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Controlled peeling of the surfaces of starch granules by gelatinization in aqueous dimethyl sulfoxide at selected temperatures

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Abstract—Microscopic examination of starch granules in 90:10 (v/v) Me_2SO–H_2O indicated that the granules were slowly being gelatinized from their surfaces. The rate of gelatinization was dependent on two variables: (1) the amount of water in Me_2SO and (2) the temperature. An increase of water in Me_2SO and/or an increase in temperature increased the rate of gelatinization and vice versa. Specific ratios of Me_2SO and H_2O (85:15–95:5) and temperatures (0–15 °C) were found to give controlled sequential peeling/gelatinization of eight kinds of starch granules in 1–12 h, with amounts of 10–25% gelatinization per hour. It was observed that the percent of starch granule remaining versus time gave curves that were linear and others that had linear parts separated by one or more abrupt changes. No two starches had a similar gelatinization curve for the same two conditions of the amount of water and the temperature. It is hypothesized that these curves reflect different structural characteristics for the individual kinds of starch granules.

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1. Introduction

Starch occurs in plant tissues as a water-insoluble granule. A common method of solubilizing starch is to heat the granules in boiling water or by autoclaving them in water at 120 °C. During the heating process, the granules swell by taking up water. The crystalline character of the granules is lost, along with the X-ray pattern and the optical birefringence (observed as the polarization cross). As the starch granules swell, they lose birefringence, indicating the break up of the granular structure. The process is called *gelatinization* and can be observed under the microscope using plane-polarized light.[1] Eventually the granules swell to a point where they burst and the amylose and amylopectin molecules go into solution.

Starch can also be gelatinized and solubilized by dispersion in dimethyl sulfoxide (Me_2SO).[2] Gelatinization and the consequent solubilization of starch granules in Me_2SO follow a very different path from gelatinization in boiling water. Starch granules do not swell in Me_2SO as they do in boiling water.[2,3] Instead, they slowly dissolve in anhydrous Me_2SO by fragmentation of the granules. Addition of water to Me_2SO greatly increases the rate and path of gelatinization, which is different from gelatinization in anhydrous Me_2SO. Potato and canna starch granules retained their shape and birefringence during dissolution in aqueous Me_2SO and fragmentation did not occur. Instead the starch was peeled from the surface of the granule, with large pieces of gel layers adhering to the smooth surfaces of the ungelatinized portion of the granules.[4]

Another method of chemically gelatinizing starch is by the use of relatively high concentrations of salt solutions to produce swelling and dissolution of starch granules.[5] Jane and Shen[6] used 4 M $CaCl_2$ to gelatinize potato starch granules and reported gelatinization from the periphery of the granule. Pan and Jane[7] used 13 M LiCl to gelatinize the starch from the surface of maize

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starch granules. In these experiments, the partially gelatinized starch was removed from the non-gelatinized portion of the granules by mechanical blending.

In the present study, we chose eight kinds of starches. Four of them included the major food starches from potato, rice, wheat, and maize. Of these, potato starch is known to be relatively easy to gelatinize. The other four starches were picked for their unusual properties. Amylomaize-VII starch contains 70% amylose and 30% amylopectin, nearly completely opposite to maize starch, which contains 28% amylose and 72% amylopectin. Waxy maize starch is further unusual, containing 100% amylopectin. Tapioca starch is also relatively easy to gelatinize and further has an unusual granule structure with a C-type X-ray pattern because it is composed of a central B-type structural core surrounded by an A-type structure. Finally, shoti starch was chosen because of its unusual granule morphology of a flat-plate-like structure, its reputation for being easily gelatinized starch, and its relatively wide use in Asia as a therapeutic material for stomach and intestinal problems.

We have obtained the controlled sequential peeling and gelatinization from the surfaces of eight types of starch granules by suspending them in Me_2SO containing different amounts of water at different temperatures. The granules were suspended in the $Me_2SO–H_2O$ solutions for varying periods of time, and the gelatinized starch was removed from the surface of the granules by the addition of *Bacillus amyloliquefaciens* alpha-amylase. After the enzyme had acted for a relatively short period of time, the enzyme and the partially hydrolyzed soluble starch were removed from the peeled granules by washing and centrifuging.

2. Experimental

2.1. Materials

Maize, potato, rice, waxy maize, amylomaize-VII, wheat, and shoti starches were freshly prepared from their various mature sources, using standard procedures,[8] but without the addition of sodium bisulfite. Mature maize seeds were obtained from Dr. Thomas Binder of Archer, Daniel, Midland in Decatur, IL. Mature amylomaize-VII and waxy maize seeds and tapioca starch were obtained from National Starch and Chemical Co., Bridgewater, NJ. Mature rice and potatoes were obtained from a local market. Shoti starch was isolated from mature *Curcuma zedoaria* tubers obtained from Dr. M. Kitaoka, National Food Research Institute, Tsukuba, Japan. Each type of starch granules was sized by using nylon filter cloths (Fisher Scientific, Pittsburgh, PA), having different size cut-off values of <5, 5–9, 10–19, 20–29, 30–40, 41–52, and >53 μm.[6] The size category with the largest percent of each starch was used in the gelatinization studies so as to give a more homogeneously sized sample. Refer to Table 1 for a summary of the properties of the individual types of starch granules used in the study. *B. amyloliquefaciens* alpha-amylase (HT-concentrate) was obtained from Miles Laboratory (Elkhart, IN, USA).

