STUDIES ON INDIGENOUS STARCHES OF PAKISTAN

Part IV.—Comparative Microphotographic Investigations in the Digestive Disintegration of Starch Granules

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(Received November 27, 1961)

The salivary digestion of heated swollen granules of shati, cassava, round potato (white and red skin), wheat and banana starches were studied on microscope slide and the course of digestion evaluated by microphotographs taken at frequent intervals following the discharge of iodine-stained blue colour. From the microphotographic observations it appeared that in case of potato, cassava and wheat starch probably the walls of the granules are ruptured by the centripetal force of the enzymes and the digestion, starting from the outer zone, gradually proceeds towards the centre. The wall of the banana starch granule, instead of being ruptured by the above process, allows the passage of the enzymes through the pores and digestion in the granules of this starch starts from the centre and proceeds towards the outer zone. Shati starch granule, because of its stronger boundary wall is not digested by any of the above processes. In this case the enzyme penetrates through a hole at the hilum end, which is formed during swelling by boiling, and the digestion then starts from the hilum end and gradually proceeds towards the other end.

Introduction

In previous communications[1-3] some chemical and physical characteristics of the starches extracted from the locally grown plants like shati (*Curcuma zedoaria*), tapioca (*Manihot utilissama*) and sweet potato (*Ipoemea batata*) were reported. In the course of thorough investigation on the starch from shati, about which nothing has yet been reported in the literature, it has been observed that this starch, unlike other tuber and root starches, possesses a high amylose content of 32%. Through spectrophotometric study it has further been determined that the percent transmission of the iodine complex of this starch at the absorption peak of 610 $\pm$ 5 was lower than those due to other two starches; and that all the values maintained reciprocally linear relationship with the amylose contents and the size of the starch granules.[2] Investigation on salivary digestion yielded lower percentage of conversion of this starch in both cooked and uncooked conditions as compared to the other two.[3] All these facts point to peculiar characteristics of the granules of shati starch for which it is less digested by saliva when taken as food. In order to throw more light on this subject it is necessary to gather some information about the condition of the enzyme action on the above starch granules and also on the nature of its surface wall which may partly be responsible for the lower digestibility by the salivary amylase.

The present communication records our attempts in this line on the photomicrographic study of the digestibility of cooked shati starch by saliva (amylase) and its comparison with other starches from bulbous sources like tapioca, and round potato (both white and red skin), from fruit sources like green banana and from cereal a source like wheat.

The first approach in this field was made by Nagel[4] in 1855 who noticed the formation of pits followed by fissures on the starch granule causing its ultimate dissolution leaving a cellulosic boundary wall when the diastase enzyme of the cell sap was allowed to act on the starch of the germinating seed. The above phenomenon could not be duplicated by Brown and Heron[5] in their *in vitro* digestion study of starch by aqueous malt extract.

In recent years, with the development of refined microphotographic technique, newer attempts in this field were made by Badenhuizen and his collaborators.[6-8] They noted that the digestion of cereal starches was characterised by the formation of cracks and canals on the surface through which the enzymes penetrate, whereas that of the tuber starch from potato is effected by the centripetal force of the enzymes on the surface without the formation of any cracks etc.

In the course of preparation of the present manuscript the authors came across a report on the photomicrographic study of the mechanically damaged wheat starch by Sandstedt and Schroeder.[9] The latter have made studies on the digestibility of the damaged and undamaged wheat starch by α- and β-amylases by adopting similar technique as has been followed in the present investigation, the only difference being in the use of cinematographic camera by these workers, whereas only an ordinary still camera has been used in the present investigation.

Moreover, in their investigation the course of enzymatic digestion was studied by the disappearance of the granule but in the present case the process was studied by the disappearance of the granule and also by discharge of blue colour due to iodine staining. In this way the technique for the photomicrographic study of enzyme reaction has been further improved by the authors as evident from the observations discussed below.

