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Surface effects in the acetylation of granular potato starch

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ABSTRACT

The occurrence of surface effects in the acetylation of granular potato starch with acetic anhydride to degrees of substitution 0.04–0.2 was studied by two different approaches. The first approach involved the fractionation of granular starch acetates into five different size classes and analysis of their acetate content. Alternatively, two narrow size fractions of potato starch acetate granules were surface-peeled by chemical gelatinization in 5 M $CaCl_2$, and the remaining cores were analyzed for acetyl content at different peeling levels. It was established that true surface peeling occurs in this medium and that the ester linkages are stable under the conditions applied. Both approaches led to the conclusion that the acetylation of potato starch granules is accompanied by a pronounced surface effect. The surface peeling method allows determination of the extent of substitution as a function of the radial position in the starch granule.

1. Introduction

Starch is widely used as a functional ingredient in food and non-food applications.[1,2] Its functionality can be enhanced by chemical,[3–5] physical,[6,7] and enzymatic modification.[8] Typically, water is used for starch processing and formulation. However, water is a marginal solvent for starch macromolecules,[9] and therefore aqueous starch systems are inherently unstable and tend to phase-separate and crystallize.[10] The storage stability of these systems can be improved by a partial substitution of the hydroxyl groups with ether or ester groups.[1–5] Chemical modification in the intact granular state is advantageous because a high starch concentration promotes the reaction efficiency. Because the degree of crystallinity of starch is rather low (typically 30%),[11] the availability of hydroxyl groups is not likely to be a limiting factor at the low degrees of modification of common granular starch derivatives. Moreover, product recovery and purification are facilitated by the insolubility of intact starch granules in cold water. As starch molecules are assembled into well-ordered structures within the granule,[10,12] the substitution pattern may not be random. Indeed, effects of the granule structure on the distribution of substituents over amylose and amylopectin,[13,14] over crystalline and amorphous domains,[14,15] and along the macromolecular chains[16–19] have been reported. The cited papers[13–19] deal with starch ethers, wherein linkages between monomer and substituent are stable. Only recently, results on the more labile starch esters have become available.[20–23]

Another aspect of reactions with starch granules, to be addressed in the present paper, is the rate of reaction compared to the rate of diffusion of the reagent into the granule. If the reaction is relatively slow, comparable extents of reaction at the granule surface and within its interior are expected. However, a significant surface effect is expected in case the reaction is fast. Apart from microscopic methods, to be discussed later in this paper, at least two other approaches have been applied for revealing surface effects in modification reactions with starch granules. The simplest one is based on the fact that a surface reaction leads to a higher degree of modification of the smaller granules within a single batch of modified starch. This approach involves fractionation of the modified starch into a number of size classes and analysis of the degree of modification of each class. The advantages of this approach are its simplicity and universal applicability. The only requirement is that the granules in the starches to be studied cover a range of sizes. However, this method is indirect and does not provide a depth profile of the degree of substitution within the granule. It was applied to demonstrate the absence of a surface effect in the methylation and hydroxypropylation of starch.[14,24] Recently, Chen et al. reported by this method a distinct surface effect in the acetylation of potato starch to a degree of substitution (defined as the average number of acetyl groups per glucose monomer) of 0.06, but they did not analyse this result in detail.[20]

The second approach is based on the discovery by Gough and Pybus that wheat starch granules display a wide range of gelatinization modes in concentrated salt solutions. These modes of gelatinization do not only depend on cation type but also depend on salt concentration.[25] Of particular interest is the gelatinization in highly concentrated aqueous $CaCl_2$. In this medium the starch granule dissolves in a layer-like fashion from the surface to the

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interior whilst retaining birefringence in its remaining core. Jane and Shen[26] were the first to recognize the analytical potential of this so-called chemical gelatinization. They applied it to the depth-profiling of unmodified potato starch granules and determined, inter alia, the distribution of amylose and natural phosphate ester groups within the granule. They also demonstrated that complex formation of starch with Ca^{2+} at high concentration is accompanied by an exothermic heat effect.[27] The latter could trigger a local gelatinization of the surface layer. A similar surface peeling effect has been discovered recently for aqueous dimethyl sulfoxide.[28] A third method involves the use of amylases. The peeling action of amylase isolated from *Bacillus firmus/lentus* on potato starch granules has been demonstrated.[29,30]

The use of chemical gelatinization for revealing surface effects in starch modification has been described for methylation by Van der Burgt et al.[31] and for acetylation by us in a preliminary communication.[32] In this paper we compare the results of our study on surface effects in the acetylation of potato starch by both methods: size classification and surface peeling of acetylated starch granules.