Table 1. Properties of the starches used in the study

Starches	Size (μm)	Percent[a]	Amylose (%)	X-ray type
Potato	>53	53.1	25	B
Rice	<5	100.0	20	A
Tapioca	10–19	81.5	17	C
Maize	20–29	70.7	28	A
Waxy maize	20–29	85.4	0	A
Amylomaize-VII	10–19	93.0	70	B
Wheat	20–29	61.3	25	A
Shoti	10–19	99.4	30	B

[a] Percent of the original starch granules having the size indicated in the first column.

2.2. Gelatinization procedure

Each of the starches (100 mg anhydrous weight, containing ~10% water) was added to 1.0 mL of 95:5 to 85:15 (v/v) $Me_2SO–H_2O$ for each of the time points that the starch was gelatinized. The starches in $Me_2SO–H_2O$ were placed into 2.0-mL capped microcentrifuge tubes that were put in a horizontal shaker at a specified temperature (0–15 °C). The microcentrifuge tubes were removed at various times from the shaker and centrifuged 2 min at 4 °C; the supernatants were removed and the starches were washed two times with 1.0 mL of water at 4 °C; 1.0 mL of 40 mM imidazole–HCl buffer (pH 6.8) with 1 mM $CaCl_2$, containing 20–100 μL of *B. amyloliquefaciens* alpha-amylase (14–70 units, where 1.0 unit equals 1.0 μmol of α-(1→4) bonds hydrolyzed per min) was added to the starch suspensions for reaction at 20 °C for varying lengths of time (see Table 2 for the amounts of enzyme and the lengths of time of reaction for each starch sample). The suspension was then centrifuged at 4 °C, and the remaining starch granules were washed with 1.0 mL of water five times, treated three times with 1.0 mL of anhyd acetone, and once with 1.0 mL of anhyd ethanol, dried at 37 °C for 18 h, and then weighed.

2.3. Light microscopy of the starch granules undergoing gelatinization

A suspension of the sized starch granules was prepared in 90:10 (v/v) $Me_2SO–H_2O$, and 20 μL was added to a microscope slide and covered with a cover glass. The slide was observed with a high dry microscope objective, with a magnification of 675 times. The granules were observed every 10–15 min intervals for 1–4 h at 20 °C.

Table 2. Percent of starch granule peeled, number of units of *Bacillus amyloliquefaciens* alpha-amylase added, and the number of minutes the enzyme was allowed to react at 20 °C before centrifugation of the remaining starch granule

Time[b] (h)	Potato starch 90:10[a] (15 °C)			Rice starch 95:5[a] (5 °C)			Tapioca starch 90:10[a] (10 °C)			Maize starch 90:10[a] (5 °C)			Waxymaize starch 90:10[a] (0 °C)			Amy-7 starch 90:10[a] (5 °C)			Wheat starch 90:10[a] (10 °C)			Shoti starch 95:5[a] (5 °C)		
	%[c]	*U*[d]	Min[e]	%[c]	*U*[d]	Min[e]	%[c]	*U*[d]	Min[e]	%[c]	*U*[d]	Min[e]	%[c]	*U*[d]	Min[e]	%[c]	*U*[d]	Min[e]	%[c]	*U*[d]	Min[e]	%[c]	*U*[d]	Min[e]
1	12.2	14	2	17.2	14	2	7.9	14	2	14.2	14	2	18.0	14	2	32.2	14	2	27.5	14	2	16.1	14	2
2	20.3	20	2	21.1	20	2	16.2	20	2	23.5	20	2	20.8	20	2	43.1	20	2	43.6	20	2	20.5	20	2
3	33.9	27	2	26.6	27	2	34.6	27	2	39.3	27	2	30.6	27	2	54.9	27	2	43.6	20	2	25.3	27	2
4	35.0	35	2	37.4	35	2	61.9	35	4	57.9	35	2	38.3	35	4	62.8	35	2	61.0	27	2	31.1	35	4
5	47.0	46	4	42.6	40	4	70.4	40	6	62.1	40	2	44.3	40	4	75.7	40	5	74.2	35	4	59.0	40	6
6	70.2	55	4	50.5	51	6	89.8	51	8	70.3	46	2	49.7	51	6	89.3	46	7	79.7	40	7	73.3	51	10
								25	4[f]											25	4[f]			
7	78.4	65	6	56.5	56	6				75.9	54	2	62.4	60	7	92.3	51	10	87.6	51	9	89.0	65	10
																	25	4[f]						
8	93.9	75	8	68.5	70	6							66.8	65	7									
9				73.0	60	8							75.3	60	10									
10				89.0	65	10							80.1	65	10									
11				92.4	70	10																		
					35	3[f]																		

[a] Volume ratio of dimethyl sulfoxide to water.