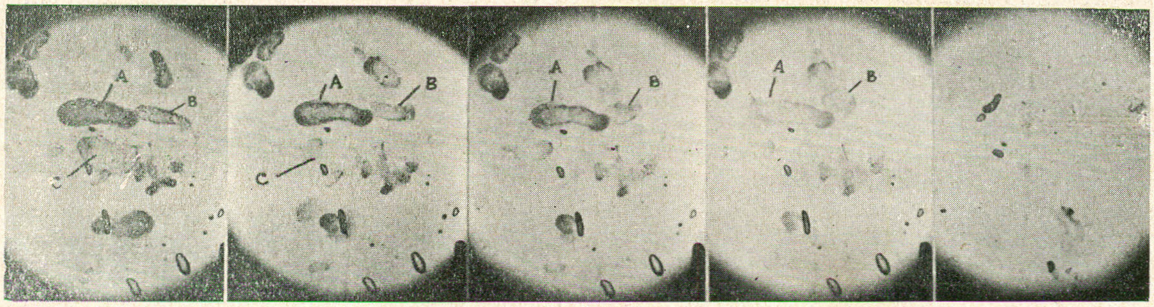
Experimental

The concentration of the starch suspension, iodine solution and of saliva, which would give reproducible results and would allow microphotographic study of the course of digestion, were first evaluated by a series of trial experiments. The digestion was studied on both raw and boiled swollen starch granules but since the rate of digestion of raw starch was very slow for differentiation under the microscope, the photomicrographic observations of the course of digestion of the boiled starch, which took only a few minutes for complete dissolution of the granules are, therefore, reported here.

One per cent alcohol-washed dry starch was suspended in water and heated for 5 minutes at constant temperature of 80°C. A drop of the above starch suspension after cooling was placed by micropipette on the slide followed by a drop of 0.1% iodine solution and a drop of 1:3 filtered saliva. One snap was taken immediately showing the start of the reaction. This was followed by further snaps at $\frac{1}{2}$ to 3 minutes intervals with the progress of digestion. The starches from the following sources were studied in the present investigation: *Curcuma zedoaria* (shati), green banana, tapioca, wheat, and white and red-skin potato.

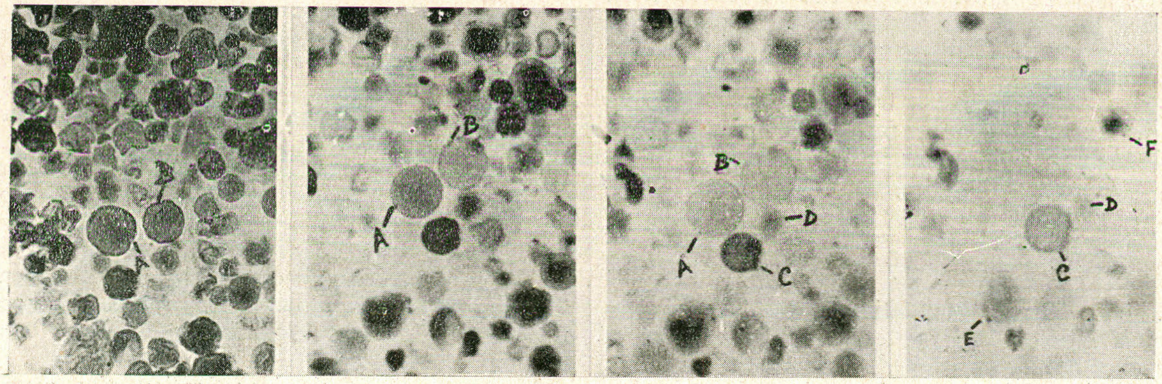
Results

Banana Starch.—Fig. 1 represents the digestion of cooked banana starch, and Fig. 1(a) shows the start of the digestion after addition of saliva and iodine. Figs. 1(b to d) represent the progress of digestion at 1-minute interval and Fig. 1(e) after a 2-minute interval. It may be



(a) (b) (c) (d) (e)

Fig. 1.—Salivary digestion of Banana starch. Fig. 1(a) represents the start of the digestion after addition of iodine and saliva. Figs. 1(b) to 1(d) represent the course of digestion after 1-minute interval and the last Fig. 1(e) after 2-minute interval i.e., after completion of total 4 minutes). Magnification each: 50×.



