

## STUDIES ON INDIGENOUS STARCHES OF PAKISTAN

Part III.—Photomicrographic and Spectrophotometric Study of the Iodine Complexes of Starches from *Curcuma Zedoaria* (Shati), *Manihot Utilissima* (Tapioca) and *Ipomoea Batata* (Sweet Potato)

M. QUDRAT-I-KHUDA, H. N. DE, NURUL HAQUE MIA AND M. RAHMAN

Food and Fruit Research Division, East Regional Laboratories, Pakistan Council of Scientific and Industrial Research, Dacca

(Received August 16, 1961)

The per cent absorption of the complex compound of iodine with starches extracted from *Curcuma zedoaria* (Shati), *Ipomoea batata* (sweet potato) and *Manihot utilissima* (Tapioca) grown here were studied within the range of wavelength from 250 to 650  $m\mu$ . The maximum absorption peak in all the cases was noted at  $610 \pm 5 m\mu$  and the minimum percent transmission values at this peak were found to be 30.4, 38.5 and 41.8 respectively for the above starches. These showed reciprocal linear relationship with the amylose contents and with sizes of the granules as determined by microphotography.

## Introduction

In a previous communication, Qudrat-i-Khuda, De and Yasin<sup>1</sup> reported the results of investigations regarding the amylose contents of the three indigenous starches from shati, tapioca and sweet potato. The method used is based on the ability of amylose fraction to bind iodine isopotentially with increase of E. M. F. and simultaneous failure of amylopectin fraction to do so. This difference in the property of binding iodine by amylose and amylopectin is also manifested in the difference in the intensity of colour produced by the iodine complex. Amylose, even in low concentration, reacts with iodine, giving an intense blue colour. The amylopectin, on the contrary, even in higher concentration gives only a faint reddish purple colour. This colour difference of iodine complex formed with amylose and amylopectin fraction in the starch granules has been studied photometrically by many workers engaged in this field.<sup>2-8</sup>

In their attempts at evaluation of a correlation between the minimum light transmission values of the iodine-starch colour with the amylose fraction, McCready and Hassid,<sup>5</sup> with the help of Klett Summerson photometer, and Kerr and Trubell<sup>4</sup> with the help of Coleman spectrophotometer have shown that the per cent transmission values of colour intensities produced by iodine with mixtures of pure amylose and amylopectin in varying proportions, maintained linear relationship with the concentration of amylose.

Since the amylose contents of the three starches reported in the previous paper by Qudrat-i-Khuda, De and Yasin<sup>1</sup> are quite different, it is expected that their colour intensities with iodine will also differ and this will ultimately influence the minimum light transmission values at a de-

finite wave length. The present work has been carried out with this object in view by adopting the techniques reported here.

The present paper also includes information on the microscopic character of these starches, their shape and size under the transmitted and polarised light so as to see how far the per cent transmission values are correlated with the size of the starch granules.

## Experimental

*Spectrophotometric Study.*—Before starting the actual experiment, the working concentration for each of the starch sample was evaluated by the verification of the Beer's law. It is seen from Fig. 1 that Beer's law holds good for the starch concentration from 0.05 to 0.3 g. per litre, although each of the starches has its characteristic transmission curve. The working concentration for these starches was selected in the range of 0.1 g. per litre (0.01%).

Iodine concentration of 0.1 g. per litre and potassium iodide to iodine ratio of 1.5 to 1 by weight as suggested by Simerl and Browning<sup>3</sup> yielded reproducible transmission curves.

Colour development of the starch solution was made by mixing this with iodine in the proportion of 10.1 in a 10-mm. cell and the per cent transmission measurement was recorded in the range of wave length from 250 to 650  $m\mu$  in an ultraviolet spectrophotometer.

*Microscopic Study.*—Photomicrography of the starches under transmitted light was performed with the help of a research microscope in a vertical position with binocular inclined eyepiece (Periplentic 10 $\times$ ) tubes, fitted with quadruple nose-

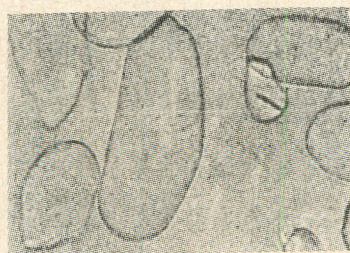
piece containing achromatic objective of N. A. 0.45 and 0.75, fitted with mechanical stage, two diaphragm condensers and a microphotographic camera. The photomicrographs were taken in 35 mm. Kodak Plux X film with green filter.