[b] Hours of peeling.

[c] Percent of starch peeled.

[d] Units of amylase added.

[e] Minutes of reaction of alpha-amylase.

[f] For the last peeling step, where there was a large amount of gelatinized starch, it was necessary to run two enzyme digestions for some of the starches to completely remove the gelatinized starch.

3. Results and discussion

3.1. Microscopy of the starch granules in 90:10 (v/v) Me_2SO–H_2O

Light microscopy of potato (>53 μm), maize (20–29 μm), and wheat (20–29 μm) starch granules suspended in 90:10 (v/v) Me_2SO–H_2O at 20 °C showed that a field of 15–20 individual starch granules, each granule slowly decreased in size over 1–4 h. A definite area of highly refractive gelatinized starch was observed surrounding each granule, and eventually the granules disappeared and a transparent gel covered the entire field, indicating that the starch was being slowly gelatinized from the surface of the granules. The birefringence of potato starch was observed, using polarized light microscopy. The polarization cross ('Maltese cross') was observed in the potato starch granules throughout the gelatinization, indicating that the structure of the starch granule was preserved during the process, further indicating that the starch was being gelatinized from the surface of the granules.

3.2. Gelatinization of potato starch granules

Potato starch granules in 90:10 (v/v) Me_2SO–H_2O at 15 °C were linearly gelatinized over 8 h with an average of ~12%/h (see Fig. 1A). Increasing the water to give 85:15 (v/v) Me_2SO–H_2O and decreasing the temperature to 10 °C gave a rapid, nearly linear gelatinization of 47% in 2 h, followed by 45% gelatinization in the third hour, giving a total of 92% gelatinization (see Fig. 1B). Decreasing the temperature to 5 °C gave 50% linear gelatinization in 4.5 h, followed by an abrupt increase in gelatinization to 86%, and then linear gelatinization in 2 h to give 99% in 7 h (see Fig. 1B). The large increase in the gelatinization between 4.5 and 5 h indicates a drastic change in the structure of the starch granules after the first 50% of the granule was removed. This was repeated twice to determine it was real. It appears that the interior 35% of the potato starch granule is less organized than the exterior 50%, different from the other starches in this study.

Potato starch granules were only gelatinized by 12% in 90:10 (v/v) Me_2SO–H_2O at 5 °C the first hour and then only 2% in 5 h (data not shown). The gelatinization of potato starch demonstrates the effects of the amount of water in the Me_2SO solution and the temperature on the rate of gelatinization and the nature of the gelatinization curve.

3.3. Gelatinization of rice starch granules

Figure 2A shows that rice starch granules were gelatinized by 23% the first hour in 95:5 Me_2SO–H_2O at 5 °C and then linearly gelatinized 80% at ~10%/h thereafter from 1 to 8 h. Increasing the water by 5 parts and decreasing the temperature 5 °C gave linear gelatinization from 18% to 74% in 1–9 h, followed by 88% and 94% after 10 and 11 h, respectively.

Increasing the temperature to 5 °C of the 90:10 Me_2SO–H_2O solution gave a rapid gelatinization of 18% the first hour, 11% the second hour, followed by a linear 38% from 2 to 5 h, giving a total of 95% gelati-