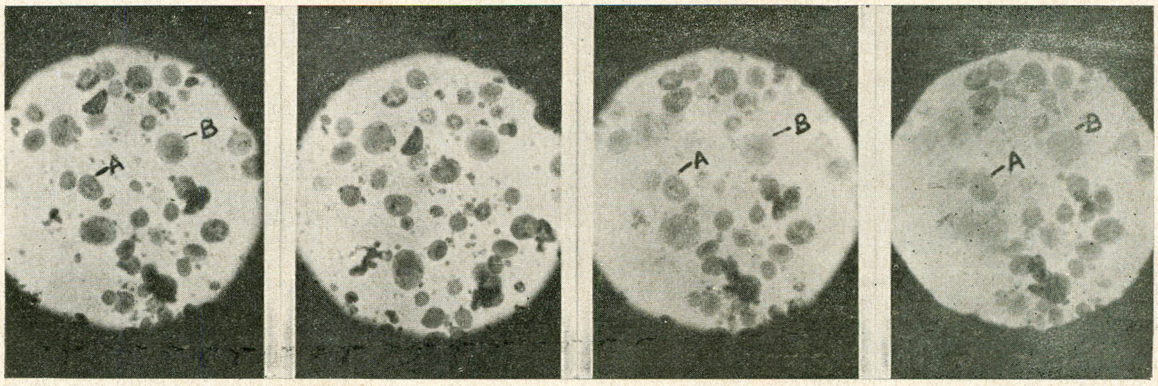
(a) (b) (c) (d)

Fig. 2.—digestion of cassava starch. Fig. 2(a) the initial stage after addition of saliva and iodine. Figs. 2(b) to 2(d) represent the digestion after 2 minutes interval. Here saliva of low concentration was used. The deep stained granules represent the unswollen starches and the light stained granules represent the swollen ones. Magnification each: 65×.

noted that intact granules A and B gradually fade and ultimately disappear after 5- minute digestion. The granule C which seems to have been ruptured during boiling is digested very quickly within 2 minutes.

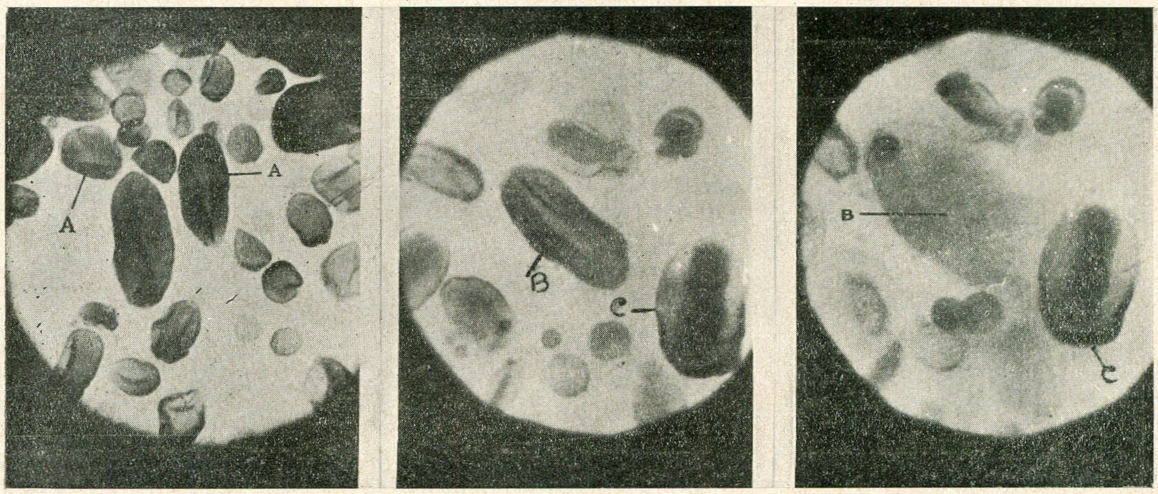
It is further noted that the digestion initiates from the centre and gradually approaches towards the wall which is also ultimately digested leaving no residue. One interesting feature is that the apex is stained darker and is not digested so easily as the central portion.