2. Results and discussion

2.1. Size classification study

2.1.1. Acetylation, size classification, and analysis of size classes

Potato starch was acetylated to a degree of substitution S_0 varying between 0.04 and 0.2 with acetic anhydride at pH 8.2 in aqueous suspension with and without the presence of sodium sulfate as a swelling inhibitor. The alkaline medium promotes the formation of a nucleophilic starch–alkali complex that rapidly attacks the anhydride.[3] The selected pH provides the optimum balance between an acceptable reaction efficiency and the necessity to avoid alkaline saponification of the ester bonds formed. Besides protecting the granules against gelatinization, sodium sulfate enhances the adsorption of alkali by starch, presumably by increasing the effective concentration of hydroxide by hydration.[3]

The granular starch acetates were fractionated into five different size classes by repeated sedimentation at five different times according to Decker and Höller.[33] This method is expected to give results that are roughly similar to the wet sieving method employed by Chen et al.[34] Tap water was used as the medium for sedimentation, which proved to be unsuitable for starch acetates with $S_0 \geqslant 0.2$ because of foaming and accumulation of part of the small granules in the foam layer. Instead, ethanol was used for starch acetate at the highest degree of substitution.

For each size class the diameter of ca. 100 granules was measured on microphotographs and the number, surface, and volume averages of the diameter d_n, d_s and d_v were calculated. These are defined as $d_n = (\sum n_i d_i)/\sum n_i$, $d_s = (\sum n_i d_i^3)/(\sum n_i d_i^2)$ and $d_v = (\sum n_i d_i^4)/(\sum n_i d_i^3)$, respectively, with n_i the counted number of particles with diameter d_i in a size class. The quality of the classification is judged from the polydispersity index d_v/d_n, which is 1 if particles of only one size are present. For sedimentation times 9, 16, and 35 min, d_v/d_n ranged between 1.19 and 1.28. It was slightly higher (1.27–1.36) for sedimentation time 4 min and for the overflow (>35 min). d_v/d_n of whole potato starch is typically 1.95 ± 0.18 as measured by a Coulter Counter. Size classification in ethanol of the two most highly substituted starches ($S_0 = 0.2$) was less satisfactory with $d_v/d_n = 1.33 \pm 0.25$ as the average for all size classes. It became increasingly difficult to suppress granule swelling and lumping at higher S_0.

All starch acetates and their size classes were analyzed for acetyl content, and the results were normalized by calculating S_d/S_0, where S_d denotes the degree of substitution of a size class with average diameter d. The results in Table 1 demonstrate that S_d/S_0 increases with sedimentation time, but depends only slightly on S_0 for low degrees of substitution. At S_0 0.2 the dependence of S_d/S_0 on sedimentation time became somewhat less strong. The results also suggest that the surface effect in the acetylation reaction is significantly enhanced in the presence of sodium sulfate. This can be explained by the fact that this salt provokes both a higher rate of the acetylation reaction and a decreased rate of diffusion of the reagents into the interior of the granule because of the inhibition of granule swelling.

Table 1
Normalized degree of substitution S_d/S_0 of size classes of starch acetates prepared with (+) and without 10% sodium sulfate (−)[a]

Sedimentation time (min)[b]	0.044 (−)	0.102 (−)	0.196 (−)[c]	0.046 (+)	0.107 (+)	0.209 (+)[c]
4/5	0.73	0.75	0.86	0.67	0.63	0.78
9/12	0.82	0.82	0.91	0.80	0.79	0.88
16/20	0.95	0.93	0.99	0.96	0.95	1.01
35/42	1.23	1.20	1.24	1.30	1.26	1.28
>35/>42	1.41	1.41	1.42	1.70	1.61	1.51

[a] S_0 of the unclassified starch acetates are displayed in the upper row.
[b] Left-hand and right-hand numbers denote sedimentation times in water and ethanol, respectively.
[c] Sedimentation in ethanol.

2.1.2. Data analysis

In analyzing these results we will first demonstrate that, in case of a pure surface reaction, there exists a simple relationship between granule diameter d and degree of substitution S_d of granules of that size. The mass and the surface area of a single starch granule with density ρ are given by $\pi\rho d^3/6$ and πd^2, respectively. One unit mass of starch with granules of diameter d contains $6/\pi\rho d^3$ granules with total surface area $6/\rho d$. In a surface reaction, S_d is proportional to the total surface area per unit of mass of the granules with diameter d and hence $S_d \sim 6/\rho d$. The same reasoning is valid for the averaged (=measured) degree of substitution S_0 of the whole product with average granule size d_0 and hence $S_0 \sim 6/\rho d_0$. Assuming size-independent proportionality constants it follows that

$$S_d/S_0 = d_0/d \tag{1a}$$

In a pure surface reaction with an infinitely thin substituted surface layer the normalized degree of substitution S_d/S_0 of granules with different size within a starch is linearly related to the reciprocal of the granule size with a slope equal to the average granule size. In order to account for a finite thickness of the substituted surface layer, the somewhat more general expression 1b was adopted as a first order approximation.

$$S_d/S_0 = A/d + B \tag{1b}$$

In case of a pure surface reaction $A = d_0$ and $B = 0$. If there is no surface effect at all, i.e., if the thickness of the substituted layer is $d/2$ for all d, then $A = 0$ and $B = 1$.

