BIOCHEMICAL STUDIES ON RICE STARCH. I. THE CHEMICAL CHANGES OF THE STARCH DURING THE GERMINATION OF RICE IN DARKNESS.

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The rice which is the chief food stuff of the Orient, is widely planted and regarded as one of the most important plants in the East.

In the cultivation of rice, the grains are usually germinated in spring, after flowering and ripening, the crops are harvested in autumn. The physiological development during the germination of the grain greatly arouses our interest, especially from the biochemical point of view. According to the authorities, the requirements for germination are sufficient water and a suitable temperature; under these conditions the embryo absorbs water and swells as other grains do, and the shoot and root sprout out through the crack of the hull. The temperature at which the grains are germinated, is said to be an optimum at 30°–35°C., while 10°C. to 13°C., and 40°C. are the lowest and highest limits.[1] Under the most favourable conditions the germination of rice becames externally evident after about two days. But the chemical changes which take place in the substance of the kernel during this process, have not yet been investigated.

The germination of other seeds was studied by many investigators from the point of view of plant physiology, and the results arrived at were summarized in the elaborate work "Vergleichende Physiologie des Keimungsprocesses der Samen (1880)" by W. Detmer. M. Berthelot's[2] investigations on this subject were carried out in collaboration with M. Andre during the periods 1883-1885, 1893-1896, following the investigations of M. Boussingault & A. Müntz.[3]

According to these investigations, the germination of seeds in darkness, which is distinct from the vegetation of plants growing in light, it was regarded that the stored substances alone were utilized, while in the latter case the transformation of the reserve materials was usually accompanied by the assimilation which results in the formation of new substances from the carbon dioxide and water in the atmosphere.

The chemical processes of germination are, therefore, not identical in different kinds of seeds as already indicated by Palladin[4] and they depend largely upon the chemical nature of the reserve materials; the loss of weight in starchy seeds such as maize and barley, during germination was explained as occurring through loss of carbohydrates. The chemical transformation of the starch in barley in the process was precisely investigated by H. T. Brown & H. Morris, estimating the products in the plant tissues by the method recommended by O'Sullivan,[5] and stated in their classical work: "Researches on the germination of some of the Gramineoe"[6] that the starch reserved in the embryo was converted into transitory starch by the hydrolytic enzymes accumulated in the germinated seeds, which by the same agency was converted into simpler sugars. Moreover, they noticed the formation of sucrose in the barley embryo together with the production of maltose, dextrose and laevulose, which was previously observed by O'Sullivan in his experimental research on barley-malt.

(1) M. Akemine, *Landw. Z.*, **63** (1914), 78; and refer, I. Nagai, "Cultivation of Rice in Japan" (1925, Japanese), 378.
(2) M. Berthelot, "Chimie vegetable et Agricole," II (1899), 7–371.
(3) M. Boussingault, "Agronomie, Chimie Agricole et Physiologie," V (1874), 50.
(4) B. E. Livingston, "Palladin's plant physiology," (1917), 175.
(5) *J. Chem. Soc.*, **45** (1884), 1.
(6) *J. Chem. Soc.*, **57** (1890), 458.

O'Sullivan has stated that the maltose is derived from the starch which unquestionably disappears during germination, but he gave no opinion as to the origin of the cane-sugar and its products of inversion.

Brown & Morris have, however, offered the suggestion for the origin of the sugar that the transformed starch is absorbed from the endosperm by the columnar epithelium of the embryo in the form of maltose which is by the more or less complicated metabolic processes of the living cells of the embryo rapidly converted into cane-sugar, and they have demonstrated the suggestion in a very striking manner, showing the ability of the growing tissue of the embryo to convert maltose into cane-sugar.

If this view is correct, we have still some difficulty in explaining in what manner the maltose is converted into sucrose.

However, there is no question as to the intimate connection between cane-sugar and starch in seeds which has been known to exist, but no chemist has made any statement concerning the conditions under which the conversion of starch into sucrose takes place.

The writer, therefore, has taken it on herself to investigate the chemical changes which may be expected to take place in the reserve starch during the germination of the rice in darkness.

I. The Germination of the Rice at 20°C.

Preparation of Materials. The seeds used in this experiments were called Asahi, produced in Zeze, near Otsu 1928 and harvested in that year and stored carefully in a straw rice-bag in our laboratory.

The grains were ground in a stone mill in the laboratory, and the resulting flour was sifted through a $\frac{1}{23}$ cm. sq. mesh sieve.

The rice seedlings were prepared by steeping the seeds in distilled water for two days at room temperature until they had absorbed about 50 per cent in weight of moisture, and were left to germinate for seven days at 20°C. in darkness. After germination had proceeded to the extent that the shoots were half an inch long, they were dried in an air current at room temperature, and ground in a stone mill until all of them were sifted through a $\frac{1}{23}$ cm. sq. mesh sieve.

Proximate Analysis. The content of moisture, the total reducing sugar, the soluble starch, (or soluble polysaccharide), starch, cellulose, pentosan (hemicellulose), the total nitrogen, the total fat and ash of each sample were determined in the following ways, and the results are shown in the table, both seeds and seedlings having a mean weight of 100 grains.

1. **Moisture.** 2 to 3 grams of each sample were dried at 105°C. in an air bath to a constant weight, the loss of weight being designated as the moisture content.

