STUDIES ON THE MOLECULAR STRUCTURES OF DECOMPOSED STARCH OF COMMON AND GLUTINOUS, WHICH PRODUCE AN IDENTICAL COLOR WITH IODINE

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Introduction

The iodine color reaction of starch has been investigated from the earlier year; compound theory by ANDREW and GOETTSCH[1] (1902), colloidal state theory by KATAYAMA[2] (1908), adsorption theory by BERZELLER[3] (1917), and colloidal particle theory by ANGELESCUS and MIRCESCU[4] (1928), had been proposed from 1902 to 1928, but no any theory could give clear explanation on the iodine color reaction of starch.

Since the spiral theory of starch molecule was recognized by RUNDLE[5] in 1943, the investigation of the iodine color reaction of starch has been developed remarkably. FRENCH, RUNDLE and KERR[6] separated the crystalline dextrines from the decomposed products of starch and investigated the iodine color reaction of these crystals. SWANSON[7] described that the iodine coloration of starch was related to non-branched chain length of the glucose residues of starch molecule. So, more attention must be paid to the iodine color reaction of starch in order to study the molecular structures of starch, common and glutinous. As is well known, glutinous starch gives a reddish brown color with iodine, whereas starch gives a blue color, but after they are decomposed, common starch gives the following color change, blue-violet-reddish violet-none, and glutinous, reddish brown-reddish violet-red-none, according to the degree of decomposition.

Therefore, by controlling the condition of decomposition, it is expected to obtain two decomposed products of common starch and

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glutinous which would produce an identical color with iodine. This paper describes the separation of two decomposed products of the identical iodine coloration and their properties, physicochemical and enzymatical.

Experiment I

Preparation of starch.

After threshing common unhulled rice and glutinous, they were ground to flour. Each flour was treated with three times its volume of 0.3% NaOH solution. Mixtures were well stirred and allowed to stand still. After standing over night, the upper liquids were siphoned off. The above process was repeated until no reactions of Biuret and Millon could be observed in starch. Then, starch were washed with water to remove NaOH and were dried in vacuum at 70°C, after treatment with alcohol and ether. Dried common starch and glutinous were extracted with warm 85% methanol of 5 times its volume for 50 hr. to remove fat and were dried in vacuum on P_2O_5 at 70°C after treatment with absolute methanol and ether.

Decomposition of common starch and glutinous by autoclaving.

Water suspensions of common starch and glutinous were poured into boiling water and were kept for 20 minutes to be made up to 1% starch solution. Then, the starch solutions were autoclaved. Conditions of decomposition are shown in table I.

TABLE I. Autoclaving of two kinds of starch.

Samples	Temperature					
	110 °C	150 °C	175 °C		190 °C	
common starch	min.	min.	min.	min.*		
glutinous starch	26	43	59	5		
common starch	20	39	61	10 *		
glutinous starch						
common starch	26	44	62		min. 95	min.* 10
glutinous starch						
common starch	31	44	64		98	15 *
glutinous starch						

Figures in minutes show the time elapsed till the temperature level presented.
* time kept in 175°C and 190°C.

After the autoclaved starch solutions were centrifuged, 5 cc of the supernatants was taken and 1 cc of 1/10 N iodine solution was added and the volume was made up to 100 cc with water. The colorations were measured by DUBOSCQ's colorimeter. From these experimental results described above, it was known that the common starch solution, autoclaved at 190°C for 15 minutes, produced similar coloration with iodine to that of the glutinous, at 175°C for 10 minutes.

Al_2O_3-chromatography of decomposed starch.[8]

Each of four decomposed starch, common and glutinous, as were shown in table I, was developed by chromatography technique, in which Al_2O_3 was used as adsorbent. After chromatography procedure was over, each column was dried at room temperature and 1/100 N iodine solution was dropped down into the column by suction. Colorations with iodine on the Al_2O_3-chromatograms are summarized in Table II.

TABLE II. Iodine colorations on the Al_2O_3-chromatograms.

condition of decomposition	starch	The first iodine color zone	The second iodine color zone	decomposed starch not adsorbed by Al_2O_3
		colors	colors	colors
at 175°C for 5 min.	common	blue	reddish violet	pale reddish brown
	glutinous	violet	violet brown	pale yellow brown
at 175°C for 10 min.	common	violet blue	reddish violet	reddish brown
	glutinous	violet red	reddish brown	bitter orange
at 190°C for 10 min.	common	violet blue	violet reddish brown	brown
	glutinous	brown	brown	pale yellow brown
at 190°C for 15 min.	common	reddish violet	reddish brown	bitter orange
	glutinous	pale brown	pale brown	pale yellow brown

Paper Chromatography.