We have plotted the results according to Eq. 1b in Figure 1, using d_s as the relevant parameter for the calculation of the abscissa because of the expected surface effect. All data points for which $1/d_s < 0.035\ \mu m^{-1}$ could be fitted by a linear curve with slope 28.7 μm and intercept 0.351 ($r = 0.985$). Inserting these values, $S_d/S_0 = 1$ for $d_s = 44.2$ μm, which is then the expected surface average diameter d_0 of the unclassified starch derivatives. This value agrees fairly well with $d_s = 43.6$ μm as the average of all size fractions with sedimentation time 16 (or 20) min in Table 1. The values of slope and intercept of the experimental curve indicate a distinct surface effect in the acetylation reaction, with a finite thickness of the acetylated surface layer. The effect of layer thickness is expected to become more significant for smaller granules. This

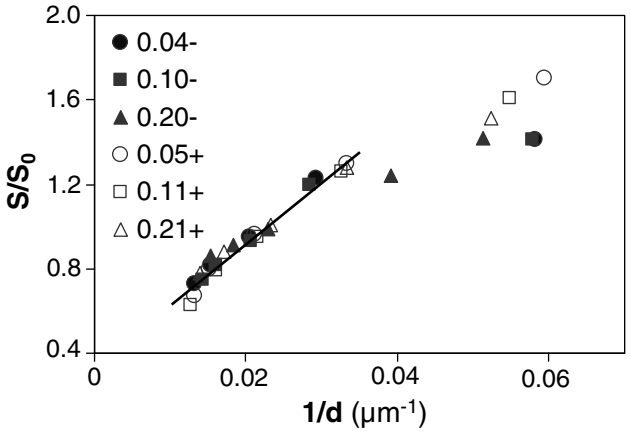

Figure 1. Plots of S_d/S_0 vs $1/d_s$ for granule size classes of starch acetates with degree of substitution S_0 denoted by the numbers. Open symbols: with sodium sulfate (+); filled symbols: without sodium sulfate (−). The line represents the linear fit according to Eq. 1b with $A = 28.7$ μm and $B = 0.351$.

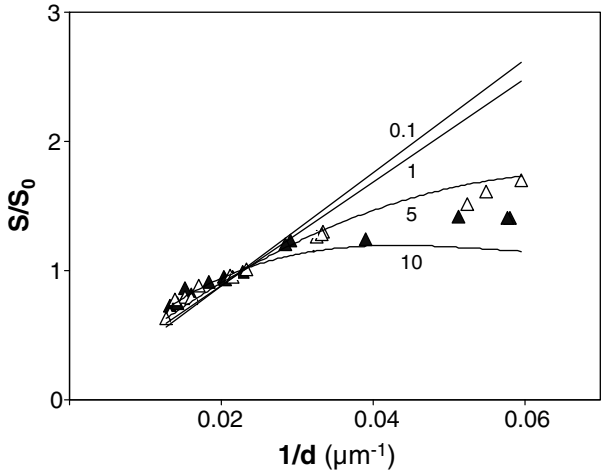

Figure 2. Calculated plots of S_d/S_0 vs $1/d$ (solid lines) for a core–shell model with substituted layer thickness (μm) as indicated. Symbols denote experimental results. Open symbols: with sodium sulfate; filled symbols: without sodium sulfate.

would explain the downward deviation from linearity of the curve for $1/d > 0.035$ μm^{-1}. It is also noticed that this deviation is stronger for acetylation in the absence of sodium sulfate. This is in agreement with Table 1 that shows that the effect of sodium sulfate on S_d/S_0 is larger for the smallest than for the largest granules.

To estimate the thickness of the acetylated layer, the crudest possible core–shell model with an unsubstituted core and a uniformly substituted shell was adopted. This is obviously a gross simplification, because in reality the degree of substitution is expected to decrease gradually from the granule surface towards the interior. It is assumed that the degree of substitution of the shell S_s and the shell thickness $\Delta d/2$ are both independent of the granule diameter. Thus, all granules have the same S_s and the variation in S_d between granules of different size is solely due to the variation of $\Delta d/d$. The first step is that we calculate the ratio S_s/S_0 of granules with surface average diameter d_0 (44.2 μm, see above) and degree of substitution S_0 for selected values of $\Delta d = d - d_c$, with d_c the diameter of the unsubstituted core. For any Δd the total amount of acetyl groups in these 'average' starch granules is proportional to $(1/6)\pi d_0^3 \rho S_0 = (1/6)\pi(d_0^3 - d_c^3)\rho S_s$. It follows that

$$S_s/S_0 = d_0^3/(d_0^3 - d_c^3) \quad (2)$$

Once S_s/S_0 is tabulated for selected values of Δd, we calculate S_d/S_0 of granules with any d for the selected Δd. The *normalized* number of acetyl groups in a granule with diameter d is proportional to $(1/6)\pi d^3 \rho(S_d/S_0) = (1/6)\pi(d^3 - d_c^3)\rho(S_s/S_0)$. This is simplified to

$$S_d/S_0 = (S_s/S_0)(d^3 - d_c^3)/d^3 \quad (3)$$

Values of S_d/S_0 calculated for tabulated S_s/S_0 corresponding to the selected Δd values are then compared to the experimental results. The value of $\Delta d/2$ that gives the best match is regarded as the most probable layer thickness. Figure 2 shows trend lines fitted to calculated data for four different layer thicknesses $\Delta d/2$ together with the experimental data. As expected, only the smaller size classes are sensitive to this type of analysis. Our results suggest a thickness of the substituted layer in granular potato starch acetates fairly close to 5 μm in case sodium sulfate had been present during the esterification, and somewhere between 5 and 10 μm if sodium sulfate had been absent.