Cassava.—The photographs of Figs. 2(a to d) represent the course of digestion of boiled cassava starch with saliva. From Fig. 2(a) it will be seen that some granules have been stained deep and some light. Those with light stain are digested quickly within 2 minutes (cf. Fig. 2(b)). The granules A and B, with slightly deep stain at the centre, disappear at the last stage after 6 minutes; whereas the granules C and D, with deep black stain, are partially digested even after 6 minutes. Similarly the granules E and F which perhaps did not swell properly and were thus stained black are not completely digested even after 6 minutes. Regarding the course of digestion it appears that probably this starts from the outer surface and proceeds towards the centre as evident from the digestion of the granules E in Figs. 2(b and c).



(a) (b) (c) (d)

Fig. 3.—The digestion of wheat starch granules. Fig. 3(a) denotes the initial stage and others the subsequent stages of digestion. Magnification each: 65×.



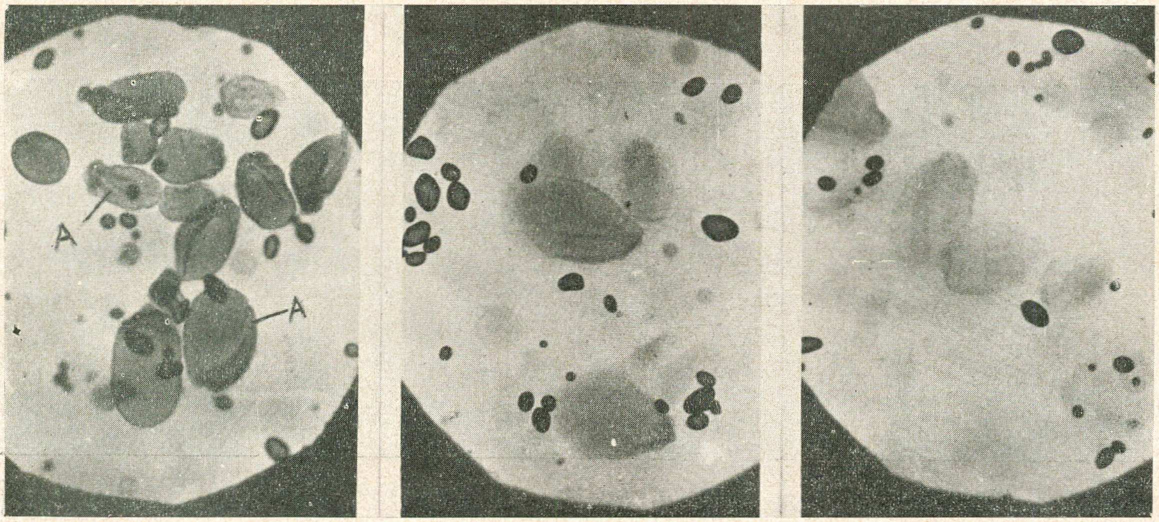
(a) (b) (c)

Fig. 4.—White skin potato starch digestion. After initial stage (Fig. 4(a) some granules were focussed in magnified condition represented by Figs. 4(b) and 4(c). Magnification each: 60×.

Wheat Starch.—The digestion of wheat is represented by Fig. 3. Unlike the previous case the digestion seems to start from the side of the wall gradually proceeding towards the centre (granules A and B). Further, black spot in this starch is noted at the centre and not at the apex, unlike that in the case of banana starch; and this vanishes at the last stage of digestion.