Each water solution of decomposed starch, common and glutinous, was examined by paper chromatography for the iodine coloration tones. Colorations with iodine on the paper chromatograms are summarized in Table III.

TABLE III. Iodine colors on the chromatograms.

conditions of autoclaving	starch	The upper iodine color zone	The lower iodine color zone
		colors	colors
at 175°C for 15 min.	common	reddish violet	violet blue
	glutinous	reddish brown	brown
at 175°C for 10 min.	common	reddish violet	reddish violet
	glutinous	bitter orange	reddish
at 190°C for 10 min.	common	reddish	reddish
	glutinous	reddish brown	reddish brown
at 190°C for 15 min.	common	bitter orange	reddish
	glutinous	reddish yellow	reddish yellow

From the results obtained above, it was known that iodine color zone of decomposed starch, common and glutinous, were arranged by Al_2O_3-chromatography as well as by paper chromatography, but the color zones by the former were divided more clearly than by the latter. Furthermore, common starch, decomposed at 190°C for 15 minutes and glutinous, at 175°C for 10 minutes, of which iodine colorations were observed similar, were found to produce not a single color but mixtures of three colorations respectively. Among these three kinds of coloration, produced by decomposed common starch, however, bitter orange coloration possessed the strongest intensity and occupied a greater part of the iodine coloration tones. This was the same with decomposed glutinous starch. This is the reason why two kinds of decomposed starch gave nearly identical iodine coloration, when examined for it by DUBOSCQ's colorimeter. Moreover, it has been known that decomposed starch, common and glutinous, whose iodine colorations are identical bitter orange, were not adsorbed by Al_2O_3 but filtered off.

Experiment II

Physicochemical properties of decomposed starch, common and glutinous, of which iodine colorations were identical bitter orange.

Preparation of decomposed starch, common and glutinous. By the

method described in the experiment I, 1% water solutions of common starch and glutinous were autoclaved at 190°C for 15 minutes and at 175°C for 10 minutes respectively. These decomposed starch solutions were cooled and centrifuged. The supernatants obtained were concentrated to 1/5 volume of the original at 40°C in vacuum and three times its volume of alcohol was added. After allowing to stand over night, the precipitates were separated by centrifugation and washed with 75% alcohol repeatedly. Then, the precipitates were dissolved in water and filtered 3 times by suction through the column, packed with Al_2O_3. 25% alcohol solutions of the filtrates were treated with charcoal and filtered. More alcohol was added to the filtrates to be 70% alcohol concentration. The precipitates were separated by centrifugation and dried in vacuum on P_2O_5 at 70°C after treatment with absolute alcohol and ether.

Two decomposed starch were dissolved in water to be 1% solution and were developed by paper chromatography technique as were described in experiment 1. The identical iodine coloration, bitter orange, was observed on the paper chromatograms.

Iodine coloration.

1 gr. of each decomposed starch preparation was dissolved in water and the total volume was made up to 100 cc, adding 1 cc, of 1/100 N iodine solution. Iodine coloration was measured by DUBOSCQ's colorimeter. It was known that iodine colorations of decomposed starch, common and glutinous, were identical bitter orange.

Solubility.

Each of decomposed starch, common and glutinous, was easily soluble in cold water and even after the solutions of decomposed starch were allowed to stand for a week or more, retrogradation never occurred.

Reducing value.

0.5 gr. of decomposed starch, common and glutinous, were dissolved in 10 cc of water, and their reducing values were determined by HANES[9] method. The results obtained are shown in following Table.

Optical rotation.

With 1% water solutions of decomposed starch, common and glutinous, optical rotation values were measured, using 200 mm tube. The results are shown in Table V.

TABLE IV. Reducing values of decomposed starch, common and glutinous.

Samples	kinds	Reducing values
decomposed starch before treatment with Al_2O_3	common	71.0
	glutinous	44.7
decomposed starch after treatment with Al_2O_3	common	82.3
	glutinous	51.7

* Reducing values are shown in mg. of maltose per. gr. of decomposed starch.

TABLE V. Optical rotation values of decomposed starch, common and glutinous.