It is worthwhile to consider the results of Chen et al.[20] These authors suggest a surface effect for amylopectin, but not for amylose, which was found to be substituted to the same extent in all granule size classes. This puzzling observation challenges the notion that the acetylation of starch granules can be described by a single diffusion and reaction rate.

2.2. Surface peeling

2.2.1. Conditions for chemical gelatinization

In their pioneering work on the depth-profiling of unmodified potato starch granules, Jane and Shen[26] applied a treatment with 4 M $CaCl_2$ at ambient temperature for different lengths of time. However, at these conditions the granules gelatinize in the Type II regime according to the classification of Gough and Pybus,[25] and a layer of gelatinized material remains attached to the birefringent core. We preferred Type IIIC gelatinization in 5 M $CaCl_2$ with complete molecular dissolution of the gelatinized material. As the gelatinization temperature range in this regime lies between 50 and 70 °C, programmed heating was applied. An additional advantage of this approach is that a predetermined degree of gelatinization can be selected by setting the final heating temperature, whereupon the process can be arrested by a temperature quench. In preliminary analytical studies we monitored the amount of gelatinization from the light transmission of a 5% starch suspension in 5 M $CaCl_2$ as a function of temperature. These experiments were performed at a heating rate of 1 °C/min on a temperature-programmed microscope hot stage having the inlet and exit apertures for the optical path connected by optical fibres to the mainframe of an immersion colorimeter. In preparative studies, 3 or 5% suspensions in 5 M $CaCl_2$ were heated at 1 °C/min on a programmed water bath to a predetermined final temperature. The amount of gelatinization was estimated after a temperature quench either from the decrease in total particle volume as measured by Coulter Counter or from the amount of dissolved carbohydrate, which was measured colorimetrically. Figure 3 shows that the relative decrease in total particle volume (before washing) is matched rather closely by the light transmission curve, provided that the latter is normalized by recalculating the transmission readings at the start and completion of the gelatinization to 0 and 100%, respectively. This correspondence justifies the use of light transmission measurements for selecting the final temperatures in the preparative studies. It is interesting to note that both the light transmission and the relative decrease in total particle volume pass through a shallow minimum in the initial stage of gelatinization, i.e., the light transmission decreases and the total particle volume increases somewhat. We ascribe this to a slight swelling of the starch granules in 5 M $CaCl_2$ at the start of the peeling process. This would also explain the higher degree of gelatinization calculated from the amount of solubilized carbohydrate. Initial light transmission results on unmodified potato starch revealed that the degree of

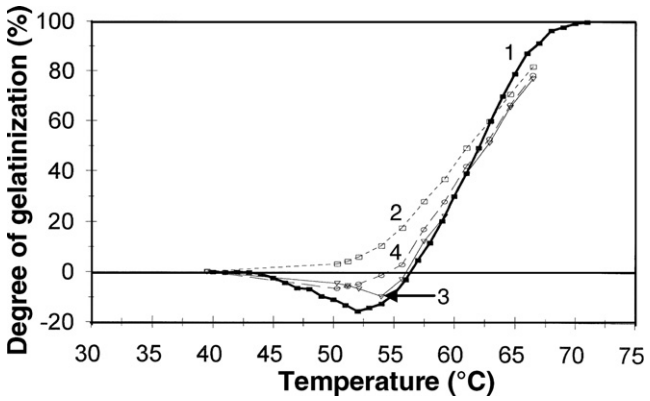

Figure 3. Chemical gelatinization profiles of potato starch granules in 5 M $CaCl_2$ as measured by light transmission (1), solubilized carbohydrate (2), total particle volume before washing (3), and total particle volume after washing (4).

gelatinization as measured by light transmission was not altered during storage after quench cooling. The gelatinization temperature range depends significantly on granule size (Fig. 4), starch water content, and concentration of $CaCl_2$ (not shown). The midpoint gelatinization temperature increased with increasing granule size and salt concentration, and with decreasing starch water content.

