# STUDIES ON INDIGENOUS STARCHES OF PAKISTAN

## Part II.—Starches from the Rhizomes of Curcuma Zedoaria, Tubers of Manihot Utilissima (Tapioca) and of Ipoemea Batata (Sweet Potato) and their In-vitro Digestion by Saliva and Taka-Diastase

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The rhizomes of *Curcuma zedoaria* (Shati), tubers of *Manihot utilissima* (Tapioca) and *Ipoemea batata* (sweet potato) yielded total starch amounting to 64.1%, 68.4% and 69.4%, respectively, on dry basis. Actual starch contents of the crude starches were found to be 93.8, 96.4 and 94.0%, respectively, for the above three types on dry basis. Tubers of *Manihot utilissima* (Tapioca), thus appears to be economically a better source of starch compared to the other two. Investigation on in-vitro digestion of the above starches in raw and cooked condition by saliva and taka-diastase shows that with cooked starch 30 to 37% conversion to maltose occurs in four hours depending on the nature of the starch and enzyme source. Although apparently tapioca by saliva and shati by taka-diastase showed maximum digestion yet the true digestion by enzymes showed maximum effect on tapioca by both saliva and taka-diastase. The increase of digestion due to cooking has also been deduced from the above results and the significance of the values has been discussed.

### Introduction

In the previous communication from these laboratories, Qudrat-i-Khuda, De and Yasin[1] reported the amylose contents of the starches extracted from the three indigenous sources of *Curcuma zedoaria* (shati), *Manihot utilissima* (Tapioca) and *Ipoemea batata* (sweet potato). It was shown there that shati starch contained higher level of amylose (31.6%), compared to those of sweet potato (22.4%) and tapioca (18.8%). The present paper reports the chemical composition of the above starch sources and of the raw starches extracted therefrom, in order to ascertain the economic value of the above sources for the manufacture of starch in this region.

The digestibility of these samples of starches by saliva and taka-diastase has also been investigated so as to judge their suitability for edible and industrial purposes. The study of digestion by salivary α-amylase will aid to select the starch most suited for edible purpose. Digestion by taka-diastase, which is also an α-amylase, will give an indication as to how far these starches will be attacked by the fungal enzymes, of which taka-diastase is one of the members, if these starches are exposed to the air. Moreover, for edible and sizing purpose cooked starches are generally used. In the present study the per cent hydrolysis by the above enzymes on both the uncooked and cooked starches has thus been investigated so as to assess how far the cooking process influences the digestion by the above enzymes.

The digestion of different starches by various enzymes has been studied in various laboratories and all these have been reviewed by Kerr,[2] Meyer,[3] Bernfield,[4] Whelan,[5] Myrback,[6] Peat[7] and others. But there is no report regarding the per cent hydrolysis of shati starch by any enzyme.

### Experimental

*Process of Extraction of Starch.*—The rhizomes of *Curcuma zedoaria* and tubers of tapioca and sweet potato, after peeling off, were crushed by a flat stone crusher and the contents were then sieved to remove the fibres. The process was repeated and the starch was then separated from the liquid extract by centrifugation. Any starch remaining in overflow water from the centrifugation was allowed to settle. The liquid was decanted off and the paste at the bottom was returned to the original extracted liquor. The raw starch obtained was dried in an oven below 80°C. and then subjected to the analysis for the different constituents as follows: (*a*) moisture, protein, ash and fat were determined by A.O.A.C. methods, and (*b*) pure starch was determined by calcium chloride dispersion method followed by Clendenning et al.,[8–10] Earle and Milner,[11] and Steiner and Guthrie.[12]

*Digestibility.*—One ml. of diluted saliva (1:100) and one ml. of taka-diastase prepared by diluting 1 ml. of Parke Davis product to 5 ml., 5 ml. of starch solution uncooked or cooked at 80°C. for 15 minutes, 2 ml. of 1% sodium chloride solution and 2 ml. of phosphate buffer at pH 6.8 were incubated in 25-ml. conical flask for 4 hours at constant temperature water bath of 37°C. From the above mixture, 0.1 ml. was immediately withdrawn before the start of the incubation and its reducing sugar in terms of maltose was estimated by the alkaline potassium ferricyanide method of Janeson and Hagedorn.[13] Similar aliquots of 0.1 ml. were removed from the incubation mixture after intervals of 15 minutes each and their maltose contents were determined in the same way as above. From these maltose values the percentages of maltose produced by digestion from 100 g. of dry starch were calculated. Dry starch content of 50 mg. of starch taken for digestion was calculated on the dry basis values as shown in Table 1. From the above maltose values the percentage of conversion or hydrolysis due to enzymatic reaction was calculated, according to the following formula adopted by Myrback and Neumuller.[14]

$$\text{Per cent conversion or hydrolysis} = \frac{\text{Maltose \%}}{2 \times 1.055}$$

It has been observed that in the case of raw starch the maltose values at 15-minute interval at certain stages were not very large. So, for the raw starch the percentage of conversion values have been calculated at 30-minute interval. For cooked starch the values at 15-minute interval are shown in Table 4. The periods at each 15-minute interval are expressed chronologically in Tables 3 and 4.