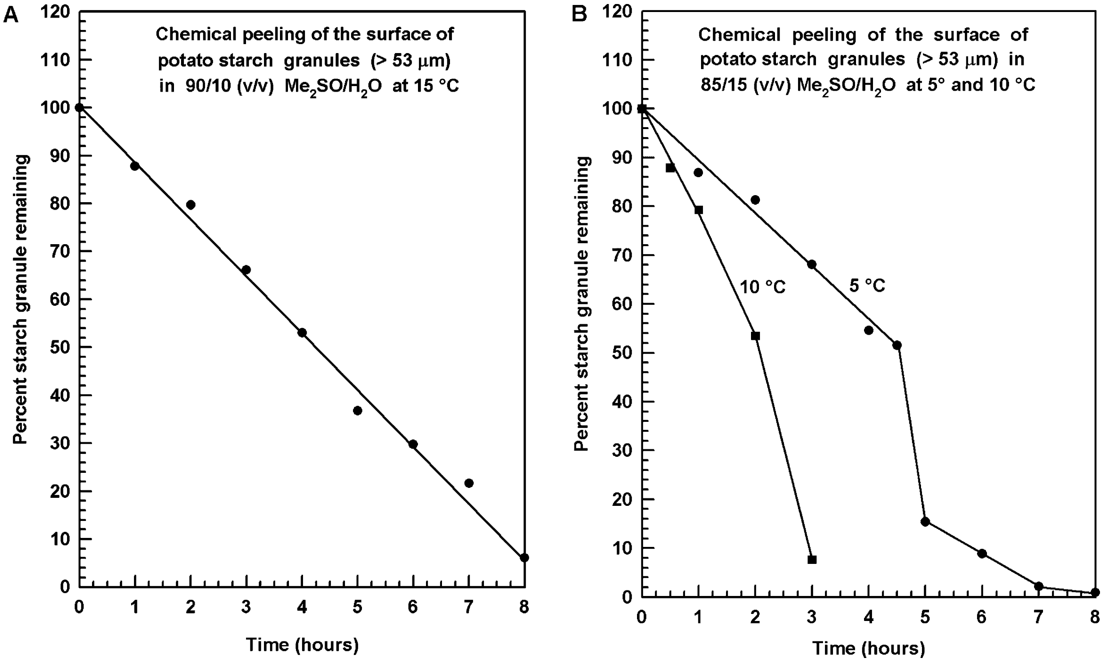

Figure 1. Controlled peeling of potato starch granules (>53 μm) by gelatinization. (A) in 90:10 (v/v) Me_2SO–H_2O at 15 °C; (B) in 85:15 (v/v) Me_2SO–H_2O at 5 and 10 °C.

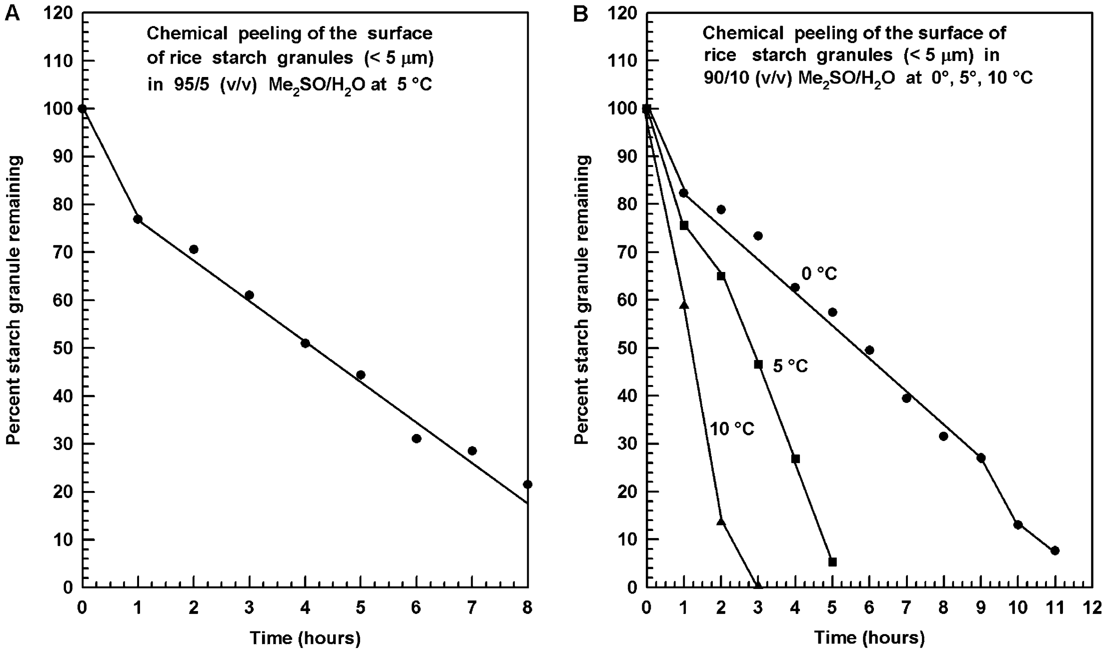

Figure 2. Controlled peeling of rice starch granules (<5 µm) by gelatinization. (A) in 95:5 (v/v) $Me_2SO–H_2O$ at 5 °C; (B) in 90:10 (v/v) $Me_2SO–H_2O$ at 10, 5, and 0 °C.

nization. Increasing the temperature to 10 °C gave a rapid 86% linear gelatinization in 2 h, followed by complete gelatinization in 3 h (see Fig. 2B).

Rice starch granules are somewhat unique in that they were gelatinized linearly or near linearly under all four of the conditions used, namely 95:5 (v/v) $Me_2SO–H_2O$ at 5 °C and 90:10 (v/v) $Me_2SO–H_2O$ at 0, 5, and 10 °C. This indicates that rice starch, the smallest (<5 µm) starch studied and probably the most homogeneous with regard to size, have a granule structure that is more homogeneous than potato starch granules and the other six kinds of starches in this study. They, however, showed a relatively rapid gelatinization the first hour for all four of the conditions, while potato starch granules did not show this for two of its linear conditions, indicating that the outer 25% of the rice starch granule was less highly organized than the inner 75%. Rice starch granules, however, did not show the rapid increase in the gelatinization at 4.5–5.0 h that potato starch granules showed in 85:15 Me_2SO/H_2O at 5 °C, indicating that the inner 75% of the rice starch granule was quite homogeneous and highly structured.

