Abderhalden and Osborne on globulin and other proteins, indicated that the difference of amino acids contents of their hydrolysis products was caused by the difference of species of plants and animals which were the origin of the proteins. A constitutional difference of protein derived from the difference of species of plants or animals was observed from a different condition of the separation of those amino acids which are considered very important in the chemistry of nutrition. These observations were, however, carried out with the proteins of those kinds of plants or animals whose systems are so unlike that, notwithstanding the same treatment in the preparation of them, there would be found a difference of their constitutions. The authors, therefore, intending to find out the difference caused by varieties, or the "Artspezifität", of pure oryzenins prepared in the same manner, carried out the following examinations with different varieties of rice. The authors(7) were able to find out the "Artspezifität" of oryzenins of the common and the glutinous rices and to justify the existence of the physico-chemical specificity of the common rice oryzenins according to the varieties. The differences between the common and the glutinous rice oryzenins are abstracted here briefly from the authors' previous report(7). The differences between the oryzenins of different subvarieties of common rice are stated in the present report.

I. The Differences between the Oryzenin of the Common and the Glutinous Rice.

The conclusions of the authors' former investigations are introduced here briefly. (The readers should refer to the Journal of the College of Agriculture, Hokkaido Imperial University. Vol. XIV, Pt. 3).

(A) The physico-chemical differences.

(1) The abundance of asymmetric carbon, and of the COR group in the molecule and the high degree of saturation cause the higher

specific rotatory power of the alkali solution of the common rice oryzenin compared with the glutinous.

(2) The common rice oryzenin is less easily soluble in alkali solution than the glutinous. This is due to the greater abundance of $R.CH\langle{}^{NH}_{CH_2}\rangle CO$ group in the former compared with the $R.CH\langle{}^{NH_2}_{COOH}$ group in the latter.

(3) For the formation of maximum precipitation of the oryzenin alkali solution by an addition of HCl solution, a smaller quantity is sufficient with common than with glutinous. This is thought to be due to the lack of alkali salt combined with the carboxyl group, in the glutinous.

(4) Employing Cohnheim's method, the quantity of HCl combining with oryzenin of the common rice was greater than that of the glutinous. This is due to the abundance of amino group which combines with HCl more easily in the former than in the latter.

(5) That the iso-electric point of the common rice oryzenin is near to the basic side and that of the glutinous is near to acidic side indicates that in the former the amino group predominates over the carboxyl group while the reverse is true in the latter.

(6) The refractive index of alkali oryzenin solution was observed. The index of the common is higher than that of the glutinous. This fact indicates that the former is constituted more densely than the latter.

(7) The velocity of fall of viscosity of the common rice oryzenin alkali solution caused by the lapse of time is greater than that of the glutinous. This seems due to the decrease of the ionized particles and the increase of the aggregated particles, i.e. the non-ionized particles.

(B) The chemical differences.

(1) The greater quantity of nitrogen in the elemental composition of the common rice oryzenin is the reason of its basic nature. The higher ratio of carbon to oxygen of the glutinous rice oryzenin as compared with that of the common rice oryzenin shows the predominance of acidity and of carboxyl groups in the former.

(2) The ash content of the common rice oryzenin is higher than that of the glutinous. There are strange tendencies that the higher sulphur content and lower phosphorous content are found in the common rice oryzenin and vice versa in the glutinous rice oryzenin.

(3) The determination of amino acids after Van Slyke indicates that the common rice oryzenin predominates in respect to quantities of ammonia, arginin and lysin nitrogen and glutinous in quantities of monoamino, histidin and cystin nitrogen. The quantity of free amino nitrogen in the common predominates over that of the glutinous.

(4) The glutinous rice oryzenin was decomposed more rapidly than the other by pancreatin. This is because the polymerization degree of the common is more complicated than the glutinous.

(5) The oryzenin alkali solution increased in quantity of free amino nitrogen and turbidity by exposure to ultra-violet ray. The velocity of increase of the glutinous was greater than that of the common.