The significant effect of particle size on the gelatinization profile necessitates the use of narrow particle size fractions in the depth-profiling of starch acetate granules. Therefore, potato starch was classified in four size fractions by sedimentation in water during 4, 9, 16, and 35 min by the method of Decker and Höller.[33] The size classes obtained by sedimentation for 9 and 35 min were recovered by filtration and dried. Particle size d_v was 54 and 28 μm, respectively, with polydispersity index slightly below 1.1. The quality of the size classification was better than with the starch acetates, presumably because the latter showed a tendency for lumping and because a lower initial suspension concentration was selected for the unmodified potato starch (Section 4.3). Both fractions were acetylated with acetic anhydride at pH 8.2 to S_0 0.05 and 0.1 in the absence of sodium sulfate. Light transmission measurements as a function of temperature in 5 M $CaCl_2$ showed that the gelatinization profiles at a given initial particle size were quite similar to the profile of unmodified potato starch and did not depend on S_0. However, microscopic examination revealed that the gelatinized layer remained attached to the birefringent core, in contrast to unmodified potato starch granules. This layer was successfully removed by treatment in a Waring blender at 1 °C after dilution with five volumes of ice-cold water. It was established in a separate experiment on unmodified potato starch that this treatment had no effect on the particle size distribution.

2.2.2. Depth-profiling

Results of the surface peeling experiments are presented in Table 2. The degree of gelatinization DG, defined as the percentage of starch solubilized by the action of $CaCl_2$, was calculated (a) from the yield of eroded granules, (b) from the decrease in particle size, and (c) from the amount of solubilized carbohydrate. DG values from the latter method were intermediate between DG from the former two and were preferred for use in the following discussion: DG from particle size is affected by the swelling effect discussed above (Fig. 3), whilst the yield of eroded granules apparently was not quantitative. Assuming a spherical shape of the original and eroded starch granules, the depth profile was calculated from the results of Table 2 by applying simple geometry. The degree of substitution S_i of layer i in the granule with volume fraction Φ_i was calculated according to

$$S_0 = \sum \Phi_i S_i \quad (4)$$

Normalized depth profiles of all acetylated size fractions are displayed in Figure 5. The distance to the granule surface was taken as the distance from the surface to the centre of each layer. It is obvious that S falls quite sharply within a few μm from the surface of the granule. This contradicts our assumed core–shell model, discussed in Section 2.1.2, that features a shell with constant S_s and a core with $S_c = 0$. One could only say that the calculated shell thickness in this core–shell model (5–10 μm) is of the same order of magnitude as the layer thickness at which the depth profile flattens out. Moreover, it appears that the depth profile does not depend on d or S_0. However, because these results were obtained on sharp size fractions that were subsequently acetylated, the latter conclusion may not apply to different size populations in an unclassified starch acetate. Figure 6 gives a pictorial representation of the layer-like distribution of acetyl groups in granular potato starch acetate. The results suggest a difference between S of the outer layer and the

Table 2
Degree of substitution S of acetylated size fractions of potato starch after surface peeling in 5 M $CaCl_2$ to various degrees of gelatinization DG

d_v 54.6 μm		d_v 28.7 μm		d_v 54.7 μm		d_v 28.9 μm	
DG (%)	S	DG (%)	S	DG (%)	S	DG (%)	S
0	0.050	0	0.058	0	0.102	0	0.105
21.0	0.030	22.9	0.042	18.7	0.070	30.7	0.077
48.0	0.024	35.3	0.038	46.1	0.054	40.8	0.073
58.2	0.020	55.7	0.034	56.5	0.050	63.4	0.065

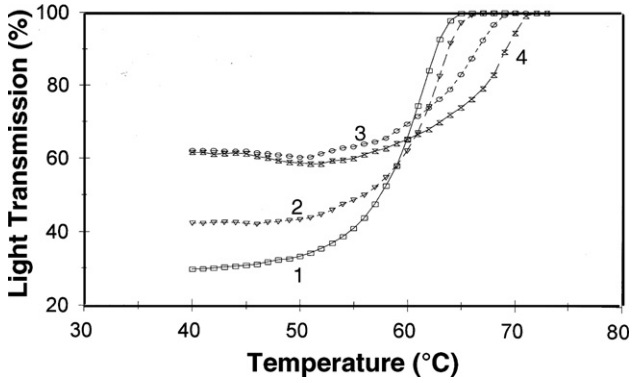

Figure 4. Effect of granule size on the chemical gelatinization of potato starch granules in 5 M $CaCl_2$. Granule diameter d_v (μm): 16.7 (1), 25.9 (2), 51.5 (3), 63.6 (4).

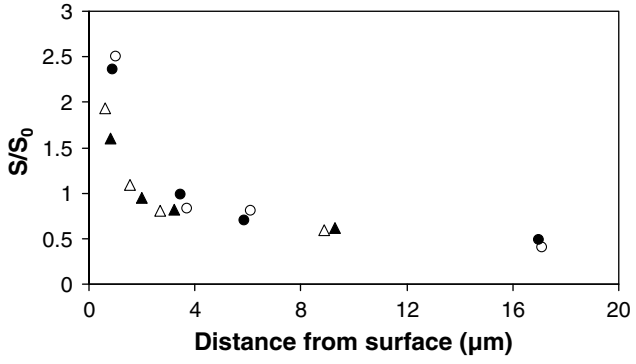

Figure 5. Normalized depth profiles of acetyl groups in potato starch acetate granules. S_0 is the degree of substitution of the size fraction. Open symbols: $S_0 = 0.05$; filled symbols: $S_0 = 0.10$; circles: $d_v = 55$ μm; triangles: $d_v = 29$ μm.