3.4. Gelatinization of tapioca starch granules

Tapioca starch granules were partially linearly gelatinized in variable amounts of 12–20%/h from 1 to 4 h in 95:5 $Me_2SO–H_2O$ at 15 °C. Then there was a break between 4 and 5 h in which there was only 2% gelatinization and then between 5 and 6 h, the granules were gelatinized by 24% (see Fig. 3). Increasing the water by 5% to give 90:10 $Me_2SO–H_2O$ at 10 °C gave a complex curve in which the gelatinization was linear at the first 2 h with 8%/h, followed by 20% gelatinization between 2 and 3 h, and 20% with a different rate between 2 and 4 h, followed by gelatinization of 8% between 4

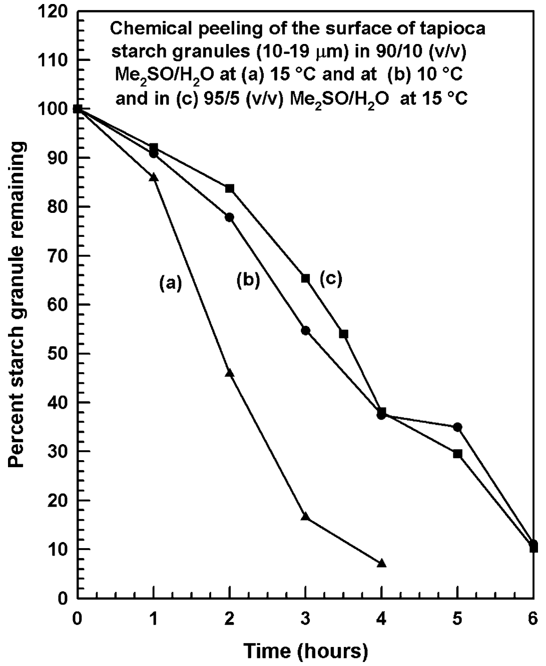

Figure 3. Controlled peeling of tapioca starch granules (10–19 µm) by gelatinization in 90:10 (v/v) $Me_2SO–H_2O$ at (a) 15 °C, (b) 10 °C and in (c) 95:5 (v/v) $Me_2SO–H_2O$ at 15 °C.

and 5 h, and another drop of 24% between 5 and 6 h, giving a total of 90% gelatinization (see Fig. 3). Increasing the temperature to 15 °C produced slow gelatinization in the first hour and 40% between 1 and 2 h, and then 30% between 2 and 3 h, followed by 10% gelatinization between 3 and 4 h, giving relatively rapid gelatinization of 94% in 4 h (see Fig. 3).

The unusual gelatinization behavior of tapioca starch in which complete linear or near-linear gelatinization was not obtained under any of the conditions studied, and the irregular percents of gelatinization most probably reflect the complex granular structure of tapioca starch. Starch granules from different sources have been classified as A-, B-, and C-type according to the type of X-ray diffraction patterns that they give.[9–11] Cereal starches, for example, rice, maize, and wheat, give the A-type pattern; tuber starches, for example, potato and shoti starches, give the B-type pattern, along with amylomaize-VII; and tapioca starch gives a C-type pattern, which results from having a central core with a B-type structure,[12] surrounded by an A-type structure. Tapioca starch has granule characteristics that are a mixture of the A- and B-types of starch granules and hence gives complex gelatinization kinetics that are a combination of the A and B types of starches.

3.5. Gelatinization of maize starch

Maize starch granules were linearly gelatinized over an 8 h period in 90:10 (v/v) $Me_2SO–H_2O$ at 15 °C (see Fig. 4). Decreasing the temperature to 10 °C gave 86% near-linear gelatinization in 3 h; decreasing the temperature further to 5 °C gave 40% linear gelatinization in 3 h, followed by a rapid increase in gelatinization of 18% in 3–4 h and then gave a slower 20% linear gelatinization from 4 to 7 h for a total of 78% (see Fig. 4).