(6) Jodoprotein was prepared after Blum and Straus and it was observed that the glutiuous combines with more iodine than common. This is due to histidin that possesses the imidazol ring.

(7) The silver salts of oryzenin was prepared. In quantity of nitrogen the common predominated while in that of silver the reverse held. This is due to the fact that the glutinous contains more carboxyl groups than the common.

(8) An acetylation of oryzenin was carried out. The nitrogen content of the common rice acetyl oryzenin predominates over that of the glutinous, but in regard to the acetyl group the reverse holds. This seems to be due to the abundance of hydroxyl group which is replaced by acetyl group in the latter.

(9) Comparing common and glutinous rice acetyl oryzenins there was a marked difference in the quantities of base, pyrrol, pyrolidin and glyoxalin, pyrrolic acid, proteol and H_2O-K_2CO_3 soluble substance produced by hydrolysis. The common was prodominant in quantities of base, pyroll, pyrolic acid and H_2O-K_2CO_3 soluble substance while the glutinous showed large quantities of pyrolidin and glyoxalin, and proteol. The glutinous rice oryzenin predominated in the quantity of histidin over the common. Comparing the constitution of histidin and glyoxalin, the identity of the two predominancies of histidin and glyoxalin contents in the glutinous was easily accepted.

II. The "Artspezifität" of the Common Rice Oryzenins.

As already stated, the differences between the common and the glutinous rice oryzenins are marked by their physico-chemical properties.

To the common rice oryzenin the authors gave the name of α-oryzenin and classified it from the glutinous rice oryzenin which was named β-oryzenin. The existence of two such types of oryzenins may be said to be one of the "Artspezifität." It must be a very interesting problem to study the same fact among the subvarieties of common rice.

Amongst the many hundred varieties of common rice, two types are distinguished generally in all parts of Japan, those employed for the fermentation of *sake* and those not so employed. For example, in Settsu, *Yamadaho* or so-called "*Banshu-Ao*" is used for fermentation and not *Shinriki* or so-called "*Banshu-Aka*"; in Tajima, *Kokuryomiyako* is selected and not *Mino*; in Akita, *Kamenoo* and not *Toyokuni*; in Etchu, *Maezawa* and not *Ohba*; in Echigo, *Bantakamiya* and *Yonemitsu* and not *Ishijiro.* That these varieties selected for fermentation must have a general "Artspezifität" is not hard to conjecture. The reason of the present investigation starts from this consideration.

(A) Materials.

The materials of the present investigation were all common rices produced during the years 1923 to 1925 in Kyushu, Kansai, Hokuriku and Tohoku provinces. The rices for fermentation are paralleled with the rices not for fermentation respectively.

TABLE I.

Varieties of common rice used throughout these experiments in determining the specificity of oryzenin.

No. of sample	Origin	Variety name	Fermentation
1	Bizen	Aka Unhulled	Unsuitable
2	Harima, Kasai Gun	Yamadaho ,,	Suitable
3	,,	Shinriki ,,	Unsuitable
4	Tajima	Kokuryomiyako Hulled	Suitable
5	,,	Mino Hulled	Unsuitable
6	Ugo, Yokotemachi	Toyokuni Unhulled	,,
7	,, ,,	Kamenoo ,,	Suitable
8	Etchu	Ohba ,,	Unsuitable
9	,,	Maezawa ,,	Suitable
10	Echigo	Ishijiro ,,	Unsuitable
11	,,	Bantakamiya ,,	Suitable
12	,,	Yonemitsu ,,	,,
13	Fukuoka	Shinriki Hulled	Unsuitable
14	,,	Itoshima ,,	Suitable
15	Kumamoto	Shinriki Unhulled	Unsuitable
16	,,	Omachi ,,	Suitable

These rices without washing with water, were powdered by means of a stone mill, sieved through a mesh of 0.5 mm diameter and stored in a sealed bottle for use.

(B) The preparation of oryzenin and its ash content.