Samples	Kinds	$[\alpha]_D$
decomposed starch before treatment with Al_2O_3	common	170
	glutinous	164
decomposed starch after treatment with Al_2O_3	common	160
	glutinous	153

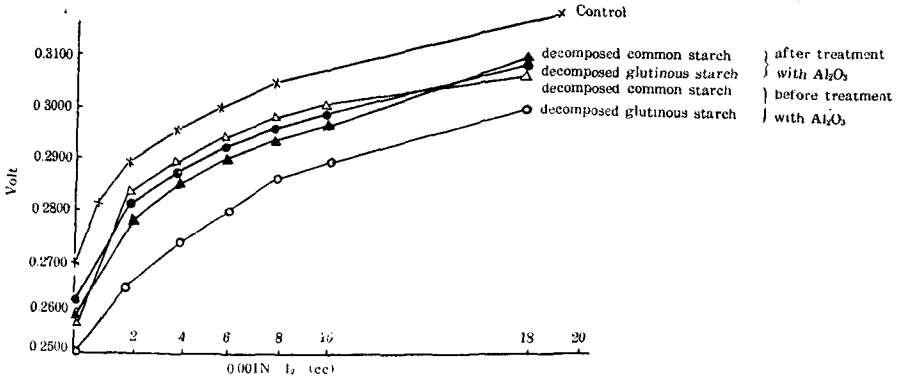

Figure 1. Iodine adsorption by decomposed starch.

Iodine adsorption.[10]

40 mg. of decomposed starch, common and glutinous, were dissolved in 100 cc of 0.05 N KI solution, containing 0.05 N potassium chloride, under well stirring. The solution were titrated potentiometrically with

0.001 N iodine solution which contained 0.005 N potassium iodide and 0.005 N potassium chloride.

Iodine amounts combined by decomposed starch, common and glutinous, are shown in figure II.

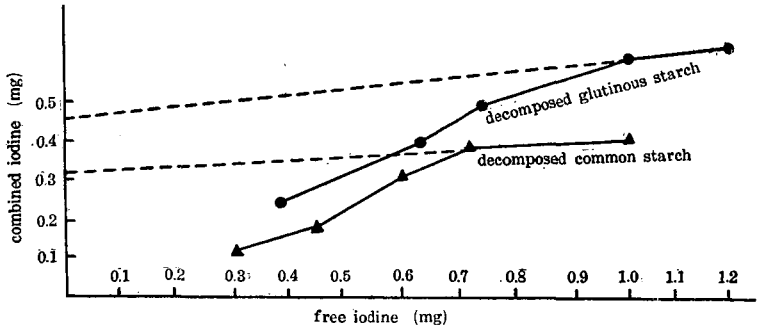

Figure 2. Combined iodine amounts by decomposed starch.

Terminal end group.[11]

After 0.2 gr. of decomposed starch, commom and glutinous, were dissolved in 5 cc of 3% NaCl solution, 5 cc of 0.73 M $NaIO_4$ solution was added to each preceeding solution. The solutions were kept at 2°C for 25 hr. and 1 cc of ethylenglycol was added. The solutions were titrated with 1/100 N $Ba(OH)_2$ solution. The results are shown in Table VI.

TABLE VI. Terminal end groups of decomposed starch, common and glutinous.

Samples	N/100 $Ba(OH)_2$ cc						average chain lengths
	0 hr.	5 hr.	10 hr.	20 hr.	25 hr.	30 hr.	
decomposed common starch	2.83	4.47	10.67	11.09	11.52	11.70	7
decomposed glutinous starch		2.04	6.59	7.58	7.90	8.24	10

Viscosity.[12),13),14)]

With 2%, 1%, 0.5% and 0.25% water solutions of decomposed starch, common and glutinous, their viscosities were determined.

TABLE VII. Viscosities of decomposed starch, common and glutinous.