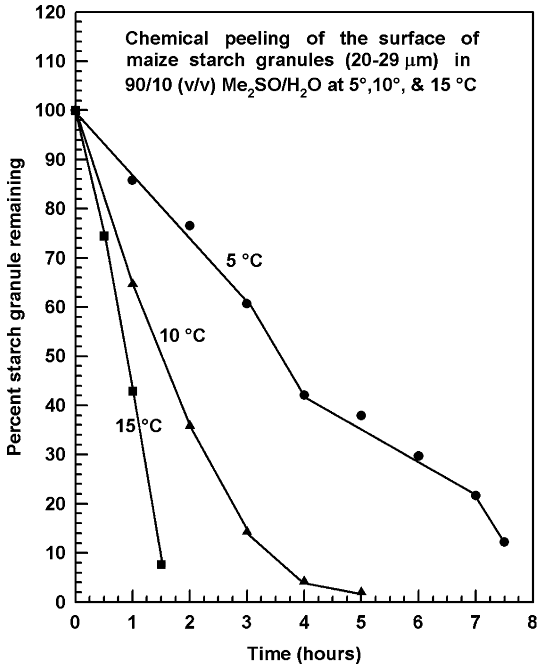

Figure 4. Controlled peeling of maize starch granules (20–29 μm) by gelatinization in 90:10 (v/v) $Me_2SO–H_2O$ at 15, 10, and 5 °C.

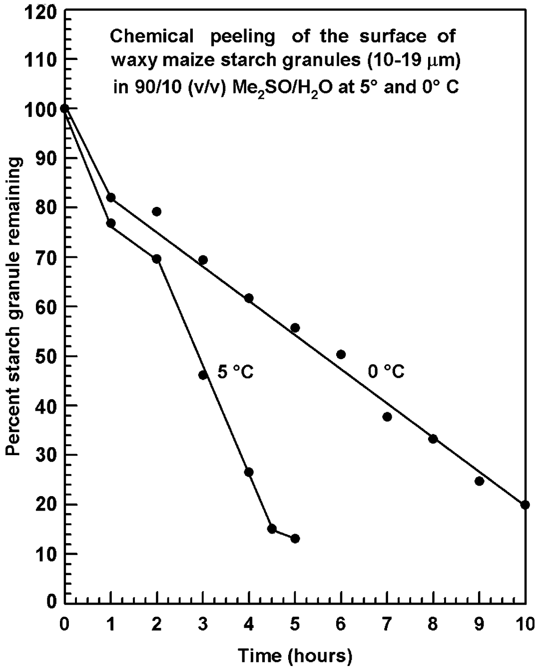

Figure 5. Controlled peeling of waxy maize starch granules (10–19 μm) by gelatinization in 90:10 (v/v) $Me_2SO–H_2O$ at 5 and 0 °C.

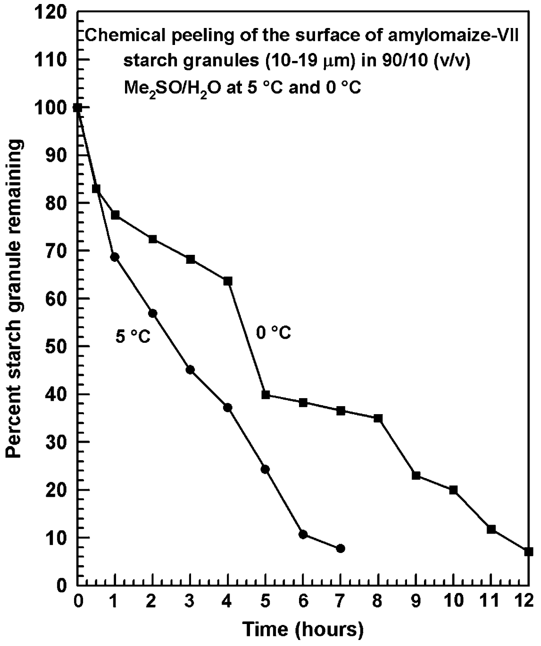

Figure 6. Controlled peeling of amylomaize-VII starch granules (10–19 μm) by gelatinization in 90:10 (v/v) $Me_2SO–H_2O$ at 5 and 0 °C.

3.6. Gelatinization of waxy maize starch granules

Waxy maize starch granules in 90:10 (v/v) $Me_2SO–H_2O$ at 0 °C were initially gelatinized 18% in the first hour, and then linearly gelatinized by 61% in 10 h with ~6%/h for a total of 89% (see Fig. 5). Increasing the temperature to 5 °C gave faster gelatinization and a more complex gelatinization/peeling curve. There was an initial gelatinization of 24%, followed by 8% gelatinization in the second hour. The gelatinization thereafter was linear for 2.5 h, giving 86% and then another 2% in the last half hour to give a total of 88% in 5 h (see Fig. 5).

3.7. Gelatinization of amylomaize-VII starch granules

Amylomaize-VII starch granules in 90:10 (v/v) $Me_2SO–H_2O$ at 5 °C gave 32% rapid gelatinization in the first hour and then 60% linear gelatinization in the following 7 h, with ~10–15%/h (see Fig. 6). Decreasing the temperature to 0 °C gave 22% gelatinization in the first hour, followed by 36% linear gelatinization from 1 to 4 h at ~6%/h. At 5 h, there was a large increase in 60%, followed by a relatively slow linear gelatinization from 5 to 8 h, with ~4%/h; there then was another increase of 12–90% at 11 h (see Fig. 6). This was also a relatively complex gelatinization process, probably due to the high amylose content of 70%.