Two hundred and fifty grams of the defatted and dried powdered material were added to 1500 ccm of 10 % NaCl solution, and shaken for thirty minutes by means of a shaking machine. The mixture was then stored in an ice box for one night. The supernatant upper solution was decanted off; the remaining solution was filtered and the residue was then washed well with water until there was no chlorine reaction in the washing. A NaOH solution was then added to the residue to make 0.2 % of alkali and volume of 1500 ccm. The mixture was shaken for thirty minutes, stood still over night in an ice box, and the extracted solution was collected by a decantation and a filtration. The solution was then filtered through a layer of paper pulp by suction to free from any traces of starch that react with iodine. The clear solution thus obtained was acidified slightly by a dilute acetic acid solution, whereupon a precipitation of protein occurred. The upper liquid was decanted off; the precipitate was then centrifuged to remove water, hung in 70 % alcohol solution, stirred very well and the solution was removed from the precipitate by means of a centrifugal machine. The same treatment was repeated until no more protein was dissolved into the alcohol solution. The protein was then washed with absolute alcohol and ether in the usual way, dried under diminished pressure over sulphuric acid, and powdered with an agate morter. The protein thus obtained did not contain any one of the water soluble, 10 % NaCl solution soluble or 70 % alcohol solution soluble proteins. The water and the ash content of the oryzenins are as follows.

TABLE II.

Water and ash contents of oryzenins prepared from the varieties suitable and unsuitable for fermentation.

Origin	Variety name	Moisture %	Ash %	Ash % (H_2O free)
Bizen	Aka Unhulled	8.6300	0.3200	0.3500
Harima	Yamadaho ,,	10.0701	0.2400	0.2660
,,	Shinriki ,,	8.1906	0.2800	0.3050
Tajima	Kokuryomiyako Hulled	9.3190	0.3600	0.3970

Origin	Variety name	Moisture %	Ash %	Ash %(H_2O free)
Tajima	Mino Hulled	9.1790	0.3800	0.4180
Ugo	Kamenoo Unhulled	2.7830	0.4400	0.4520
,,	Toyokuni ,,	2.7550	0.5600	0.5750
Etchu	Maezawa ,,	8.4279	0.2200	0.2402
,,	Ohba ,,	8.6707	—	—
Echigo	Ishijiro ,,	9.0164	0.2400	0.2638
,,	Bantakamiya ,,	6.5041	0.2200	0.2354
,,	Yonemitsu ,,	6.3953	0.2200	0.2350
Fukuoka	Shinriki Hulled	7.9409	—	0.3459
,,	Itoshima ,,	8.8178	—	0.2742
Kumamoto	Shinriki Unhulled	7.6779	—	0.4874
,,	Omachi ,,	7.3554	—	0.4102

From the table, the ash content of the oryzenin of the rice suitable for fermentation is always less than that of the unsuitable in case they are produced in the same part. The ash content of the glutinous rice oryzenin was 75–80 % of the common as already reported[7]. The fact is thought to be due to the constitutional difference of the two kinds of oryzenins. The same comparison is again calculated in Table III.

TABLE III.

Comparison between ash contents of various oryzenins obtained from rice varieties suitable and unsuitable for fermentation.

Name	Yamadaho	Shinriki Aka	Kokuryomiyako	Mino	Kamenoo	Toyokuni
Ash	0.266	mean 0.327	0.397	0.418	0.452	0.575
Ratio	ca. 81.2	100.0	ca. 95.0	100.0	ca. 78.7	100.0
Name	Maezawa	Ohba	Ishijiro	Bantakamiya	Yonemitsu	
Ash	0.2402	—	0.2638	0.2354	0.2350	
Ratio	—	—	100.0	ca. 89.25	ca. 89.08	
Name	Shinriki	Itoshima	Shinriki	Omachi		
Ash	0.3459	0.2742	0.4874	0.4102		
Ratio	100.0	ca. 84.5	100.0	ca. 84.5		

It can be seen that the ash content of the oryzenin of the rice suitable for fermentation is always 79–95 % of that of the unsuitable.

(C) The sulphur and the phosphorus content of the oryzenin.