Samples	Kinds	C %	η sp	η sp/C gm.
Decomposed starch before treatment with Al_2O_3	common	1	0.09	1.46
		0.5	0.03	0.97
		0.25	0.01	0.64
	glutinous	1	0.08	1.29
		0.5	0.02	0.65
		0.25	0.01	0.64
Decomposed starch after treatment Al_2O_3	common	1	0.07	1.13
		0.5	0.02	0.649
		0.25	0.005	0.324
	glutinous	1	0.04	0.648
		0.5	0.014	0.146
		0.25	0.005	0.324

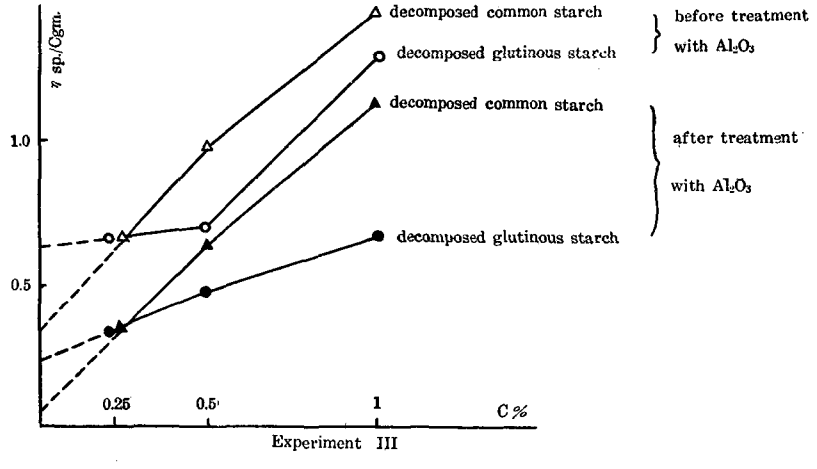

Figure 3. Intrinsic viscosities of decomposed starch.

Preparation of α-amylase.

Separation of α-amylase from commercial malt amylase was carried out mainly according to HOLMBERGH's[15] and OHLSSON's[16] method, as following; 10 gr. of malt amylase was dissolved in 200 cc of water and filtered. Alcohol was added to the clear filtrate to be 40% alcohol concentration and precipitates were centrifuged off. The supernatant

solution was filtered three times repeatedly through the column, stuffed with dry potato starch, to remove β-amylase. The filtrate was tested for α-amylase activity as following; 5 cc of enzyme solution was added to 1 cc of 1% soluble starch solution, containing 5 cc McIlvaine buffer of which pH values are 3.4 and 5.4. One drop of 1/10 N iodine solution was added to the mixtures after incubation for 7 hr. The results are as following.

Table VIII.

Samples	Iodine colorations
Mixture at pH 3.4	Violet red
Mixture at pH 5.4	none

From the experiment proceeded above, however, it was known that α-amylase preparation contained trace of β-amylase. After pH value of α-amylase solution was ajusted to 5.3, the enzyme solution was heated for 20 minutes at 70°C and filtered. Alcohol was added to the filtrate to be 70% alcohol concentration. After centrifugation, the test for α-amylase activity was carried out with the filtrate by the same method as was described above.

Table IX.

Samples	Iodine colorations
Mixture at pH 3.4	blue
Mixture at pH 5.3	none

From the result, it is considered that no β-amylase is contained in α-amylase preparation. Furthermore, purity of α-amylase preparation was examined by hydrolysis of soluble starch.

10 cc of 0.1% enzyme solution was added to 100 cc of 0.1% soluble starch, containing 10 cc of McIlvaine buffer (pH 5.8) and the mixture was incubated at 37°C. 5 cc of the mixture was sampled at intervals and hydrolysis degree of starch was determined by Hanes' method.

The curve obtained above is similar to that by pure α-amylase. So it was known frome these experiments that α-amylase preparation contained no β-amylase.

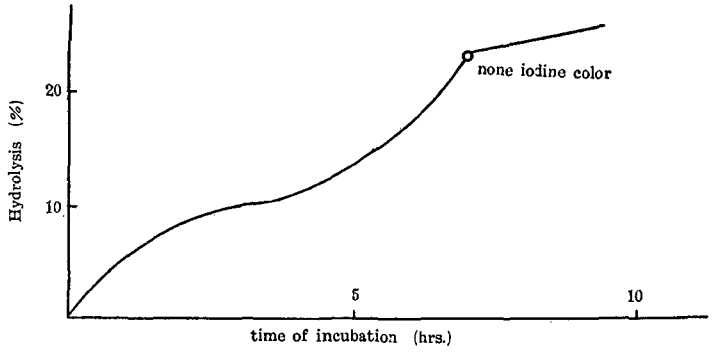

Figure 4. Hydrolysis of soluble starch by α-amylase.

Hydrolysis of two decomposed starch by α-amylase preparation.[17),18)]

20 cc of 1% α-amylase solution was added to 100 cc of 0.1% decomposed starch, containing 10 cc of McIlvaine buffer (pH 5.8) and the mixture was incubated at 37°C. Reducing values of the mixture were determined by Hanes' method at intervals. The results are shown in following figure.