3.8. Gelatinization of wheat starch granules

Wheat starch granules in 90:10 (v/v) $Me_2SO–H_2O$ at 10 °C gave near-linear gelatinization from 0.5 to 4 h of 16–18%/h and then smaller percents between 4 and 6 h (see Fig. 7). Decreasing the temperature to 5 °C gave 16% gelatinization in the first hour, followed by a relatively slow linear 38% gelatinization for 1–5 h; then this was followed by a rapid increase of 16% between 5 and 6 h, with linear gelatinization to 62% in 8 h (Fig. 7).

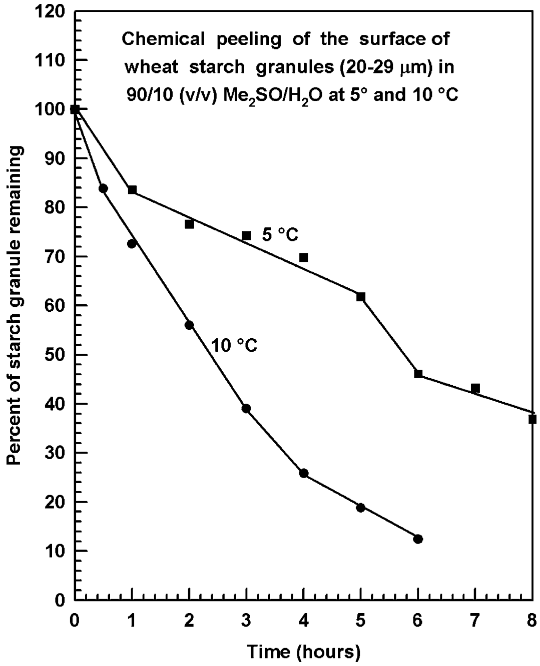

Figure 7. Controlled peeling of wheat starch granules (20–29 μm) by gelatinization in 90:10 $Me_2SO–H_2O$ at 5 and 10 °C.

3.9. Gelatinization of shoti starch granules

Shoti starch granules in 90:10 $Me_2SO–H_2O$ at 10 °C gave complete gelatinization in less than 5 min. This starch has an unusual granule morphology that consists of a relatively flat plate in which the third dimension has a thin lenticular shape[13] and is known to gelatinize easily. Because of its unusual morphology, and the result obtained above, it appeared that the partially controlled gelatinization of shoti starch might not be possible. We, however, reduced the temperature to 0 °C and found that partial gelatinization could be obtained in 4 h. Shoti starch was gelatinized by 15% in 1 h, 20% in 2 h, 38% in 3 h, 67% in 4 h, and thereafter rapidly and completely gelatinized in 4.25 h (see Fig. 8). The water content of the Me_2SO solution was then decreased by 5%, and the gelatinization of shoti starch was studied in 95:5 $Me_2SO–H_2O$ at 5 °C. Under these conditions, there was 16% gelatinization in the first hour, followed by a 34% near linear gelatinization from 1 to 4 h and then rapid gelatinization to 60% at 5 h, followed by a slower linear gelatinization to 88% in 7 h (Fig. 8).

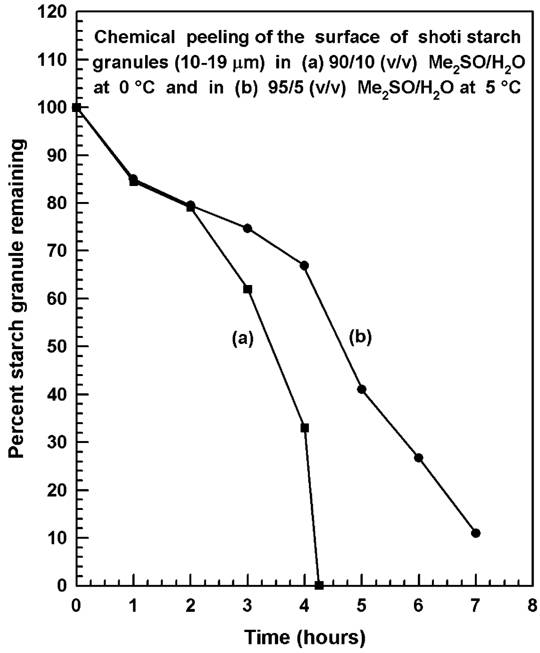

Figure 8. Controlled peeling of shoti starch granules (10–19 μm) by gelatinization in (a) 90:10 (v/v) $Me_2SO–H_2O$ at 0 °C and in (b) 95:5 (v/v) $Me_2SO–H_2O$ at 5 °C.

3.10. Concluding discussion

The study shows that the aqueous Me_2SO gelatinization process of starch granules was dependent on two variables: (1) the amount of water from 5% to 15% in the Me_2SO solution and (2) the temperature from 0 to 15 °C. The rate of gelatinization increased as the amount of water was increased and/or as the temperature was increased. An increase in 5 parts of water or an increase in 5 °C dramatically increased the rate of gelatinization and vice versa.