The sulphur was estimated by Denis-Benedict's method and the phosphorus by a molybdic-NaOH titration method described by Hawk[8]. The results are indicated in Table IV.

TABLE IV.

Sulphur and phosphorus contents of oryzenins prepared from various rices which are suitable or unsuitable for fermentation.

No. of sample	For fermentation	Sulphur content		Phosphorus content	
		Air dry %	H_2O free %	Air day %	H_2O free %
1	Unsuitable	—	0.9586	0.09983	0.1087
2	Suitable	—	0.8284	0.09443	0.1050
3	Unsuitable	—	1.0400	0.11570	0.1265
4	Suitable	—	0.8120	0.10793	0.1189
5	Unsuitable	—	1.0905	0.11570	0.1274
6	,,	—	0.7039	0.10570	0.1108
7	Suitable	—	0.6491	0.10390	0.1069
8	Unsuitable	—	—	—	—
9	Suitable	0.9541	1.0418	0.10570	0.1154
10	Unsuitable	0.8827	0.9702	0.12952	0.1423
11	Suitable	0.9019	0.9647	0.11333	0.1222
12	,,	0.9047	0.9665	0.11603	0.1240
13	Unsuitable	—	0.8664	—	0.0959
14	Suitable	—	0.8321	—	0.0824
15	Unsuitable	—	0.8455	—	0.1002
16	Suitable	—	0.7996	—	0.0877

The oryzenin of the unsuitable rice seems to contain more sulphur and phosphorus than that of the suitable, however, the difference is not very remarkable.

(D) The rotatory power of the oryzenin alkali solution.

The rotatory power[9] 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. Though the detailed connection between the rotatory power and the structure is not distinct, the compounds of the same system enlarge their rotatory power by an increase of the side chains or of CH_3, H and OH groups or markedly by an existence of COR group. The authors, therefore, as in the case of the difference of the common and the glutinous rice oryzenins, carried out the following examinations.

One-tenth gram of water free oryzenin was dissolved in 10 or 15 ccm of $^1/_{10}$ normal or $^1/_{25}$ normal NaOH solution and the specific rotatory power of the solution was observed by means of a Haensch and Schmidt's half shadow saccharimeter.

TABLE V.

Rotatory power of the alkali solution of various oryzenins.

	No. 2	No. 3	No. 4	No. 5	No. 8	No. 9
Ventzke degree	-3.50	-4.00	-3.40	-3.90	-3.80	-2.60
Sp. rot. power	-67.28	-75.61	-64.88	-74.41	-88.30	-67.47

	No. 10	No. 11	No. 12	No. 13	No. 14	No. 15	No. 16
Ventzke degree	-1.60	-1.30	-1.40	-1.45	-1.25	-1.50	-1.30
Sp. rot. power	-83.04	-67.47	-72.66	-75.25	-64.87	-77.85	-67.47

The specific rotatory power of the rices suitable for fermentation is lower than that of the unsuitable rices, being between 64.88 and 72.66 while that of the latter is between 74.41 and 88.30. This is the remarkable difference between the two oryzenins.

(**E**) As stated in the former report[7], the isoelectric point of oryzenin is to be inferred from the change of surface tension of its alkali solution by an acid titration. One-tenth gram of water free oryzenin was dissolved in 10 ccm of $^1/_{50}$ normal NaOH solution. The solution was stood still over a night for the completion of solution. One ccm of the solution was diluted with 9 ccm of redistilled water, to which mixture was then added $^1/_{100}$ normal HCl solution. The surface tension of the mixture was measured by Noüy's torsionmeter. The figures of Table VI are calculated on the basis of water whose surface tension is 75 dyn/sq. cm.

TABLE VI.

Surface tension of oryzenin alkali solutions remaining after the addition of stated quantities of HCl.