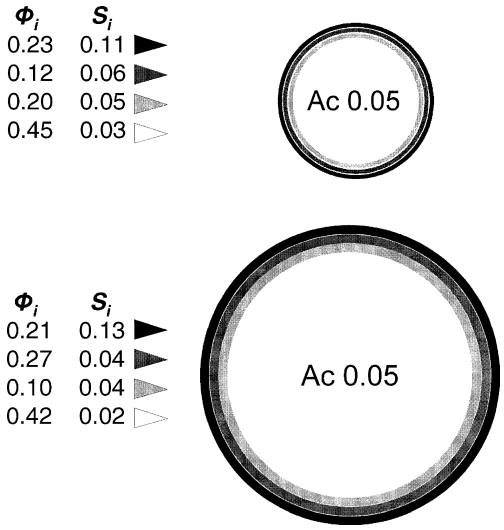

Figure 6. Cross-section of potato starch acetate granules with $S_0 = 0.05$ and d_v = 29 μm (upper) and 55 μm (lower). Indicated are volume fraction Φ_i and degree of substitution S_i of layers from granule surface to interior.

granule interior by a factor of ca. 5. The outermost 20–25% of the granule volume accounts for about half of the total number of acetyl groups. The associated layer thickness amounts to only 1–2 μm.

This supports earlier reports based on transmission[35] and scanning electron microscopy[36] and on confocal scanning laser microscopy[37] that most of the reaction in phosphated or sulfated starch granules occurs in narrow layers near the granule periphery or around cavities. However, in starch phosphates prepared by soaking the starch granules in a reagent solution followed by filtration, drying, and heating, a uniform distribution of phosphate groups throughout the granules was observed.[38] These contrasting results suggest that the occurrence of surface effects is indeed related to diffusion versus reaction rates in granular starch.

2.2.3. Role of artefacts

When proposing chemical gelatinization in 5 M $CaCl_2$ as a method for making depth profiles of granular starch derivatives, we have to ensure that artefacts do not play a role. It has to be ascertained (a) that the chemical gelatinization of starch acetate granules in 5 M $CaCl_2$ indeed proceeds in a layer-like fashion independent of initial granule size, and (b) that the expected decrease in S is not due to the instability of the ester linkage in the medium.

Initial attempts to examine the surface peeling action of 5 M $CaCl_2$ on a whole potato starch gave indecisive results: the effects on the particle size distribution were too weak. Hence we decided to work with bimodal populations prepared by mixing two sharp size fractions. In case of pure surface erosion, the change in a bimodal distribution must fulfill the following requirements at any DG, except at high DG:

$$(d - d_0)_{ns} = (d - d_0)_{nl} \quad (5)$$

$$(d - d_0)_{vs} = (d - d_0)_{vl} \quad (6)$$

$$SD_{ns} \text{ and } SD_{nl} = \text{constant} \quad (7)$$

Here SD denotes the standard deviation of the size of a subpopulation, whereas the subscripts n and v refer to number and volume averages, l and s to the large and small granule subpopulations, and 0 to the initial state. We confirmed the validity of Eqs. 5–7 for unmodified potato starch (not shown). Here we present only the results for acetylated potato starch. To this aim, the coarse and fine size fractions with S_0 0.1 were mixed at a mass proportion of 4:1 and heated in 5 M $CaCl_2$ to four different final temperatures. After removal of the gelatinized layer and further cleanup, the wet eroded granules were subjected to particle size analysis. Because of lack of material, this part of the work was done with batches of potato starch acetate prepared at slightly different conditions. The results in Table 3 demonstrate the validity of Eqs. 5–7 with deviations becoming apparent at the highest DG, because then the smallest granules have started to disappear completely. This gives confidence in that 5 M $CaCl_2$ acts indeed by layer-to-layer surface erosion.

Table 3
Changes in particle size characteristics of a bimodal mixture of small and large acetylated size fractions of potato starch due to chemical gelatinization with 5 M $CaCl_2$[a]

DG[b] (%)	$(d-d_0)_{ns}$ (μm)	$(d-d_0)_{nl}$ (μm)	$(d-d_0)_{vs}$ (μm)	$(d-d_0)_{vl}$ (μm)	SD_{ns} (μm)	SD_{nl} (μm)
0	0	0	0	0	3.7	7.1
27.7	3.2	3.2	2.9	3.1	3.8	7.1
50.5	6.6	6.5	5.7	5.9	4.1	7.3
66.3	10.2	9.8	8.4	8.5	4.4	7.5
79.9	13.8	14.4	11.3	12.0	4.2	8.0
81.3	14.1	15.7	11.8	12.7	4.0	8.3

[a] The meaning of symbols is given in the text in conjunction with Eqs. 5–7.
[b] Degree of gelatinization calculated from decrease of d_v.

