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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 $d_s = 44.2\ \mu m$, which is then the expected surface average diameter d_0 of the unclassified starch derivatives. This value agrees fairly well with $d_s = 43.6\ \mu m$ 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