Sample \ ccm of HCl added	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8
No. 1	58.2	58.2	58.2	58.0	59.0	59.5	**60.1**	59.5	58.7
No. 2	60.1	60.1	59.5	60.8	**62.0**	60.8	60.8	59.5	—
No. 3	59.5	59.5	59.9	59.0	60.1	**62.0**	60.8	60.8	—
No. 4	60.1	60.1	59.5	59.0	**61.2**	60.1	59.5	—	—
No. 5	60.1	60.1	59.5	59.5	59.5	60.1	**62.0**	60.1	—
No. 8	60.1	60.1	59.5	59.0	59,5	60.1	**62.0**	60.1	59.5
No. 9	59.5	59.5	58.2	59.0	60.1	**62.0**	60.1	60.1	59.5

ccm of HCl added / Sample	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8	1.9
No. 6	60.1	60.1	59.5	59.5	59.5	59.0	59.5	**61.2**	60.8
No. 7	60.1	60.1	59.5	59.5	59.0	59.5	**61.2**	60.8	60.1
No. 10	60.1	60.1	59.5	59.5	59.5	60.1	60.8	**61.2**	—
No. 11	60.1	60.1	59.5	59.5	60.1	60.8	**61.2**	60.8	—
No. 12	60.1	60.1	59.5	59.5	60.1	60.8	**61.2**	60.8	—
No. 15	60.1	60.1	59.5	60.7	60.8	61.0	**62.0**	61.0	60.5
No. 16	60.1	60.1	59.6	60.8	61.0	**62.0**	61.0	60.8	—

By the table, it is observed that the oryzenin of the rices suitable for fermentation indicate a maximum surface tension at a smaller titration number of HCl solution than that of the unfermentable.

(**F**) Another factor[7] that is suitable for the determination of the isoelectric point of oryzenin is the change of the turbidity of its alkali solution by an acid titration. By the same treatment as in the former experiment, the turbidity of the alkali solution was determined by means of a Dubosque colorimetric nephelometer. The figures are the length of the solution and the smaller the figure, the higher its turbidity.

TABLE VII.

Turbidity of oryzenin alkali solutions indicated by depths remaining after the addition of stated quantities of HCl.

ccm of HCl added / Sample	1.0	1.1	1.2	1.3	1.4	1.5	1.6	1.7	1.8
No. 1	60 —	— 31	60 —	— 22	60 —	— 14	**35** —	— 60	—
		60 —	— 45	60 —	— 50	60 —	— **57**		
No. 2	60 —	— 37	60 —	— 22	**52** —	— 60		—	—
		60 —	— 48	60 —	— **11**	48 —	— 60		
No. 3	60 —	— 38	60 —	— 31	60 —	— **15**	34 —	— 60	—
		60 —	— 37	60 —	— 24	**52** —	— 60		
No. 4	60 —	— 44	60 —	— 22	**54**	60		—	—
		60 —	— 37	60 —	— **13**	44 —	— 60		
No. 5	60 —	— 55	60 —	— 38	60 —	— 25	**15** —	— 60	—
		60 —	— 42	60 —	— 25	60 —	— **18**		
No. 8	60 —	— 55	60 —	— 52	60 —	— 41	**55** —	— 60	
		60 —	— 43	60 —	— 37	60 —	— **28**	50 —	— 69
No. 9	60 —	— 55	60 —	— 43	60 —	— **18**		—	—
		60 —	— 35	60 —	— 35	**50** —	— 60		

Sample \ ccm of HCl added	1.1	1.2	1.3	1.4	1.5	1.55	1.60	1.65	1.70
No. 6	60	48	60	58	60	18	60	**41**	
		60	42	60	22	60	15	**36**	60
No. 7	60	44	60	41	60	32	**45**	60	—
		60	55	70	49	60	**15**		
No. 10	—		60	58	60	45	60	**16**	
		60	55	60	52	60	42	**32**	60
No. 11	—		60	50	60	35	**40**	60	—
		60	52	60	42	60	**18**		
No. 12	—		60	48	60	28	**45**	60	—
		60	58	60	32	60	**18**		
No. 15	—	60	52	60	35	60	50	60	30
			60	55	60	40	60	40	—
No. 16	—	60	30	60	32	**45**	60		
			45	60	55	**13**	53	60	

It is also observed that the oryzenin of rice suitable for fermentation shows a maximum turbidity at a smaller titration number of HCl solution than that of the unsutable.