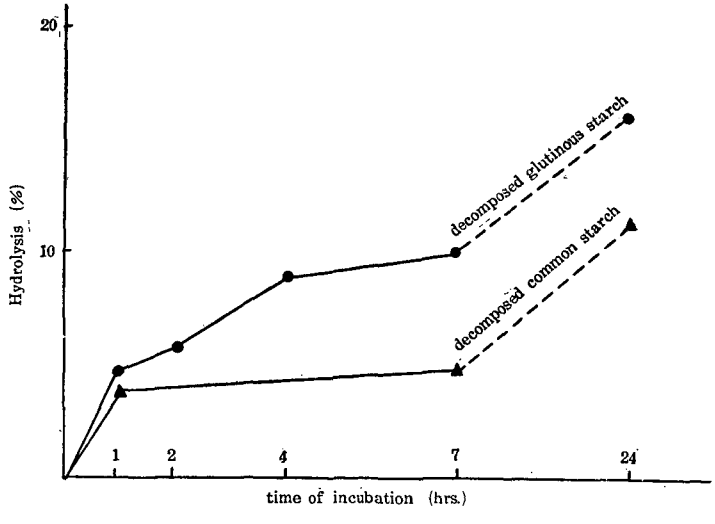

Figure 5. Hydrolysis of decomposed starch by α-amylase.

Decomposed glutinous starch was hydrolysed by α-amylase more easily than decomposed common starch, and hydrolysis of decomposed glutinous starch appeared to stop practically after 4 hr. This was the

same with decomposed common starch. Furthermore, hydrolysis degree of original starch, common and glutinous, were examined and following results are obtained.

Figure 6 showed that remarkable difference was not seen in the hydrolysis degree by α-amylase between original common starch and glutinous and after 23 hr., hydrolysis degree of each starch was about 36 %. As is well known, common starch contains two constituents, amylose and amylopectin, and glutinous starch contains amylopectin only, but no remarkable difference between the hydrolysis degrees of these two starch by α-amylase were observed, whereas remarkable difference was observed between the hydrolysis degrees of decomposed starch, common and glutinous.

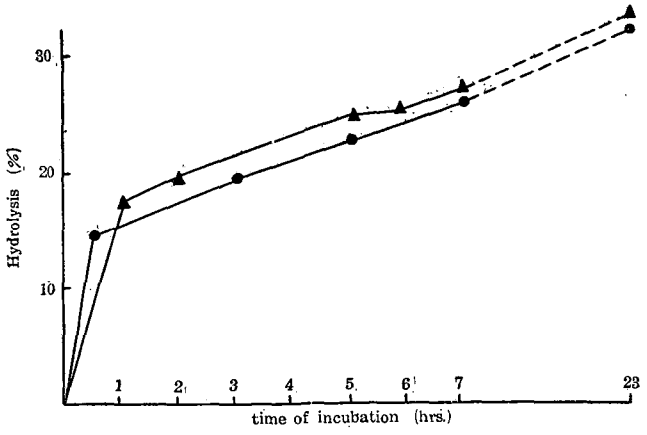

Figure 6. Hydrolysis of original starch, common and glutinous.

Hydrolysis degrees of decomposed starch, common and glutinous, by α-amylase were conspicuously lower than that of original starch, after 23 hr. incubation as are shown in following table.

TABLE X. Hydrolysis of original starch and decomposed starch by α-amylase.

Samples		hydrolysis (%)
original starch	common	36.2
	glutinous	34.5
decomposed starch	common	11.3
	glutinous	15.9

From the experimental results described above, it may be considered that molecular chain residues which are attacked by α-amylase are also decomposed by autoclaving, and as common starch was decomposed by autoclaving more than glutinous starch was, decomposed glutinous starch contains much more chain residues which are attacked by α-amylase than decomposed common starch.

Incubation with β-amylase.[20),21)]

β-amylase was prepared by OHLSSON's method as following; 20 gr. of commercial malt amylase was dissolved in 200 cc of water. pH value of clear water solution of enzyme was ajusted to 3.3 with acetic acid.