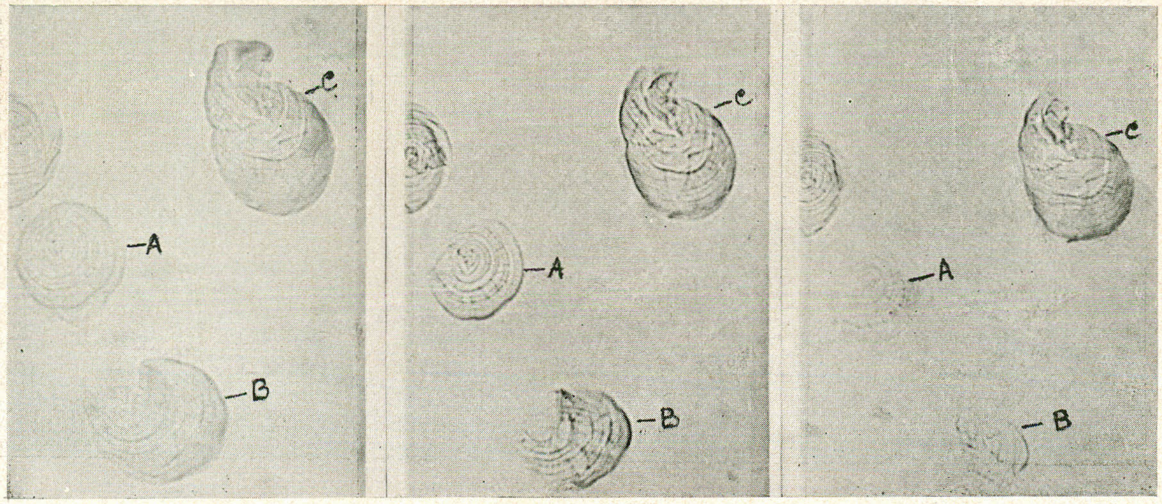
White-Skin Potato Starch.—Figure 4(a) indicates the start of the digestion and Figs. 4(b and c) are two different positions on slide showing the course of digestion in some granules. Here are also cracks on the walls marked by white lines, noted on some granules (A). Study of the granule B indicates the probable rupture of the cell for which the contents of the granule diffuse out and a black zone of wider area is formed. The granules A on the contrary are not ruptured. In the granules of this starch also, the digestion starts from the surface wall and proceeds towards the centre and gradually the black spot at the centre fades out (granule C of Figs. 4(b and c)).

Red Skin Potato Starch.—The photographs of Fig. 5 indicate the location of some black



(a) (b) (c)

Fig. 5.—Digestion of red skin potato starch. After intitial stage (Fig. 5(a)) digestion of some granules at magnified condition were photographed (Figs. 5(b) and 5(c)). Magnification each: 60×.



(a) (b) (c)

Fig. 6.—Digestion of shati starch. A few of the granules magnified for showing the course of digestion in three stages at Figs. (a), (b) and (c). Magnification each: 60×.

spots almost in every granule but in different positions—some cracks indicated by white lines appear on the cell wall (granules A). Figures 5(a and b) indicate the course of digestion which starts from the cell wall and proceeds towards the centre.

Shati Starch.—A very interesting feature of salivary digestion was noticed in case of shati starch as represented by Fig. 6. It will be observed from the picture that the granules consist of striations with dented rods axially arranged converging towards the hilum located eccentrically. Out of the three granules focussed, two (A and B) have been attacked by the enzyme, where digestion starts from the hilum end and gradually proceeds towards the wider side, dissolving the striated layer one after another until the whole granule disappears. Close observation will show the penetration of the enzymes in the above two granules A and B through the hole formed at the hilum and by swelling effect due to boiling. Unless this hole is formed the enzyme cannot enter the granules as is evident in case of granule C which shows the absence of the rupture at the hilum end and formation of a hole ultimately leading to its stabilisation against enzyme attack.