It can, therefore, be seen that the isoelectric point of the oryzenin of the rice suitable for fermentation is more acidic than that of the unsuitable and naturally that the latter is more basic than the former.

(G) The silver salts of oryzenin.

The combination and the salt formation of protein and its decomposition products with heavy metal have been reported hitherto by many investigators[10]. The silver salt of casein made by an addition of silver nitrate solution to ammonium solution of casein was investigated by Hirschstein[11]. The authors[12] have already observed in the case of silver contents of the salt the difference between the common and the glutinous rice oryzenins. Under the same conditions, the following examination was carried out with the oryzenins of the rices suitable and unsuitable for fermentation. Eight-tenths gram of oryzenin was dissolved in 50 ccm $^1/_{25}$ normal NaOH solution. The solution was neutralized with nitric acid using phenolphthalein as an indicator. Eight ccm of 20 % silver nitrate solution was added to the mixture to form a precipitate. The precipitate was filtered, washed, dried and weighed in the usual way. Using 0.2 g of the dried dark brownish precipitate total nitrogen was estimated according to Kjeldahl's method. Four-tenths gram of the substance was fused with a fusing mixture, dissolved in nitric acid and the silver of the solution was determined as silver chloride by the gravimetric method. The results are tabulated in Table VIII.

TABLE VIII.

Nitrogen and silver content of silver salts of oryzenin. The ratio between them.

Sample	No. 1	No. 2	No. 3	No. 4	No. 5	No. 10	No. 11	No. 12
Nitrogen %	16.525	15.836	14.684	15.492	13.771	16.771	11.705	15.836
Silver %	4.2906	5.1939	3.9143	5.0434	2.9357	5.6456	5.3258	5.7961
Ag/N × 100	25.9	32.7	26.6	32.5	21.3	32.5	45.5	36.6

The oryzenin of the rice suitable for fermentation combines with larger quantities of silver than that of the unsuitable, showing the predominance of the quantity of carboxyl group of the former in its molecule as compared with the latter.

(H) The kinds and quantities of amino acids found in oryzenin.

Employing the same treatment as described in former report[7], the amino acids and their percentages found in the oryzenins of the two classifications of rice were determined and calculated.

TABLE IX.

Amounts of various amino acids found in oryzenins derived from rice both suitable and unsuitable for fermentation.

Sample	Total N	Ammonia N	Melanin N	Monoamino N	Diamino N	Arginin N	Histidin N	Lysin N	Cystin N
No. 1	18.1082	1.7363	0.3999	10.1728	5.8002	3.5551	0.8691	1.1674	0.2089
No. 2	17.0941	1.4733	0.3225	9.7254	5.5296	3.2236	1.2171	0.8554	0.2335
No. 3	17.8863	1.6542	0.3618	9.9805	5.8896	3.7209	1.0388	0.9240	0.2061
No. 4	17.6106	1.5610	0.3296	9.8560	5.7644	3.3894	1.4339	0.7096	0.2351
No. 5	18.4566	1.6130	0.3823	10.3167	6.1447	3.6192	1.3827	0.9626	0.1852
No. 6	18.1949	1.7804	0.4773	9.0727	6.5345	4,2566	1.2632	0.8179	0.1968
No. 7	17.0663	1.6137	0.4001	8.8474	6.2051	3.9851	1.4165	0.5750	0.2285
No. 8	16.6869	1.7022	0.3627	9.8154	4.8066	2.9018	1.2687	0.4539	0.1822
No. 10	18,3021	1.9718	0.4382	10.0508	5.8412	4.0184	1.0020	0.6354	0.1866
No. 11	17.1988	1.8456	0.4264	9.7733	5.1555	3.2064	1.3000	0.4416	0.2055
No. 12	17.036	1.7747	0.3549	9.9590	4.9475	3.0664	1.2525	0.4380	0.1906
No. 13	17.6532	2.1298	0.3155	8.5417	6.6667	3.3188	1.5477	1.6247	0.1755
No. 14	17.2081	1.8632	0.2825	9.8321	5.2303	3.3910	1.1036	1.5493	0.1865
No. 15	17.8056	1.9666	0.3682	8.7462	6.7246	3.9886	1.1178	1.6182	0.1844
No. 16	17.3399	1.7756	0.3363	9.1642	6.0639	3.6222	0.8435	1.4102	0.1880

TABLE X.