*Apparent and True Conversion Values.*—It has been found that the initial maltose value before digestion (0 hr.), is not very significant in uncooked starches yet it shows some increase specially in case of shati starch after being cooked. Moreover, these initial maltose values did not show much difference whether treated with taka-diastase or saliva. These initial maltose values are apparently included in the subsequent values obtained at various stages of digestion. So, the percentages of hydrolysis calculated from the above maltose values do not indicate the "true" per cent hydrolysis values as effected mainly by enzyme activity. These have, therefore, been termed as the "apparent" per cent conversion values and thus expressed in Tables 3 and 4 which also include the initial value at 0 hr. The "true" per cent hydrolysis values at the final stage of hydrolysis after 4 hrs. digestion have been calculated by deducting the 0-hr. value from the apparent values of Tables 3 and 4 and these are presented in Table 5 so as to correlate as to how far the hydrolysis, directly affected or controlled by the above enzymes, are influenced by the nature of the starches.

The improvement in the per cent hydrolysis values due to cooking treatment has been deduced

TABLE 1.—THE COMPOSITION OF SHATI RHIZOMES, TAPIOCA AND SWEET POTATO TUBERS AND STARCHES EXTRACTED FROM THESE SOURCES.

| Constituents % | Shati Rhizome | Shati Starch | Tapioca Tuber | Tapioca Starch | Sweet Potato Tuber | Sweet Potato Startch |
|---|---|---|---|---|---|---|
| Moisture | 77.2 | 11.49 | 73.4 | 8.96 | 67.70 | 11.3 |
| Protein | 2.22 | 0.38 | 2.07 | 0.61 | 1.58 | 0.48 |
| Fat | 0.03 | 0.14 | 0.04 | 0.20 | 0.086 | 0.33 |
| Ash | 0 52 | 0.45 | 0.62 | 0.49 | 0.88 | 1.28 |
| Reducing sugar | 0.76 | 0.84 | 0.54 | 0.84 | 1.48 | 0.86 |
| Starch | 14.64 | 83.02 | 18.2 | 87.84 | 22.42 | 83.48 |
| | (64.1) | (93.8) | (68.4) | (96.4) | (69.4) | (94.0) |

The figures within the parentheses indicate the values on moisture-free basis.

TABLE 2.—STARCH PERCENTAGE ON DRY BASIS COMPARED WITH STARCH OF OTHER COUNTRIES.

| Starch | Moisture % | Starch % on moist basis | Starch % on dry basis | References |
|---|---|---|---|---|
| Tapioca tuber | 73.4 | 18.2 | 68.4 | Present observation |
| | 63.7 | 25.19 | 69.4 | Mamboo Leo East African variety-Raymond et al.[15] |
| | 70.25 | 21.45 | 72.1 | Eynon and Lane[16] |
| Tapioca starch | 8.96 | 87.84 | 96.4 | Present values |
| | 11.3 | 88.0 (including fibre) | 99.4 | Brautlecht[17] |
| | 12-16 | 81-89 | — | Radley[18] |
| | 10-18 | 81-89 (including fibre) | — | Eynon and lane[16] |
| Sweet potato tuber | 67.70 | 22.42 | 69.4 | Present observation |
| | 68.3 | 21.0 | 66.2 | Brautlecht[17] |
| Sweet potato starch | 11.3 | 83.48 | 94.0 | Present observation |
| | 15.85 | 81.30 | 96.61 | Japan variety Brautlecht[17] |
| Shati rhizomes | 77.2 | 14.64 | 64.1 | Present observation |
| Shati starch | 11.49 | 83.02 | 93.8 | Present observation |

by deducting the apparent and the true values of the uncooked starches from the cooked ones and these are shown in columns K and L of Table 5.

**Results and Discussion**

*Composition of Starches and their Sources.*—The results of analyses of the rhizomes of *Curcuma zedoaria* and tubers of sweet potato (*Ipoemea batata*) and tapioca (*Manihot utilissima*) and of their starches are shown in Table 1. In order to assess the economics of these sources for the starch production, all the values have been expressed on dry basis and are presented in Table 2 for comparison with other such values reported in the literature. It would appear that the actual starch content of the tubers of tapioca and of sweet potato, and of their crude starches do not differ much from the values obtained from similar varieties produced in other countries. The results on 'shati' cannot be compared with any other value as this is of local origin and no work has yet been reported in the literature on this plant product.