Photomicrographs under polarized light were taken with the help of rotating polarising condenser and an analyser, Periplentic eyepiece 10 ×, achromatic objective 45 : (N.A., 0.65) and with

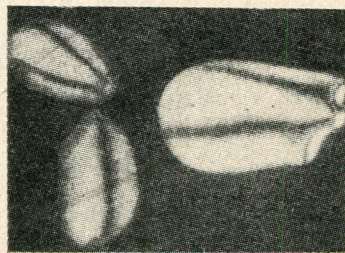
the same camera with micro-attachment and film as used above.

### Results and Discussion

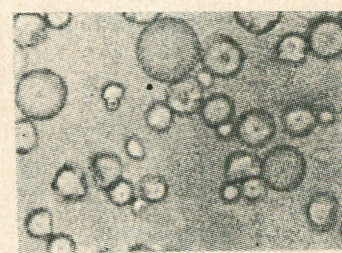
*Per cent Transmission Values of Starch Iodine Complex.*—From Fig. 2 it would appear that all these starches showed similar pattern of absorption peak at the narrow limit of wavelength of  $610 \pm 5\mu$ . The minimum per cent transmission values, as



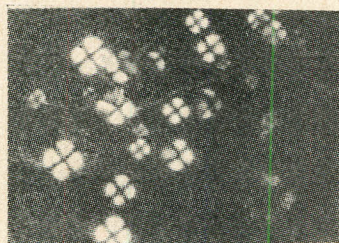
Shati starch. Mag., 400×



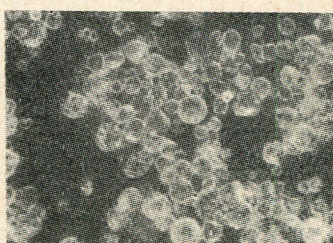
Shati starch under polarised light. Mag., 450 ×



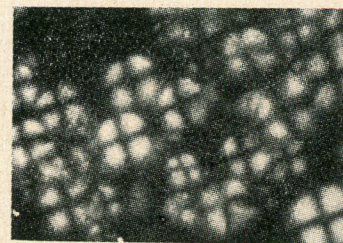
Tapioca starch. Mag., 400×



Tapioca starch under polarised light. Mag., 270×

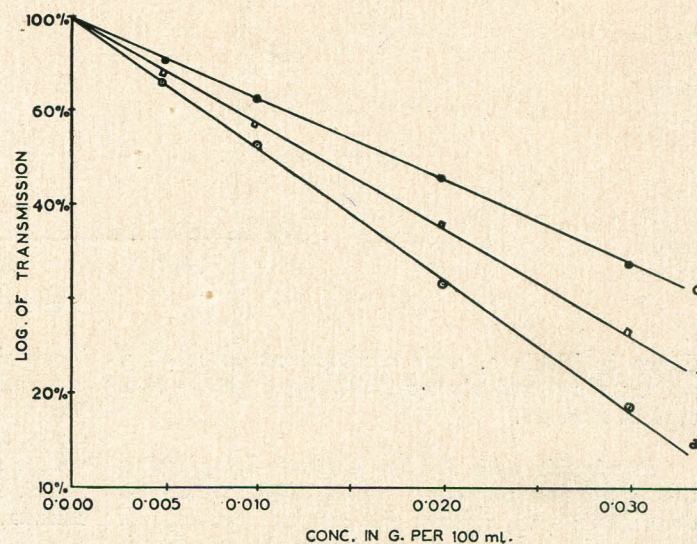


Sweet potato starch under phase contrast. Mag., 200×



Sweet potato starch under polarised light. Mag., 450×

Fig. 1.—The validity of Beer's law (at 550 m $\mu$ ) with (a) shati starch, (b) sweet potato starch (c) tapioca starch.