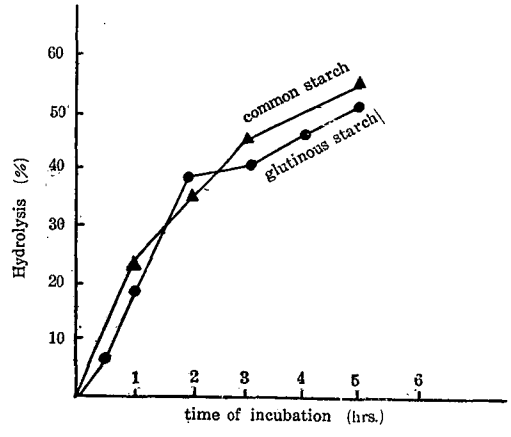

Figure 7. Hydrolysis of original common starch and glutinous by β-amylase.

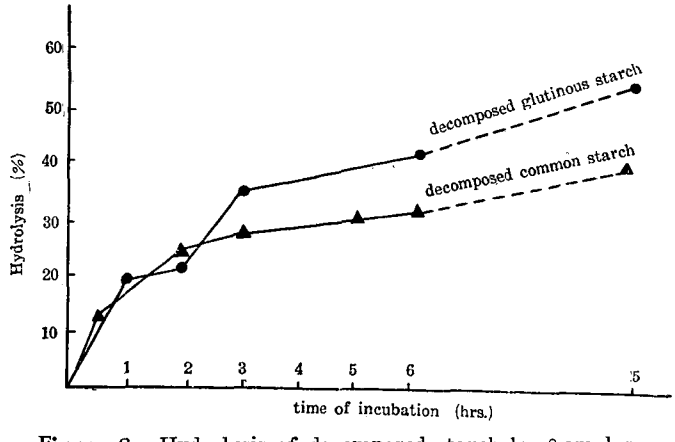

Figure 8. Hydrolysis of decomposed starch by β-amylase.

The enzyme solution was kept at 0°C for 15 minutes. After centrifugation, pH value of supernatant was raised to 5.3 with NH_4OH. This procedure was repeated 2 times. The enzyme solution was used in following experiment. Limit of hydrolysis of soluble starch by this enzyme was 53.9%. Original starch and decomposed starch, common and glutinous, were incubated with β-amylase.

From these results, it was known that decomposed glutinous starch was attacked by β-amylase more than decomposed common starch.

Incubation with malt amylase.

0.5 gr. of commercial malt amylase was dissolved in 100 cc of water and the supernatant solution was used as enzyme solution. 500 mg. decomposed starch, common and glutinous, were dissolved in 100 cc of water and the solution was incubated at 37°C with 5 cc of enzyme solution, adding 10 cc of McIlvaine buffer (pH 5.8). Reducing value was determined at intervals by Hanes' method. The results are shown as following.

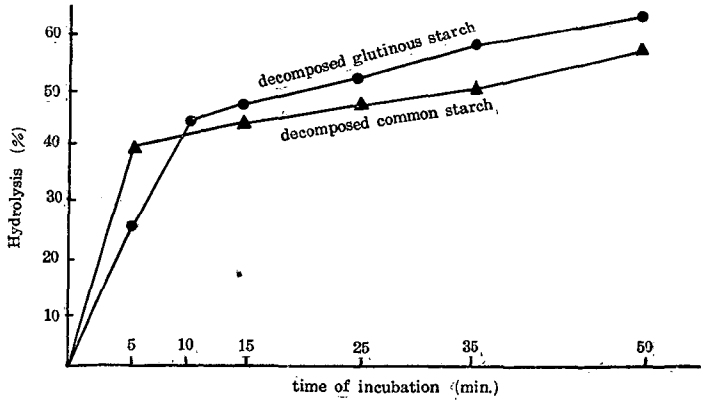

Figure 9. Hydrolysis of decomposed starch by malt amylase.

Discussion

It is well known that common starch contains two constituents, amylose and amylopectin, whereas glutinous starch contains amylopectin only, but when they are decomposed by autoclaving, types of decomposed starch, common and glutinous, may be influenced by the following three possibilities:

1. α-1-4 linkages in starch molecule are decomposed more easily than

α–1–6 linkages.

2. α–1–4 linkages in starch molecule are more resistant to the decomposition by autoclaving than α–1–6 linkages.

3. α–1–4 linkages are decomposed by autoclaving with the same rate as α–1–6 linkages.

Whichever of the three possibilities it may occur, non-branched decomposed starch common and glutinous, as well as branched decomposed starch, will be produced. Therefore, it is sure that decomposed starch, common and glutinous, contain both types of amylose and amylopectin. But the following results were obtained by the studies on the properties of decomposed starch which were separated and purified by Al_2O_3-chromatography as were described in the experiment III.