While comparing the total yield of starch and the actual starch content from the above three sources it appears that on dry basis, tubers of tapioca and sweet potato yield more of total and the actual starch as compared to that extracted from ther hizomes of *Curcuma zedoaria.* Comparatively, which of these two sources will be more suited for the commercial exploitation, will be best judged after collection of sufficient data about their acreage yield, cost of fertilisation, irrigation etc.

*In Vitro Digestibility.*—From the results detailed in Table 3, it would appear that the per cent conversion of the three starches in the uncooked condition, by both saliva and taka-diastase, in-

TABLE 3.—*In-vitro* DIGESTION OF UNCOOKED SHATI, TAPIOCA AND SWEET POTATO STARCHES BY SALIVA AND TAKA-DIASTASE.

| Starch | Enzymes | Apparent per cent conversion values at different periods of digestion* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 2 | 4 | 6 | 8 | 10 | 12 | 14 | 16 |
| Tapioca | Saliva | 0·38 | 1·3 | 1·9 | 3·0 | 3·8 | 5·0 | 6·2 | 7 | 8 |
| | Taka-diastase | 0·38 | 0·8 | 1·2 | 1·7 | 1·9 | 2·4 | 2·7 | 2·9 | 3·1 |
| Shati | Saliva | 0·40 | 1·2 | 1·8 | 2·6 | 3·0 | 3·2 | 3·4 | 3·6 | 3·8 |
| | Taka-diastase | 0·40 | 0·7 | 1·2 | 1·7 | 1·9 | 2·2 | 2·4 | 2·6 | 2·8 |
| Sweet potato | Saliva | 0·38 | 1·0 | 1·4 | 2·4 | 3·2 | 3·6 | 4·0 | 4·3 | 4·6 |
| | Taka-diastase | 0·38 | 0·7 | 1·4 | 2·0 | 2·4 | 2·8 | 3·0 | 3·1 | 3·2 |

*The chronological numbers indicate the periods at 15-minute intervals.

TABLE 4.—*In-vitro* DIGESTION OF COOKED SHATI, TAPIOCA AND SWEET POTATO STARCHES BY SALIVA AND TAKA-DIASTASE.

| Starch | Enzymes | Apparent conversion values at different periods of digestion* | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| Tapioca | Saliva | 1·18 | 17·5 | 23·5 | 26·5 | 27·8 | 28·4 | 30·0 | 30·8 | 31·5 | 32·1 | 33·0 | 33·7 | 34·4 | 35·2 | 35·8 | 36·5 | 37·0 |
| | Taka-diastase | 1·18 | 9·4 | 13·0 | 16·4 | 18·6 | 21·0 | 22·0 | 23·1 | 24·2 | 25·0 | 25·8 | 27·2 | 28·5 | 29·5 | 30·4 | 31·0 | 31·5 |
| Shati | Saliva | 3·19 | 9·5 | 14·5 | 19·6 | 21·4 | 23·7 | 25·3 | 27·0 | 29·0 | 30·4 | 32·0 | 33·0 | 33·6 | 34·1 | 34·4 | 34·8 | 35·1 |
| | Taka-diastase | 3.19 | 8.0 | 13.0 | 16.8 | 20.1 | 21.8 | 23.7 | 25.5 | 25.6 | 27.2 | 27.8 | 28.4 | 29.1 | 29.7 | 30·9 | 31·5 | 32·0 |
| Sweet potato | Saliva | 1.40 | 15.0 | 22.1 | 24.2 | 26.0 | 27.2 | 28.1 | 29.0 | 29.8 | 30.6 | 31.3 | 31.8 | 32.6 | 33.1 | 33.6 | 34.0 | 34.5 |
| | Taka-diastase | 1.40 | 11.4 | 16.0 | 18.6 | 21.8 | 24.0 | 25.5 | 26.4 | 27.0 | 27.6 | 28.2 | 29.0 | 29.5 | 30.0 | 30.2 | 30.5 | 30.5 |

*The chronological numbers indicate the periods at 15-minute intervals.