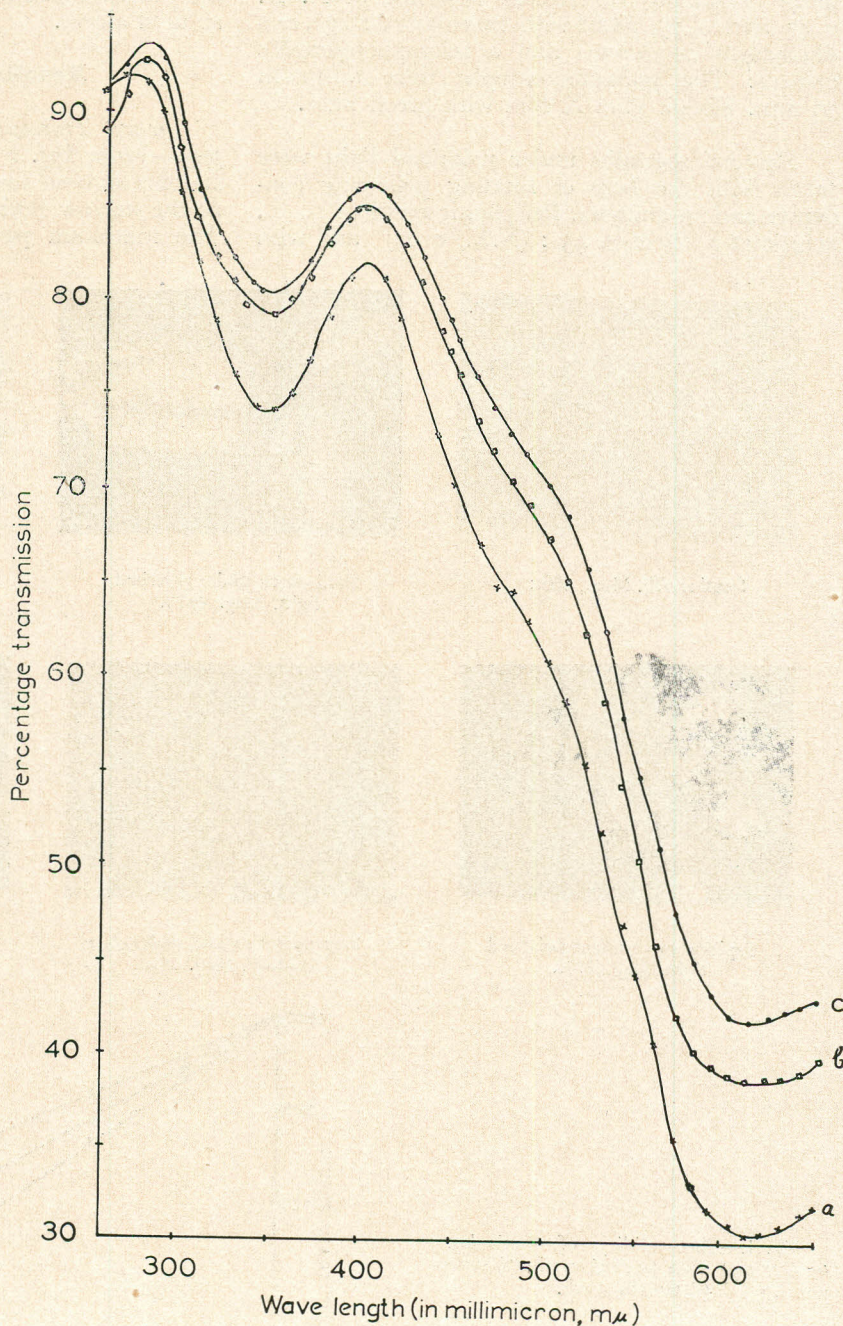


Fig. 2.—Absorption curves for iodine complexes with (a) shati starch, (b) sweet potato starch and (c) tapioca starch.

shown in Table 1, were recorded at 30.4, 38.5 and 41.8 respectively for shati, sweet potato and tapioca starches.

*Relationship with Amylose Content.*—By plotting the minimum per cent transmission values as described above, against the amylose contents of the starches reported previously by Qudrat-i-Khuda, De and Yasin,<sup>1</sup> a linear curve (Fig. 3)

is obtained which indicates the dependency of the iodine-complex colour intensity on its amylose fraction. In a reverse way the linearity of the curve as in Fig. 3 confirms the amylose content values as evaluated by potentiometric iodine titration method reported previously by Qudrat-i-Khuda, De and Yasin. From the measurement of minimum per cent transmission values of iodine complex of any other starch by following

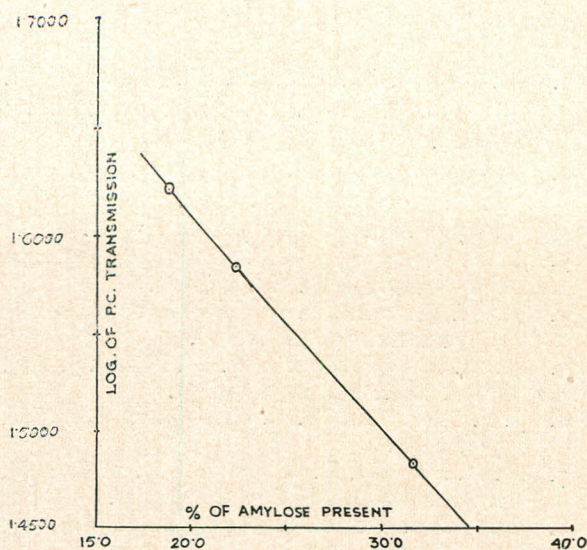


Fig. 3.—The relationship between the amylose content and log of p.c. transmission.