Discussion

It will be noted from the above photomicrographic study that the granules of starch extracted from different sources are attacked by the salivary amylase at different foci. The granules of red skin and white skin potato, wheat and cassava starches indicate the digestion starting from the surface. Perhaps in such cases the enzymes rupture the cell wall by centripetal force as postulated by Badenhuizen on this work on potato starch, and then initiate the digestion rapidly layer after layer proceeding towards the centre. The banana starch, on the other hand, shows the digestion gradually proceeding from the centre towards the wall. Perhaps in this case the enzyme instead of completely rupturing the cell wall by centripetal force, as in the case of the above starches, might have gained access within the granules through the pores of the surface wall, which might have expanded due to swelling by heating and acted as permeable membrane to the enzymes.

The surface boundary wall of the shati starch seems to be so constituted that it is neither ruptured by the centripetal force of the enzymes nor allow passage of the enzyme through the pores even after swelling due to heating. In this starch the enzyme digestion may occur only if the hilum end may be ruptured by heating forming a hole through which the enzyme will then enter and start digestion. Similar appearance of hole was noted by Bear and Samsa[10] in many granules of swelled potato starch at the hilum end. The importance of these holes in the process of enzymatic dissolution is now well realised from the present investigation. However, the above characteristic nature of the boundary wall of the shati starch granule may be one of the reasons for its poorer digestibility by saliva as compared to cassava and other starches as previously reported.[3]

In many of the iodine stained starch granules as discussed above, it has been noted that deep stained granules are digested more slowly than the light stained ones. This is due to differential dextrinisation of the granules due to heating for which some dextrinised to a greater degree, are swollen more, stained light but digested quickly. Less dextrinised granules, on the other hand, swell less but are stained deep and digested rather slowly. Sanstedt and Schroeder[9] in their work on raw wheat starch have, however, reported that damaged starches are stained deep with iodine and digested quickly. In that case the starch granules due to mechanical damage was extruded from the cell and exposed to iodine which ultimately stained those deeper. For such exposure of the free starch granules, the darker portions were, therefore, digested quickly in their experiment.

In the light of the above findings it may be further pointed out that the rupture of the granule wall by the centripetal force of the enzymes, penetration through the pores and rupture at the hilum end forming a hole for passage of the end will depend on the extent of swelling of the starch granules. If the granules are not properly swollen, the enzymes will not properly operate through any of the above routes and thus the granules will remain undigested. This is clearly pictured in many of the photographs above in which the deep stained unswollen (or not fully swollen) granules remain unattacked by the enzymes even when the other granules swollen properly disappear within a short time. This offers an explanation as to the lower digestion of crude starch as compared to boiled ones where the boundary wall changes to a characteristic feature amenable to enzyme digestion of the granule contents.

References

1. M. Qudrat-i-Khuda, H. N. De and M. Yasin, Pakistan J. Sci. Research, **12** 171 (1960).
2. M. Qudrat-i-Khuda, H. N. De, Nurul Haque Mia and M. Rahman, Pakistan J. Sci. Ind. Research, **5**, 62 (1962).

3. M. Qudrat-i-Khuda, H. N. De and J.C. Debnath, ibid., **5**, 30 (1962).
4. C. Nagel, *Die Starkekorner* (Zurich, 1858), referred to by H.I. Brown and G. Heron (loc cit.).
5. H. T. Brown and G. Heron, J. Chem. Soc., 35 1872.
6. N. P. Badenhuizen, Res. Trav. Vierland, **36**, 559 (1939).
7. N. P. Badenhuizen, Protoplasma, **32**, 440 (1938).
8. N. P. Badenhuizen, Handbuch der Pflanzen Physiologie, **6**, 137 (1958).
9. R. M. Sandstedt and Helen Schroeder, Food Technology, **14**, 257 (1960).
10. R. S. Bear and E.G. Samsa, Ind. Eng. Chem., **35**, 721 (1943).