MICROMANIPULATIVE STUDIES ON GELATINIZED STARCH GRANULES

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(WITH TWO FIGURES)

Gelatinized starch granules are generally considered to be comprised of sacs or outer membranes of starchy material enclosing a more or less fluid colloidal starch dispersion [see reviews by Radley (10) and Kerr (6)]. NägeI (9) and Meyer (8) considered the membrane to be preexistent in the native, ungelatinized granule. Alsberg (1) and Badenhuizen (2, 3) on the other hand, concluded that the membrane is formed as an artifact by the swelling and coalescence of the more resistant lamellae. Experimental results reported by Hess and Rabinowitsch (5) and further discussed by Roberts (11) indicate that, however it may be formed, the outer membrane of the gelatinized granule is tough and can be punctured by a needle, releasing a fluid colloidal suspension with which the sac is filled.

During the course of an investigation at this Laboratory of the factors affecting starch paste viscosity, micromanipulative studies were made of the degree to which gelatinized granules of corn, potato, tapioca, and glutinous corn starch could be stretched. Several hundred granules of each kind of starch were observed, each granule being stretched until broken. The main purpose of the study was to determine whether length of paste might be correlated with ability of the individual unbroken granules to stretch. (A long paste has a small yield value and low mobility.) Information was also obtained on the degree to which starch granules could be stretched after pasting at different temperatures and for different lengths of time. Some effects of chemical pretreatment of starch granules upon their subsequent ability to stretch were also noted.

The starches used in these experiments included commercial tapioca starch, laboratory-processed glutinous corn starch, and both commercial and laboratory-processed corn and potato starches. Nitrogen and ash contents of these starches, determined as described by MacMasters and Hilbert (7), are given in table I. The laboratory-processed samples of corn starches were prepared by the method described by Cox, MacMasters, and Hilbert (4).

Methods

In order to move and stretch the starch granules, fine glass needles were used in a pair of Fitz micromanipulators which permitted movement in three planes. Gelatinized starch granules were stretched at room temperature and at approximately 40° C. For stretching at room temperature a glass micromanipulator cell of conventional type was used. The gelatinized starch was suspended in a small drop of water on a cover slip and the slip
### TABLE I

**Description of Starch Samples Used**

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Starch</th>
<th>Method of Preparation</th>
<th>Percentage on Dry Basis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>N</td>
</tr>
<tr>
<td>1</td>
<td>Tapioca</td>
<td>Commercial</td>
<td>0.01</td>
</tr>
<tr>
<td>2</td>
<td>Glutinous corn</td>
<td>Laboratory 0.1% SO₂ steep</td>
<td>0.04</td>
</tr>
<tr>
<td>3</td>
<td>Corn</td>
<td>Commercial SO₂</td>
<td>0.05</td>
</tr>
<tr>
<td>4</td>
<td>Corn</td>
<td>Laboratory H₂O</td>
<td>0.03</td>
</tr>
<tr>
<td>5</td>
<td>Potato</td>
<td>Commercial No steep, SO₂ processed</td>
<td>0.01</td>
</tr>
<tr>
<td>6</td>
<td>Potato</td>
<td>Laboratory H₂O</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Inverted over the opening in the center of the glass cell. A similar micro-manipulator cell heated by water circulated from a constant temperature bath was employed for samples stretched at 40° C.

Individual starch granules were stretched by placing two micro-needles upon the granule and slowly pulling the needles apart. The needles were placed near the center of the granule, then as they were pulled apart, they slipped over the surface of the granule until near the granule periphery. Thus stretch was measured throughout essentially the whole length of the granule. Considerable care was necessary in placing the needles upon a granule so as not to puncture it prior to stretching. When granules were biaxial, as in potato starch, they were pulled so that the stretch would be along the long axis. For the starches which had approximately spherical granules, alignment was necessarily at random. The needles were pulled apart slowly. Rapid or jerky movement of the needles caused the granules to break before the maximum degree of stretch was reached. When the granules were stretched and broken, the granule fragments would snap back around their respective needles. Measurements were made with a microscope equipped with a calibrated ocular micrometer. The percentage stretch of each granule was calculated.

\[
\text{Percentage stretch} = \frac{\text{Stretched diam.} - \text{original diam.}}{\text{Original diam.}} \times 100.
\]

Percentage of stretch within any one sample varied considerably. Twelve corn starch granules from a sample pasted for 10 minutes in a boiling water bath and stretched as soon as they had cooled to room temperature (25° C.) gave 55 to 222 per cent. stretch, average 127. Another 13 granules, prepared and stretched in an identical manner gave 100 to 207 per cent. stretch, average 150. For 140 corn starch granules, including the 25 already mentioned, the average stretch was 157 per cent. These granules were chosen at random from 11 different samples. These data were analyzed statistically and it was found that the probability is about 99 per cent. that the mean of the sample, 157, is within 10 of the mean of the population of starch granules; i.e., it is practically certain that the mean of the stretch of starch...
granules taken from similar starch pasted under similar conditions will fall within the range of 147–167 per cent.