Ratios between the various amino acids.

Sample	Total N	Ammonia N	Melanin N	Mono-amino N	Di-amino N	Arginin N	Histidin N	Lysin N	Cystin N
No. 1	100.00	9.5889	2.2084	56.1779	32.0308	19.6329	4.7995	6.4468	1.1519
No. 2	100.00	8.6188	1.8866	56.8933	32.3477	18.8580	6.1200	5.0041	1.3660
No. 3	100.00	9.2484	2.0228	55.7996	32.9292	20.8031	5.8078	5.1660	1.1519
No. 4	100.00	8.6931	1.8716	56.7022	32.7376	18.6785	8.1422	4.0294	1.3145
No. 5	100.00	8.7394	2.0731	55.8971	33.3228	19.6092	7.4916	6.9426	1.0034
No. 6	100.00	9.7302	2.6234	48.9189	35.9683	23.3919	6.9426	4.4408	1.0816
No. 7	100.00	9.4555	2.3444	51.2554	36.3588	23.3507	8.3000	3.3692	1.3389
No. 8	100.00	10.2008	2.1735	58.8204	28.8046	17.3897	7.6029	2.7201	1.0919
No. 10	100.00	10.7730	2.3924	54.9718	31.9201	21.9545	5.4745	5.4715	1.0195
No. 11	100.00	10.7310	2.4792	56.8255	29,9643	18.6432	7.5587	2.5676	1.1949
No. 12	100.00	10.4168	2.0832	58.4885	29.0414	17.9995	7.5521	2.5710	1.1188
No. 13	100.00	12.0643	1.7872	48.3848	37.7655	18.7995	8.7670	9.2032	0.9941
No. 14	100.00	10.3216	1.6419	59.1433	30.3744	13.8946	6.4133	9.0032	1.0832
No. 15	100.00	11.9448	2.0679	49.1205	37.7667	22.4008	6.2878	9.0882	1.0356
No. 16	100.00	10.2400	1.9389	52.8504	34.9708	20.3127	4.8645	8.1327	1.1486

A definite difference in the amino acids from the oryzenins of the two types of rice is observed from the table, i.e. the suitable for fermentation is deficient in quantities of ammonia, melanin and lysin nitrogen and is predominant in those of histidin, and cystin nitrogen while the reverse is observed in the unsuitable.

(I) Free amino nitrogen of oryzenin.

For the purpose of the investigation Sörensen's method(14) was adopted rather than Van Slyke's because of Wilson's statment(13) that the former is preferable. The method has been described in former report(7).

TABLE XI.

Free amino nitrogen found in the various oryzenins.

Sample	ccm of 1/5 normal HCl	Amino nitrogen (g.)	% of protein	% of total N
No. 1	0.55	0.00154	2.0963	11.7313
No. 2	0.45	0.00126	1.7514	10.2456
No. 3	0.50	0.00140	1.9163	10.5826
No. 4	0.45	0.00126	1.7361	9.8583
No. 5	0.50	0.00140	1.9269	10.4402
No. 6	0.48	0.00134	1.7606	6.6763

Sample	ccm of $\frac{1}{5}$ normal HCl	Amino nitrogen (g.)	% of protein	% of total N
No. 7	0.43	0.00121	1.5502	9.0775
No. 8	0.65	0.00182	2.2750	12.7630
No. 9	0.55	0.00154	1.9250	11.5360
No. 10	0.90	0.00252	3.1500	17.6052
No. 11	0.75	0.00210	2.6250	15.2633
No. 12	0.70	0.00196	2.4500	14.3813
No. 13	0.53	0.00148	1.855	10.8037
No. 14	0.48	0.00134	1.680	9.7628
No. 15	0.48	0.00134	1.680	9.4352
No. 16	0.43	0.00120	1.505	8.6794

The quantity of free animo nitrogen found in the oryzenin of rice suitable for fermentation is less than that of the unsuitable. This is the reason why the latter exceeds in its basic nature.