Investigating iodine colorations on the chromatograms as were shown in the experiment I, amylose type polysaccharide of decomposed starch, common and glutinous, seemed to be adsorbed by Al_2O_3, prior to amylopectin type. Iodine adsorption curves of decomposed starch, common and glutinous, not adsorbed by A_2IO_3 were similar to that of amylopectin. Retrogradation of decomposed starch were hardly seen.

Decomposed glutinous starch was more easily hydrolysed by α- or β-amylase than decomposed common starch, as were shown in the experiment III.

From these results described above, it is known that decomposed starch, common and glutinous, contain only polysaccharide of amylopectin type.

Values of (η sp/cgm) were 0.19 for decomposed glutinous starch and 0.05 for decomposed common starch. If STAUDINGER's law may be applied to viscosities of decomposed starch, common and glutinous, molecular weight of decomposed glutinous starch would be larger by 3.8 folds than that of decomposed common starch. In addition to this, following results seem to indicate the larger molecular weight of decomposed glutinous starch than that of decomposed common starch. As is well known, molecular weight of glutinous starch is larger than that of common starch, and the present decomposed common starch was prepared under the condition of higher temperature and longer time in comparison with decomposed glutinous starch.

Reducing value of decomposed common starch was greater by 30% than that of decomposed glutinous starch.

Iodine amount, combined by decomposed glutinous starch, was

larger than that by decomposed common starch.

Decomposed glutinous starch was easily hydrolysed by amylase more than decomposed common starch (starch of larger molecular weight seems to be hydrolysed by amylase prior to smaller molecular). From the results described above, it is considered surely that molecular weight of decomposed glutinous starch is larger than that of decomposed common starch. Measuring by periodate method, the authors found that the average chain length of decomposed glutinous starch was longer than that of decomposed common starch and the value was 10 for decomposed glutinous starch and 7 for decomposed common starch. Taking account of the discussion described above, branching degrees of decomposed starch, common and glutinous, are p/7 and 3.8 p/10 respectively. (p is designated as polymerisation degree of decomposed common starch). These values show that branching degree of decomposed glutinous starch is greater by 2.6 folds than that of decomposed common starch.

The enzymatical studies are now to be discussed. The experimental result that decomposed glutinous starch was more easily hydrolysed by α-amylase than decomposed common starch, seems to indicate that molecular linkages, which are attacked by α-amylase, may remain in the molecule of decomposed glutinous starch more than in the molecule of decomposed common starch and these molecular linkages may result from branching of original starch. β-amylase hydrolysed decomposed glutinous starch much more than decomposed common-starch. This result appears to be in conflict, regarding that branching of decomposed glutinous starch is greater than that of decomposed common starch.

This discrepancy, however, could be resolved clearly by the determination of the average chain length of both decomposed starch.

Decomposed starch, common and glutinous, produced an identical iodine color but, as was shown in enzymatical experiment, remarkable difference was found between the molecular structures, especially their chain lengths of decomposed starch, common and glutinous.

Consequently, it is likely that iodine color reaction of starch is related not only to iodine amounts, combined by starch, but also to the molecular structure of starch, especially to the chain length and stereochemical configuration of starch.

Summary

1. Decomposed starch, common and glutinous, were prepared by autoclaving at 190°C for 15 minutes and at 175°C for 10 minutes respectively, which produced an identical color with iodine.

2. Physicochemical properties of decomposed starch, common and glutinous were investigated, such as reducing value, viscosity, optical rotation, iodine adsorption and terminal end group.

3. Molecular structures of decomposed starch, common and glutinous, were examined enzymatically with α- and β-amylase.

4. From the results obtained, it was known that decomposed starch, common and glutinous, which produced an identical color with iodine after treatment with Al_2O_3, contained polysaccharide of amylopectin type only and decomposed glutinous starch possessed larger molecular weight, higher branching degree, longer chain length, and larger amount of combined iodine, in comparison with decomposed common starch.

5. α- and β-amylase hydrolysed decomposed glutinous starch much more than decomposed common starch. This result shows that molecular structure of decomposed starch, especially average chain lengths, are related to hydrolysis degree by amylase.

6. Consequently, iodine color reaction of starch is related not only to iodine amount combined by starch, but also to the molecular structure of starch, especially to chain length and stereochemical configuration.

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