2. **Alcohol Extract.** An 80% solution of alcohol was added to 2 to 3 gr. of each sample, and allowed to stand overnight and then heated at 70°C. in a water-bath for four hours, filtered, washed, and this process was repeated till the washings contained no more reducing sugar. The filtrate and washings combined together and concentrated under diminished pressure to a small volume, which brought down the final volume to 100 c.c.

(a) Total Reducing Sugars. Of 50 c.c. of the solution, the total reducing sugar was estimated by determining the reducing power of the solution and calculated as *d*-glucose on an ash and moisture free basis.

(b) Soluble Polysaccharides. After 50 c.c. of the extract above mentioned was heated with a 3 % sulphuric acid solution on a direct flame for 3 hours, diluted to 100 c.c., neutralized with sodium carbonate and filtered. The reducing sugars in this filtrate were estimated by determining the reducing power of Fehling's solution and calculated as *d*-glucose on an ash and moisture free basis. The difference between the content of the reducing sugars in the solution after and before the hydrolysis was designated as a soluble polysaccharide.

3. **Starch.** About 0.2 to 0.4 gram of the sample which might be expected to contain reducing sugar, soluble polysaccharide and starch were heated to hydrolyse the starch and polysaccharides into *d*-glucose by using 50 c.c. of 3 % sulphuric acid for 4 hours on a direct flame. The acid solution was diluted to exactly 100 c.c., neutralized with sodium carbonate and filtered, The content of reducing sugars in the filtrate was estimated in the usual way, from which the content of reducing sugars and soluble polysaccharides was subtracted and the remainder was assumed to be the starch-content.

4. **Pentosans.** The presence of pentosans in the seeds and seedlings was confirmed by transforming them into furfural; for the estimation of the sugar 1 to 2 grams of the sample were subjected to distillation with 12 % HCl, and the content of furfural distilled was determined by means of phloroglucin.

5. **Cellulose.** The amount of cellulose was determined by the method of Cross and Bevan.

6. **Total Fat.** The content of the fat in the samples was determined by the usual way by extracting it with petroleum ether.

7. Total Nitrogen and Protein. The total nitrogen of 0.1 to 0.2 gr. of the sample was estimated by Kjeldahl's method, and the protein content in the samples was calculated by multiplying 6.25 with the total nitrogen content.

8. Total Ash. The samples were ignited carefully at a low temperature and the crude ash, thus obtained, was dissolved in water, filtered, and the residue was heated in an electric furnace to a constant weight. The filtrate was dried in a water-bath, and then to constant weight at 105°C. The two ashes combined together were designated as the total ash content.

The analytical results of the seeds and seedlings are shown in the following table:

	Seeds	Seedlings	Gain or loss during germn.
Dry wt. of 100 grains	3.1 gr.	2.3 gr.	–.8 gr.
Ash	.136	.098	–.038
Total nitrogen	.039	.031	–.008
Fat	.066	.042	–.024
Protein	.255	.200	–.055
Reducing sugars	0	.088	+.088
Soluble polysaccharides	.048	.220	+.172
Starch	2.184	1.234	–.950
Cellulose	.213	.224	+.011
Pentosan	.162	.123	–.039

In the above table, the content of fat, protein, reducing sugar, soluble polysaccharides, starch, cellulose and pentosan was calculated on a moisture and ash free basis.

Discussion.

As will be seen by the results shown in the above table, the loss of weight occurs during the germination of rice seeds as other investigators have already observed in experiments on the germination of other seeds, due to the transformation of the reserve starch into other substances while the amount of total fat remains almost unchanged.

As a matter of fact, 0.950 gr. of starch were lost per 100 grains of rice seeds on the one hand, and on the other 0.260 gr. of soluble sugars calculated as *d*-glucose were found to be formed during germination, and accordingly 0.716 gr. of the starch had disappeared from the seeds; in other words, they are consumed by being converted into carbon dioxide and water for the germination. Thus, the fate of the starch in the seeds during germination would be considered in the following manner; one part of starch was transformed by hydrolysis into soluble polysaccharides and simple sugars of unknown nature, and one part of the sugars was then decomposed by oxidation into carbon dioxide and water, similar to the function of respiration.

$$(C_6H_{10}O_5)_n \xrightarrow{+H_2O} C_6H_{12}O_6 \xrightarrow{+O} CO_2+H_2O.$$

The energy liberated in these transformations of starch was evidently utilized for the germination of the seeds. The main source of the energy would be supplied by the complete combustion of the simple sugar molecule in the plant tissues since the heat of formation of both the soluble polysaccharides and the simple sugars would be negligibly small compared with that of the combustion of the sugar molecule, as will be seen in the following equations:

$$C_6H_{12}O_6+6\ O_2=6\ CO_2+6\ H_2O+674{,}000\ \text{cal.}$$

$$C_{12}H_{22}O_{11}+12\ O_2=12\ CO_2+10\ H_2O+1{,}350{,}000\ \text{cal.}$$

$$C_6H_{10}O_5+H_2O=C_6H_{12}C_6+5{,}900\ \text{cal.}$$

Thus, the energy which was required for the germination of one grain was estimated approximately from the quantity of diminished starch based on the assumption stated above to be 3000 cal.

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