(J) The change of oryzenin alkali solution.

In the former report[7], it was stated that the oryzenin alkali solution increases its free amino nitrogen content when exposed to ultra-violet ray and that between the common and the glutinous rice oryzenins the velocity of the change is remarkably different. The same experiment was repeated with the oryzenins of the rices suitable and unsuitable for fermentation with the following results.

TABLE XII.

Effect of exposing oryzenin alkali solutions to ultra-violet ray in increase of free amino nitrogen.

Duration of Exposure / % of / Sample	30 min		1 hour		$1\frac{1}{2}$ hours	
	protein	total N	protein	total N	protein	total N
No. 1	4.0028	22.379	5.1465	28.7734	8.1945	45.8144
No. 2	4.2822	25.0507	3.5266	32.3316	9.0676	53.0512
No. 3	4.1372	22.8472	5.3631	29.6169	8.4277	46.5408
No. 4	4.4368	25.1938	5.7099	32.4231	9.1822	52.7080
No. 5	4.1392	22.4267	5.4725	29.6509	8.5555	46.3548
No. 6	4.1392	22.1205	5.3186	28.6817	8.2530	45.6589
No. 7	4.3201	25.3137	5.5082	32.2753	8.6402	50.7273
No. 8	—	29.6171	—	25.5258	—	41.2342
No. 9	—	24.1207	—	29.3644	—	50.3389
No. 10	—	21.5176	—	26.4076	—	37.1666
No. 11	—	24.4203	—	31.4848	—	46.8073
No. 12	—	23.6026	—	30.8171	—	43.3200
No. 13	3.200	19.8259	4.200	23.7910	5.025	28.4643
No. 14	4.025	23.3901	4.830	28.0682	5.950	34.5767
No. 15	2.975	16.3082	3.675	20.6396	5.025	28.2205
No. 16	3.325	19.1754	4.025	23.2124	5.025	28.9794

The table shows that the alkali oryzenin solution of the rice suitable for fermentation is changed more easily than that of the unsuitable by the action of ultra-violet ray. The amount of the change was greater, the longer the exposure to the ray. This is due to the ease of decomposition of the former as compared with the latter.

III. Conclusion.

The above described examinations of the physico-chemical nature carried on with 16 samples of the common rices produced in 7 parts of Japan conduct to a definite "Artspezifität." The differences observed are as follows.

(1) The ash content of the oryzenins of the rices suitable for fermentation are always inferior to that of the unsuitable, the content of the former being between 79 and 95 % of that of the latter. The oryzenin of the unsuitable rices seems to contain more sulphur and phosphorus than of those suitable for fermentation. However, the difference is not very striking.

(2) The specific rotatory power of the oryzenins of the rices suitable for fermentation is lower than that of the unsuitable, the former being between −64.88 and −72.66, while the latter is between −74.41 and −88.30. This is the most remarkable difference of the two oryzenins.

(3) The isoelectric point of the suitable rice oryzenins is more acidic than that of the unsuitable.

(4) The oryzenins of rices suitable for fermentation combine with larger quantities of silver than the unsuitable, showing the predominance of the quantity of carboxyl group in the molecule of the former compared with that of the latter.

(5) The oryzenin of rices suitable for fermentation is deficient in the quantities of ammonia, melanin and lysin nitrogen but is predominant in histidin and cystin nitrogen.

(6) The content of free amino nitrogen of the oryzenin of rices suitable for fermentation is inferior to that of the unsuitable, but the alkali solution of the former is changed more easily than that of the latter when exposed to the ultra-violet ray. This is due to the ease of decomposition of the former compared with the latter.

These physico-chemical and chemical differences are considered as the "Artspezfität" of the oryzenins of the two classifications into which the common rices are usually divided.

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