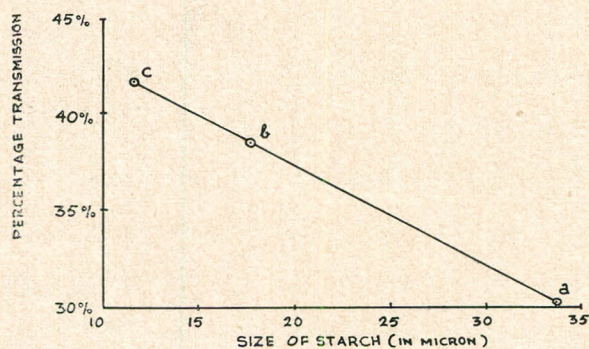


Fig. 4.—The relationship between the size of the starch granules and the percentage transmission of (a) shati starch, (b) sweet potato starch and (c) tapioca starch at wave length 610  $m\mu$ .

the above procedure, its amylose fraction can be evaluated from the above linear graph (Fig. 3), provided the absorption curve shows the peak at  $610 \pm 5\mu$ . For absorption peak at any other wave length this graph may not be applicable for the determination of amylose values.

**Relationship with Starch Size.**—It has been reported by Paloheims and Antila<sup>9</sup> that the minimum per cent transmission depends also on the size of starch granules. They noted that large granule starch showed larger absorption than those of small granules. The average size of the three starches as shown in Table I and discussed in the following seems to maintain an inverse relationship with the per cent transmission values and this is represented by the linear curve as in Fig. 4.

TABLE I.—RELATIONSHIP OF THE PER CENT TRANSMISSION OF IODINE COMPLEX WITH STARCHES FROM *Curcuma zedoaria* (SHATI), *Manihot utilissima* (TAPIOCA) AND *Ipomea batata* (SWEET POTATO) WITH THEIR AMYLOSE CONTENTS AND SIZE OF THE GRANULES.

Starch	Minimum transmission %	Amylose content %	Size of the granules in $\mu$	
			Range	Average
<i>Curcuma zedoaria</i> (Shati)	30.4	31.6	15.5-52.0	33.8
<i>Ipomea batata</i> (sweet potato)	38.5	22.4	5-30.0	17.5
<i>Manihot utilissima</i> (tapioca)	41.8	18.8	7-16	11.0

From these results it would appear that the colour intensity formed by starch with iodine depends not only on the amylose fraction but also on their sizes to a considerable extent.

Starches from other indigenous sources are now under investigation so as to explore the relationship between the size of the granules, their amylose content and the per cent transmission of their iodine complexes.

**Microscopic Character of Shati Starch.**—The granules with tapered elliptical shape resemble mostly the sago starch. The size varies from 15.5 to 52.0  $\mu$  with an average value of 33.8  $\mu$ . The hilum is eccentric and some striations are noted on the surface. Under the polarised light, two distinct black lines radiating from the hilum divide the granules into four distinct portions.

**Sweet Potato.**—The granules are variable in shape and size. Some are round and some show polygonal structure. The size varies from 5 to 30.0  $\mu$  with an average of 17.5  $\mu$ . Hilum is concentric and radiating fissures from the hilum are noted in some granules. Distinct black lines crossing the hilum are observed in the polarised light. But the pattern of the cross of the black lines under the polarised light is also variable due to variable shape of the granules.

**Tapioca Starch.**—The granules are mostly round and very few show oyster shapes like shell and kidney. The size varies from 7-16  $\mu$  with an average of 11.0  $\mu$ . Hilum is concentric with some faint

fissures radiating from this. In polarised light, the distinct black lines crossing the hilum at the centre are seen.

From the above microscopic structures of the starches it would appear that starch extracted from the sweet potato of this region is of the same size and shape as reported in the literature. Starch from the locally grown tapioca almost resembles in shape with those grown in other regions but their average size is less than that reported by Kerr<sup>4</sup> and Brautlecht.<sup>10</sup>

The microscopic structure of the shati is not reported anywhere else but its shape and size and other characteristics resemble with those of sago starch discussed above.

#### Acknowledgement

The authors express their sincerest thanks to Dr. S. Siddiqui, Chairman, P.C.S.I.R. for his interest in this work.

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