creases gradually at a slower speed with the progress of incubation period without showing any sharp increase followed by a break of the curve. By both the above α-amylose sources the maximum hydrolysis is effected in the tapioca starch and minimum in the shati starch. When these starches were digested after cooking (Table 4) the per cent conversion increased considerably by both saliva and taka-diastase. The maximum per cent conversion by saliva after 4-hour incubation was found to be 37, 35.1 and 34.5 for tapioca, shati and sweet potato starches, respectively. In case of taka-diastase, the order of the superiority in per cent conversion was changed, shati showed the maximum digestion to the extent of 32.0 in this case. These results suggest that tapioca in the cooked condition will be better digested by saliva, and shati starch, on the contrary, will be more attacked by the fungal α-amylase like taka-diastase. The above relationship, however, does not remain valid if the above "apparent" per cent conversion values are expressed in terms of the "true" values as effected only due to enzymatic hydrolysis. From the study of results in Table 5 it would appear that dispersion of raw starch in cold water caused the production of 0.811 to 0.852% of maltose as a result of 0.38 to 0.40% conversion. Due to cooking, the above values in case of shati starch showed marked increase of 6.74% maltose due to 3.2% conversion. The other two starches did not show such high increase of the maltose formation and consequently of the per cent hydrolysis values. The "true" per cent hydrolysis values after 4-hour digestion, calculated by deducting the above blank initial zero hour conversion values from the corresponding apparent values, show that by both saliva and taka-diastase the maximum hydrolysis occurs in the tapioca starch and minimum in the shati starch, sweet potato starch standing in between the two (columns F and J of Table 5). This shift in the order of the superiority of enzyme digestion by saliva and taka-diastase on the three starches, when their "apparent" conversion values are converted to "true" conversion values, is mainly due to the influence of the per cent conversion value of these starches by their enzymes at the initial stages, i.e., at 0 hr. before digestion had started, which, however, cannot be discounted. For ordinary study of comparative digestion by saliva and taka-diastase or by any other enzyme, the "apparent" values have to be considered since the blank hydrolysis values, which have a great bearing on the ultimate digestion, are included in the above apparent conversion values.

*Increase of Per Cent Conversion Due to Boiling Treatment.*—From the results presented in columns I and J of Table 5 it would appear that the cooked samples show a wide range of variation from 30.5 to 37 in case of apparent values and from 28.8 to 35.8 in case of true values. When the effect of cooking on conversion values of starch is only to be considered, which may be deduced by deducting the values of uncooked samples from

TABLE 5.—THE RELATIONSHIP BETWEEN THE 'APPARENT' AND 'TRUE' CONVERSION VALUES AND THE INCREASE OF THESE VALUES DUE TO COOKING OF THE STARCHES.

| | | Uncooked Starch | | | | Cooked Starch | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Starch | Enzymes | Maltose % at 0 hr. | Apparent % conversion at 0 hr. | Apparent % conversion after 4-hr. digestion | True % conversion after 4-hr. digestion (E-D) | Maltose % at 0 hr. | Apparent % conversion at 0 hr. | Apparent % conversion after 4 hrs. | True % conversion after 4 hrs. (I-H) | Increase in apparent % conversion value due to cooking (I-E) | Increase in true % conversion values due to cooking (J-F) |
| (A) | (B) | (C) | (D) | (E) | (F) | (G) | (H) | (I) | (J) | (K) | (L) |
| Tapioca | Saliva | 0 811 | 0.38 | 8 | 7.62 | 2.50 | 1.18 | 37 | 36 82 | 29 | 28.2 |
| | Taka-diastase | 0 811 | 0.38 | 3.5 | 3.12 | 2.50 | 1.18 | 31.5 | 30.32 | 28 | 27.2 |
| Sweet potato | Saliva | 0.827 | 0.39 | 4.6 | 4.21 | 2.97 | 1.40 | 34.5 | 33.1 | 29.9 | 28.89 |
| | Taka-diastase | 0.827 | 0.39 | 3.3 | 2.91 | 2.97 | 1.40 | 30.5 | 29.1 | 27.2 | 26.19 |
| Shati | Saliva | 0.852 | 0.40 | 3.4 | 3.0 | 6.74 | 3.2 | 35.0 | 31.8 | 31.6 | 28.80 |
| | Taka-diastase | 0.852 | 0.40 | 2.8 | 2.4 | 6.74 | 3.2 | 32.0 | 28.8 | 29.2 | 26.20 |

the cooked ones, it would appear from the results presented in columns K and L of the same table, that the increase of the apparent and true per cent conversion values only, due to cooking, shows a narrow range of variation of nearly 1.2% between the minimum and the maximum values. Thus it is evident that the wide range of variation in the per cent conversion values, both apparent and true, of the cooked samples of different starches, is not mainly due to the effect of cooking, but to the initial large variation in the per cent conversion value of the uncooked samples, which ultimately influences the values of cooked ones. It is further noted from the cooked tapioca starch that salivary digestion caused 5.5% more conversion than taka-diastase digestion. But when the effect of cooking only is considered it is noted from columns K and L of the same table that salivary digestion causes only one per cent more conversion than the taka-diastase digestion. Similar is the case with the other starches also. It may be similarly argued that the apparently large difference in the conversion values between the taka-diastase and salivary digestion of the cooked samples is mainly due to difference in the action of these enzymes on the uncooked starches, the effect of which ultimately influences the values of cooked samples. Although theoretically, as discussed above, there is no such difference between the taka-diastase and salivary digestion values when the effect of cooking treatment only is considered, nevertheless, from ultimate digestion point of view the conversion values of the cooked starches, which reasonably include those of the uncooked ones also, have to be taken into account.

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