BIOCHEMICAL STUDIES ON RICE STARCH. II. TEMPERATURE EFFECTS ON THE GERMINATION OF RICE SEEDS IN DARKNESS.

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In the previous experiment, the chemical changes of rice starch during germination were studied. In the experiment, the germination of seeds was operated at 20°C. In practice, however, the most favorable temperature for the germination is said to be 35°C. for the purpose of obtaining strong and healthy seedlings.

So the writer now has studied further, the germination of seeds under different temperatures, viz., 20°C., 35°C. and 40°C. in order to observe the temperature effects on the chemical transformations of starch during the process.

The rice seeds were soaked in water for 2 days and allowed to germinate in darkness at the different temperatures above-mentioned, till the shoots were half an inch long. Then they were dried in an air current at room temperature and ground in a stone mill. The content of moisture, reducing sugars, soluble polysaccharides and starch was determined by the usual methods and the results are shown in the following table:

Table 1.

	Germinated at	Dry wt. per 100 grains gr.	Loss	Reducing sugars %	Soluble poly-sacch. %	Starch %	Loss in starch %
Seeds		3.1		0	1.6	73	0
Seedlings	20°C.	2.3	0.8 gr. (26%)	3.9	10.0	56	- 3
	35°C.	2.2	0.9 gr. (30%)	2.7	7.3	53	-10
	40°C.	2.3	0.8 gr. (26%)	2.5	3.5	58	- 9

As will be seen in the above table, the weight loss was most noticeable in the seedlings, the loss differing with the temperature of germination, and greater at 35°C. than at either 20°C. or 40°C. When the chemical constituents of seeds and seedlings were compared, the content of simple sugars and soluble polysaccharides in the seedlings of the three cases was all found to have increased inversely to the decrease of the starch-content. The loss of starch among the seedlings was greatest in the seedlings germinating at 35°C., while the loss among those germinating at 40°C. and at 20°C. is quite regular.

The fact that the proportion of soluble polysaccharides and reducing sugars in seedlings which germinated under different temperatures is not the same, indicates that the hydrolysis of starch into sugars in seedlings depends greatly upon the surrounding conditions, especially the temperature under which the seeds were germinated. The formation of reducing sugars and soluble polysaccharides from starch is favonrable at 20°C., while the

inversion of polysaccharides into simple reducing sugars is comparatively great at 40°C.

In order to learn the chemical nature of the sugars which occur in seedlings, by the transformation of starch during the germination, the separation and identification of these sugars, if possible, were undertaken.

About 500 grams of the rice seedlings ground in a stone mill, were steeped in 80% alcohol for about 12 hours then diluted with distilled water to make a 50% alcohol solution, and heated in a water bath at 70°C. for 5 hours with occasional stirring. After cooling, it was filtered and the residue was again treated with 50% alcohol in a water bath. This process was repeated 5 or 6 times till the filtrate contained no more reducing sugar. The extracts and washings were combined together and then concentrated under diminished pressure to small volume, and a sufficent quantity of basic lead acetate was added, the precipitate thus formed was filte ed off and to the filtrate hydrogen sulphide gas was passed to remove the excess of lead in the solution. After filtering off the lead sulphide, the solution was concentrated to small volume and alcohol was added to make an 80% alcohol solution. The precipitate (A) thus formed was separated by decantation, dissolved again in a little water, and reprecipitated with an 80% alcohol solution, washed with absolute alcohol and ether, and dried in a desiccator over sulphuric acid.

The clear decanted liquid and the washings were combined together and concentrated to small volume under reduced pressure and the precipitate (B) was obtained by means of a 90% alcohol solution.

The filtrate separated from the precipitate (B), was evaporated to a thick syrup, absolute alcohol was added, on which the precipitate (C) was formed, and this was washed with ether and dried in a desiccator.

The alcoholic filtrate was evaporated to a syrup in vacuo, dissolved in methyl alcohol and ether was added to form the precipitate (D). Thus, the soluble dextrins, mono- and poly-saccharides, which occur in the seedlings, were extracted and separated approximately by fractional precipitation into the four crops of the following properties:

The first precipitate (A) or the 80% alcohol precipitate was a lustrous white powder, easily soluble in cold water. The second precipitate (B) or the 90% alcohol precipitate was a slightly yellowish white powder, easily soluble in cold water. Both the third precipitate (C) or the absolute alcohol precipitate, and the fourth precipitate (D) or the ether precipitate was a light yellowish powder with a sweet taste, and easily soluble in cold water.

Of these crops, the rotatory power and reducing power of the aqueous solution were determined on the one hand, and on the other the colour

reaction with iodine, the ketose test and the formation of osazone with phenyl hydrazine were studied, and the experimental results are shown in the accompanying table:

Table 2.

	Germinated at	$(\alpha)_D$	R.P.	Iodine test	Pinoff's test	Osazone test
(A)	20°C.	163-164	4-5	Red Brown		
	35	173	5	,,		
	40	173	6	,,		
(B)	20°C.	159	10	Brown		
	35	163	22	,,		
	40	123	23	,,		
(C)	20°C.	59	25	Nil	+	Malto- and gluco-osazones
	35	86	42	,,	+	
	40	50	49	,,	+	
(D)	20°C.	56	29	,,	+	Malto- and gluco-osazones.
	35	78	55	,,	+	
	40	38	50	,,	+	

As will be seen in the above table, the first precipitate (A) from three cases, seem to consist equally of a mixture of dextrins which usually can be obtained from starch by hydrolysis with acids or diastase. The second of the precipitates (B) were ascertained from their properties to consist of dextrin with some maltose, the proportion of the latter in the precipitate was increased with the germinating temperature. The third and fourth precipitates (C & D) were supposed to consist mostly of simple sugars with some maltose. To our interest, these two precipitates in each case, contain some cane sugar its occurrence being indicated by the rotatory power and reducing power and also the Pinoff test. According to the literature, Kuhneman has isolated sucrose from germinated barley and the same sugar was also confirmed by O'Sullivan and Brown & Morris to occur in the same germinating seeds, though the mechanism for the formation of the sugar was obscure. As a matter of fact, the content of sucrose was rich in the seedlings at 20°C. among three cases, glucose and fructose were predomi-

nant in the other cases which germinated at the highest temperature, and the formation of the disaccharide such as maltose was favoured at the middle temperature, 35°C.

According to Brown & Morris, the growing tissues of the barley embryo has the ability to convert maltose into cane-sugar but not glucose into sucrose. It seems, therefore, that maltose formed by the action of the hydrolytic enzymes from starch, will convert into sucrose by the intramolecular transformation on the one hand, and on the other, into glucose and fructose by inversion, and the former reaction is favoured at the lower temperature, and at the higher the latter reaction prevails.

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