For this study, we defined an optimum controlled gelatinization process of starch granules as one that was of moderate rate and linear over an 8–10 h, with a relatively constant percent per hour. Potato starch granules in 90:10 $Me_2SO–H_2O$ at 15 °C, thus, had the best gelatinization process of the eight starches in which it was completely linear and there was an average amount of gelatinization of ~12%/h (Fig. 1A). Rice starch granules, with the exception of an initial rapid 23% gelatinization in the first hour, also were linearly gelatinized in 95:5 $Me_2SO–H_2O$ at 5 °C, to give 79% in 8 h, with ~10%/h (see Fig. 2A). Waxy maize starch in 90:10 $Me_2SO–H_2O$ at 0 °C, again with the exception of an initial rapid 18% gelatinization in the first hour, was linearly over a 9 h to give 80% gelatinization (see Fig. 5). Amylomaize-VII in 90:10 $Me_2SO–H_2O$ at 5 °C, again with the exception of an initial rapid 32% gelatinization in the first hour, was linearly gelatinized in 5 h to give 90% gelatinization (see Fig. 6).

Similar to rice, waxy maize, and amylomaize-VII starch granules, many of the starches had a 15–25% rapid gelatinization in the first hour, followed by a slower more controlled linear gelatinization process for 4–5 h, for example, rice starch in 90:10 $Me_2SO–H_2O$ at 0 °C, wheat starch in 90:10 $Me_2SO–H_2O$ at both 5 and 10 °C, amylomaize-VII starch in 90:10 $Me_2SO–H_2O$ at 10 °C, shoti starch in 90:10 $Me_2SO–H_2O$ at 0 °C and in 95:5 $Me_2SO–H_2O$ at 5 °C. The faster gelatinization in the first hour of the process suggests that in the outer portions of the granule, the starch molecules are farther apart from each other, and, therefore, there is less intermolecular association, giving less organization than in the inner parts of the granule, and the granule therefore is more easily gelatinized in these outer parts.

Another phenomenon that was observed was a rapid increase in gelatinization after linear gelatinization for several hours, as was observed for potato starch after 4.5 h in 85:15 $Me_2SO–H_2O$ at 5 °C, for maize starch after 3 h in 90:10 $Me_2SO–H_2O$ at 5 °C, for wheat starch after 5 h in 90:10 $Me_2SO–H_2O$ at 5 °C, for amylomaize-VII starch after 4 h in 90:10 $Me_2SO–H_2O$ at 0 °C and after 8 h at 0 °C, and for shoti starch after 4 h in 95:5 $Me_2SO–H_2O$ at 5 °C.

The opposite phenomenon was also observed in which there was a decrease in the gelatinization for 1 h after linear gelatinization for several hours. This was observed for tapioca starch after 4 h in 95:5 $Me_2SO–H_2O$ at 10 °C and for amylomaize-VII starch after 6 h in 90:10 $Me_2SO–H_2O$ at 5 °C and after 9 h in 90:10 $Me_2SO–H_2O$ at 0 °C.

For the eight types of starches studied, the amount of water in the Me_2SO and the temperature were used to control the rate and the form of the gelatinization process. In most instances, a combination of these two variables could be found in which the rate of gelatinization was linear or was a combination of two linear rates that were interrupted by a faster or slower rate. No two starches had a similar gelatinization curve with the same two conditions of the amount of water and the temperature. This reflects differences in the ways in which the starch molecules are packed together by association with each other to form crystalline structures in the granule.

As mentioned in Section 1, the use of 100% Me_2SO does not give the same kind of gelatinization of starch granules as observed in the aqueous solutions of Me_2SO, and the process is much slower. Me_2SO is known to be an effective hydrogen-bond breaker and functions in gelatinizing starch granules by breaking the intermolecular hydrogen bonds between the molecules. By itself it *penetrates* into the granule and slowly produces fragmentation of the granule by breaking hydrogen bonds. The addition of 5–15 parts of water to the Me_2SO rapidly facilitates the gelatinization of the starch granule by hydrating the starch chains at the surface of the granules, as the Me_2SO is breaking the hydrogen bonds.

The use of *B. amyloliquefaciens* alpha-amylase to remove the gelatinized starch from the non-gelatinized starch granules greatly facilitated the process of cleanly removing it from the granule under mild conditions that did not alter the structures in the remaining parts of the starch granule. *B. amyloliquefaciens* alpha-amylase requires either 1000-fold more enzyme or 1000 more minutes of reaction time to hydrolyze starch granules than to hydrolyze gelatinized starch.[14] Hence, the amylase did not hydrolyze the starch granules that remained from the partial gelatinizations. Potential use of the controlled peeling of starch granules are in the study of the structures of the various parts of the granule and in the location of the active enzymes for the synthesis of starch in the granules.

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