Results and Discussion

NATURE OF THE INTERIOR OF STARCH GRANULES

ROBERTS (11), in a résumé of work which he had carried out earlier with Hess (5), reported that with an ultramicroscope Brownian movement could be seen within gelatinized starch granules to which alcohol or other organic solvent had been added. BADENHUIZEN (3) reported that upon pricking an untreated swollen starch granule with a needle the granule did not crumple nor did any material flow out from it. Repetition of the work of these two authors confirmed the results of both. The apparent conflict in their results can be attributed to the use by ROBERTS of an organic solvent which causes precipitation of the colloidal material within the liquid contents of the granule.

Gelatinized potato starch stained with approximately 1 per cent. I$_2$KI solution appeared to have a firm gelatinous center (fig. 1a) around which there was a clear transparent margin. When these granules were stretched and broken, the break was clean (fig. 1b).

When 0.01 per cent. solution of I$_2$KI was used, the interior of the granule was not precipitated and the break was ragged. It was reported by BADENHUIZEN (3) that the iodine staining hardened the granules and made them...
much more difficult to cut than unstained granules. The clear margin which surrounds the dark-stained center of the granule is attributed by Baden-
Huizen (3) to the thinness of the granule in this region.

In order to determine whether reorientation of the carbohydrate mole-
cules within the starch granules could be brought about by stretching swollen starch granules, a commercial corn starch (No. 3, table I) was gelatinized in a boiling water bath and stretched between crossed Nicol prisms. The gran-
ules were stretched parallel to the axis of the polarizer and no birefringence
was observed. The micromanipulator needles were then set approximately
30° from the axis, but still no birefringence was exhibited by the stretched
granules. Although it is known that in many instances the addition of I₂KI
solution will bring about a return of birefringence to gelatinized starch
granules (unpublished data), when granules were stained with iodine solution
and subsequently stretched between crossed Nicol prisms, no birefringence
was seen.

**Effect of Temperature at Time of Stretching**

The length to which granules could be stretched at 25° C. was not sign-
ificantly different from that to which similar granules could be stretched
at 40° C. This was true for each starch studied regardless of pasting tem-
perature and time.

**Effect of Pasting Temperature**

A significant increase was found in the percentage stretch of granules
with increasing temperature of pasting for both corn and potato starches.
Corn starch granules pasted at 80° C. showed a mean stretch of 106 per
cent. compared with 152 per cent. for those pasted in a boiling water bath.
In the case of potato starch the difference between samples pasted at 80° C.
and 90° C. was highly significant, the respective averages of stretch being
140 and 185 per cent.

**Effect of Pasting Time**

Results of the statistical analysis of data (table II) collected on com-
mercial corn starch (No. 3, table I) pasted at 80° C. for 10, 30, and 60
minutes and for 72 hours showed that the percentage of stretch in samples

<table>
<thead>
<tr>
<th>STARCH/STATISTICAL MEASURE</th>
<th>Time of Pasting</th>
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<tbody>
<tr>
<td></td>
<td>10 min.</td>
</tr>
<tr>
<td>Corn starch</td>
<td></td>
</tr>
<tr>
<td>Mean stretch (%)</td>
<td>94</td>
</tr>
<tr>
<td>No. in sample</td>
<td>12</td>
</tr>
<tr>
<td>Standard dev.</td>
<td>23</td>
</tr>
<tr>
<td>Potato starch</td>
<td></td>
</tr>
<tr>
<td>Mean stretch (%)</td>
<td></td>
</tr>
<tr>
<td>No. in sample</td>
<td></td>
</tr>
<tr>
<td>Standard dev.</td>
<td></td>
</tr>
</tbody>
</table>
pasted 30 minutes is significantly greater than that in samples pasted for all other lengths of time used (table III). No difference was shown among samples pasted for 10 minutes, 60 minutes, and 72 hours. From the available data, it is impossible to say at what time between 10 minutes and 1 hour maximum stretch would occur.

Although the data for potato starch (No. 5, table I) are incomplete, granules pasted for 30 minutes at 80° C. were found to stretch considerably more than granules from a similar sample pasted for 72 hours (table II). There was a highly significant difference in the length to which granules pasted for these two time intervals would stretch before breaking.

### TABLE III

**Significance of difference of stretch between samples of corn starch pasted for varying lengths of time at 80° C.**

<table>
<thead>
<tr>
<th>Time of pasting</th>
<th>Time of pasting</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>10 minutes</td>
</tr>
<tr>
<td>10 minutes</td>
<td></td>
</tr>
<tr>
<td>30 minutes</td>
<td>....</td>
</tr>
<tr>
<td>60 minutes</td>
<td>....</td>
</tr>
<tr>
<td>72 hours</td>
<td>....</td>
</tr>
</tbody>
</table>

* Indicate 5 per cent. level of significance.
† No significant difference.
‡ Approaches 1 per cent. level of significance.