Table 4
Mass balance of bound acetyl in the chemical gelatinization of an acetylated size fraction of potato starch with $S_0 = 0.102$ and $d_v = 54.7$ μm

DG[a] (%)	S granules[b]	S solubilized[c]	S_0 calcd[d]	S solubilized calcd[e]
0	0.102	0	0.102	0
18.7	0.070	0.231	0.100	0.237
46.1	0.054	0.155	0.101	0.158
56.5	0.050	0.136	0.099	0.142

[a] Degree of gelatinization calculated from amount of solubilized carbohydrate.
[b] Degree of substitution of surface-peeled granules.
[c] Degree of substitution of solubilized carbohydrate.
[d] Calculated from solubilized carbohydrate, S granules, and S solubilized.
[e] Calculated from solubilized carbohydrate, S_0 and S granules.

A second potential artefact stems from saponification of the acetyl groups under the conditions of chemical gelatinization. This possibility was investigated by establishing a mass balance of bound acetyl and carbohydrate in the granules and the solubles. A loss of total bound acetyl would indicate saponification. Bound acetyl content is determined by adding a known excess of alkali and measuring the consumption of alkali due to saponification of the ester linkages. Free acetyl is excluded from the analysis by adjusting the pH to neutral prior to the standard alkali addition. This analysis was performed on all four size classes. Results are presented for one sample in Table 4. The other samples gave similar results. The total amount of bound acetyl in granular and dissolved starch remained constant irrespective of the degree of solubilization. Moreover, if saponification had occurred, ever larger amounts of alkali would have been required to adjust the sample pH to neutral, which was not observed. This leads to the conclusion that the ester linkages are stable under the conditions of depth-profiling.

3. Conclusions

A pronounced surface effect in the acetylation of granular potato starch with acetic anhydride has been revealed by two different methods, size classification and surface peeling. This suggests that, under the conditions employed, the rate of diffusion of the reagent and/or catalyst into the granule is slower than the reaction rate. The surface effect is stronger in the presence of sodium sulfate, presumably because this salt enhances the reaction and suppresses

granule swelling. The results of the size classification study are in agreement with a surface reaction with a finite thickness of the acetylated layer. Assuming the simplest possible core–shell model with an unsubstituted core and a uniformly substituted shell, a shell thickness of 5–10 μm was calculated. Surface peeling by chemical gelatinization with 5 M $CaCl_2$ enabled a detailed depth-profiling of acetylated potato starch granules. This method revealed a strong gradient of acetyl groups within a few μm from the granule surface: about half of the acetate groups are located within 1–2 μm from the granule surface, which is in agreement with microscopic studies on other starch esters. This refutes the idea of a uniformly substituted shell. If narrow size fractions of potato starch are acetylated, the surface effect does not depend on granule size and degree of substitution. However, this may not be the case for different size populations within a single batch of starch acetate.

4. Experimental

4.1. General methods

Distilled or demineralized water was used throughout, except if indicated otherwise. Dry matter content was calculated from the mass loss on heating in a freely ventilating oven (130 °C, 2 h). Carbohydrate content of supernatant solutions was determined with the anthrone colorimetric assay.[39] The $CaCl_2$ content of blank and standard solutions was adjusted to those of the analyte solutions. The effect of acetyl on this assay was negligible.

For the determination of the degree of substitution S a known amount of starch acetate was suspended in water in a sealable vessel. The pH of the magnetically stirred suspension was adjusted to neutral on phenolphthalein and a known amount of aqueous 0.1 M NaOH was added. The vessel was sealed and the suspension was stirred (2 h, ambient) to saponify the acetyl groups. Excess NaOH was back-titrated with standard aqueous 0.1 M HCl, and results were corrected by a blank determination without starch. The amount of bound acetyl of starch dissolved in 5 M $CaCl_2$ was analyzed in the supernatant solution in a similar way. Because the effective alkali concentration is lower in this case, experiments with starch acetates with known S were performed to verify that saponification is quantitative also under these conditions. It was also established that $CaCl_2$ does not interfere with the analysis. S was calculated from the bound acetyl and carbohydrate contents in the supernatant.

Granule size distributions of fractions obtained for the size classification study were determined by taking photomicrographs of dilute suspensions and measuring the longest projection on a horizontal line across the photograph of the diameter of ca. 100 granules. Size averages were calculated by the formula presented in Section 2.1. In the surface peeling study, size distributions were measured in a Coulter Counter Multisizer II in a counting medium (Isoton) with a counting tube of 200 μm. Stock suspensions were treated in an ultrasonic bath for 1 min. Average diameters d_n and d_v from Coulter Counter data are number and volume median diameters, respectively.