**Relation of Stretch to Starch Paste Viscosity**

One of the objects of this investigation was to determine whether there is any correlation between paste viscosity and the ability of individual granules to stretch. There is known to be a rough correlation between length of paste, tackiness, and paste viscosity. For example, corn starch which has a short paste has a low paste viscosity and tapioca and potato starch which have long tacky pastes have a high paste viscosity. In concentrated pastes, in which the granules might be in close contact with each other, the ability of individual granules to stretch might contribute materially to the length of the paste. From the data at hand, however, it would appear that there is no correlation between the ability of a starch granule to stretch and the paste viscosity of the starch.

The MacMichael viscosity (at 95° C., 20 r.p.m.) of 3 per cent. suspensions pasted at 95° C. for 10 minutes with stirring at 120 to 160 r.p.m. is 375, 270, 95, and 140 C.P. for glutinous corn, tapioca, corn and potato starches, respectively. Glutinous corn starch or tapioca starch pasted for 10 minutes in a boiling water bath could be stretched to about 3 times or 200 per cent. of the original granule length (fig. 2) as compared with 157 per cent. for corn and 145 per cent. for potato starches.

The stretch of pasted granules of corn starch which had been processed with sulfur dioxide (No. 3, table I) was compared with the stretch of simi-
larly pasted granules of the same kind of starch processed with distilled water (No. 4, table I). The latter had higher viscosity. There was no more difference in stretch between these two starches than there was among granules within either sample. A portion of commercial corn starch (No. 3, table I) was shaken for one hour in N/32 NaOH solution and subsequently washed free of alkali. This treatment increased the paste viscosity of the starch. The degree of stretch of the pasted granules was, however, the same as for the untreated starch. Treatment of corn starch with dilute solution of either acid or alkali did not alter its potentiality for being stretched.

**COHESIVENESS OF STARCH GRANULES**

Sulfur dioxide treatment had a marked effect on potato starch granule properties. Potato starch processed with water (No. 6, table I) was very difficult to handle with a micromanipulator because of the stickiness of the
gelatinized granules and their tendency to adhere to the cover slip. When a sample of this starch was washed with 0.2 per cent. SO\textsubscript{2} solution, dried at 40° C. and subsequently gelatinized and stretched, there was no tendency for the granules to adhere to the cover slip. The treatment also lowered the paste viscosity.

Micromanipulative tests were made on 3, 4, and 5 per cent. pastes of commercial corn starch to determine how easily granules could be separated from the main body of the paste. It was very easy to disengage granules from the 3 per cent. paste. Whenever 2 or 3 granules pulled away together they could be readily separated from one another. In the case of the 4 per cent. paste, it was somewhat harder to separate granules from the paste and from each other than in the 3 per cent. sample. When a microneedle was fastened onto a granule near the edge of the 5 per cent. paste and a pull exerted, a large piece of the paste would separate from the main portion of the sample. It was very difficult to disengage individual granules from this gel.

These samples were again examined after having stood overnight at about 5° C. In every instance it was more difficult to separate the granules than it had been when the freshly prepared material was used. It was also more difficult to engage a granule with the microneedles after the paste had stood overnight, as the granules became hard and rubbery. Reheating the paste to 100° C. did not facilitate the separation of an individual granule from the paste nor was it easier to fasten a microneedle onto a granule. The changes which had taken place during refrigeration were therefore not reversible.

Similar tests were made upon potato starch pastes. The 3 per cent. paste was very elastic and although the granules would undergo considerable stretch before pulling away from the paste, individual granules could be separated fairly easily. The 4 per cent. paste was very cohesive but individual granules could be pulled away from the main body of the paste. The 4 per cent. and 5 per cent. pastes were almost identical.

Summary

1. A micromanipulative study was made of starches, including corn, glutinous corn, tapioca, and potato to determine whether length of paste might be correlated with ability of the individual granules to stretch without breaking. The data show that there is no apparent correlation between these two phenomena.

2. Starch granules pasted at 100° C. can be stretched to a greater extent than similar granules pasted at 80° C. for the same length of time.

3. When identical corn starch samples were pasted at a given temperature for 10, 30, and 60 minutes, and 72 hours, a significantly greater stretch was exhibited by the samples pasted for 30 minutes than for the others, all of which were essentially alike in ability to stretch.

4. Pretreatment of starch granules with 0.2 per cent. SO\textsubscript{2} or Na\textsubscript{32}NaOH did not cause any appreciable change in the percentage of stretch of the
granules. These treatments respectively decreased and increased the paste viscosity.

5. Stretching the gelatinized granules did not bring about realignment of the molecules sufficient to cause a return of birefringence.

6. Individual granules could be pulled away from the main body of a 3 per cent. corn starch paste with ease. Potato starch granules would undergo more stretch than corn starch granules before separating but they, too, came away with ease. It was difficult, however, to separate single granules from a 4 or 5 per cent. corn or potato starch gel.

The authors are indebted to CAROL M. JAEGEr for statistical analyses and to members of the Analytical and Physical Chemical Division for analytical data.

**LITERATURE CITED**


2. BADENHUIZEN, N. P. Growth and corrosion of the starch grain in connection with our present knowledge of the microscopical and chemical organization. Protoplasma 33: 440–468. 1939.