4.2. Acetylation of granular potato starch

Acetylation was performed with 50–200 g of potato starch (AVEBE) with continuous stirring at ambient temperature. A magnetically stirred starch suspension (40% w/w, wet basis) in water or in aqueous Na_2SO_4 (10% based on total suspension weight) was adjusted to pH 8.2. Acetic anhydride was delivered from a motor-driven syringe at a rate of 6 mL/h. The pH was maintained at 8.2 during the reaction with aq 3% NaOH using a titrator/pH-stat. The reaction was terminated as soon as the approximate degree of substitution, calculated from the consumption of acetic anhydride and NaOH, had attained the desired value. The suspension was acidified to pH 6–6.5 and filtered. The reaction product was washed with water and air-dried.

4.3. Size classification of starch granules

The granular starch acetates were fractionated into five different size classes by repeated sedimentation in tap water or ethanol at five different times according to Decker and Höller.[33] The essential feature of this method is that a starch suspension is subjected to successive sedimentation treatments of increasing duration in a set of graduated cylinders in such a way that each cylinder n has its own fixed sedimentation time t_n. This is achieved by transferring the supernatant of the suspension in cylinder $n-1$, having settled for a time t_{n-1}, to the sediment in cylinder n to be left for settling during a time t_n after mixing. The sedimentation in each cylinder can be repeated several times by diluting the sediment in the first cylinder with fresh medium and repeating the transfer of supernatant to the neighbouring cylinders. The supernatants of the terminal cylinder are collected in a vessel (overflow). Water was unsuitable as a sedimentation medium for starch acetates with $S_0 \geqslant 0.2$ because of foaming and accumulation of part of the small granules in the foam layer, so ethanol was used for starch acetate at the highest degree of substitution. Sedimentation was performed in graduated cylinders (height ca. 17 cm) on 100 g of starch acetate at an initial suspension concentration of 20% (wet basis). Sedimentation times were 4, 9, 16 and 35 min in water or 5, 12, 20 and 42 min in ethanol. The number of sedimentation cycles was 10 for each sedimentation time. Size fractions were recovered by filtration and were air-dried. Size classification of unmodified potato starch was performed in water in a similar way, at a 1-kg scale in bins (height 20 cm) and an initial suspension concentration of 10% (wet basis).

4.4. Chemical gelatinization

Chemical gelatinization profiles on an analytical scale were recorded in a home-made temperature-programmed light intensity meter (LIM). The LIM is based on a microscope hot stage (Mettler FP82) having the inlet and exit apertures for the optical path connected to the mainframe of an immersion colorimeter by optical fibres. A starch suspension (5% in aqueous 5 M $CaCl_2$) was put on a microscope slide with a well and covered with a normal microscope slide. The suspension was heated at 1 °C/min by a Mettler Central Processor FP800. Intensity of transmitted light (measured without filter) and temperature were recorded simultaneously with a two-pen recorder. The transmission of 5 M $CaCl_2$ was set to 100% prior to the measurement. Strictly standardized operation conditions were required for obtaining reproducible results.

Starch acetate samples for gelatinization on a preparative scale (6 g dry basis) were gently ground with a mortar and pestle and treated several minutes in an ultrasonic bath in a small volume of aq 5 M $CaCl_2$ to disintegrate aggregates. Aq 5 M $CaCl_2$ was added to obtain a starch concentration of 3% (dry basis), and the suspension was heated at 1 °C/min with stirring (100 rpm) on a programmed water bath to a selected final temperature previously determined with the LIM. Good agreement between heating bath and LIM temperatures was obtained by programming the bath thermostat by an external thermocouple immersed in the starch suspension rather than directly by the bath temperature itself. The suspension was quench-cooled by adding five volumes of ice water. Gelatinized layers around granule cores were removed (starch acetates only) by treatment of the suspension with a

Waring blender cooled at 1 °C for 15 min (large granule fractions) or 30 min (small granule fractions). In this way the maximum temperature was limited to 10 °C. The suspension was centrifuged (3000 rpm, 15 min), and the sediment containing the peeled granules was washed 4 times with water, suspended in ethanol, filtered on glass (Nr. 3), and dried in a ventilating oven at ambient temperature. At high degrees of gelatinization DG ($\geqslant$75%) two batches had to be prepared in order to provide sufficient material. The supernatant and the first washing fluid (150 mL) were combined and diluted with water to 1 L. DG was calculated from the carbohydrate content of this dilution.

The data of Figure 3 were obtained under somewhat different conditions. The concentration of (unmodified) starch in 5 M $CaCl_2$ was 5% (wet basis) and total sample mass was 20 g. After attaining the selected temperature, the gelatinized suspension was diluted with ice water (75 mL). An aliquot (300 μL) was diluted with Isoton (175 mL) and analyzed for granule size and total granule volume (=before washing) with the Coulter Counter. The remaining suspension was centrifuged (15 min, 3000 rpm), the sediment was washed 3 times, diluted, and analyzed for granule size and total granule volume (=after washing). Supernatant and washing fluids were combined and diluted to a known volume. This dilution was assayed for carbohydrate content. The light transmission data in Figure 3 were obtained as described above. Light transmission data were converted into DG values by setting DG = 0 and 100% before and after gelatinization, respectively.

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