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Topochemical effects in the methylation of starch

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ABSTRACT

The patterns of substitution in methylated starches, prepared in granular suspension, in the semi-dry state, and in solution, have been determined. There is evidence that the methyl groups are distributed uniformly throughout the interior of the starch granules. Methylation probably occurs exclusively in the amorphous domains. Consequently, the amylose fraction of granular starch is etherified preferentially, in contrast to methylation in solution. The physical state of the starch has no influence on the distribution of substituents amongst HO-2,3,6. The patterns of substitution are influenced markedly by the integrity of the granular structure.

INTRODUCTION

Starch is the most important storage polysaccharide in higher plants, and is deposited as a mixture of amylose and amylopectin in small semi-crystalline granules (1–100 μm in diameter) which are insoluble in cold water[1,2]. Starch derives its industrial value from its ability to form highly viscous dispersions by swelling of the granules in hot water[3,4] and as a raw material for chemical or physical modification[5–7].

Esterification or etherification of only a small proportion of the hydroxyl groups is usually sufficient to modify the properties of starch. The degree of substitution (d.s.) denotes the number of substituents per monomer unit. These modification reactions are carried out usually on aqueous suspensions of granules in the presence of a catalyst and (optionally) an inhibitor of swelling. The advantages of this procedure are a high concentration of starch and easy purification of the product. More recent techniques involve reactions in a semi-dry state[8]. In view of the semi-crystalline nature of starch[1], the question arises as to whether or not the substituents are introduced randomly. Comparison of the distribution of substituents in modified granular starch with those of derivatives of starch prepared in solution is an obvious approach, but has received little attention.

It is important to note that the structural features of the starch granule may affect the pattern of substitution in different ways. Therefore, such investigations must deal with distributions of substituents at the surface and in the interior of the granule, in the amylose and amylopectin fractions, in the crystalline and amorphous domains, amongst HO-2,3,6 of single glucose residues, and along the macromolecular chains.

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Some of these aspects have been studied and involve surface effects[9–11], amylose–amylopectin[12,13], monomer composition[12,14–17], and monomer sequence[18,19].

A systematic investigation of one type of starch derivative prepared in different ways should give information on all of the aspects mentioned above and should be complementary to (ultra)structural studies[20–25]. For instance, the crystalline structure of cellulose affects the reactivity of the different hydroxyl groups in the monomer unit[26].

It is a common experience in the starch industry that derivatives prepared in different ways, but with the same d.s., exhibit different properties. We now report on the patterns of substitution in methylated starches with a low d.s., prepared by reactions in suspension, in the semi-dry state, and in solution.

EXPERIMENTAL

Materials. — Methylated starches were prepared from potato starch (AVEBE B.A., Veendam); 2-, 3-, and 6-*O*-methylglucose were commercial products, and di- and tri-*O*-methylglucoses were kindly provided by Mr. J. Muetgeert (TNO, Delft).

Methylation of starch. — The procedure of Hjermstad and Kesler[27] was followed, whereby a slurry of granular starch was reacted with methyl sulphate in the presence of a catalytic amount of sodium hydroxide. After neutralisation, the product was washed with aqueous ethanol. Methylation in the semi-dry[8] state involved treatment of a mixture of potato starch (moisture content 20%) with sodium hydroxide and methyl chloride at elevated temperature. The product was washed free from salts with aqueous 80% ethanol, but it was impossible to remove the last traces of alkali even after neutralisation (HCl in aqueous ethanol). Methylation in solution involved treatment of aqueous 5% starch, prepared by autoclaving at 140°, with methyl sulphate at room temperature and a constant pH of 11. After neutralisation, the starch derivative was precipitated with methanol, then washed with aqueous and anhydrous methanol. All products were washed well with acetone in order to remove alcohol which interferes with the methoxyl determination.

Size classification. — Granular methylated starches were fractionated[28] into five sizes by sedimentation of 10% suspensions in ethanol in measuring cylinders (500 mL, height 17 cm). Sedimentation times were 2, 5, 12, and 20 min. Each fraction was passed through 10 sedimentation cycles. The fractions and the material in the overflows were collected by filtration, washed with acetone, and dried.

Methylated starch, prepared under semi-dry conditions, contained an appreciable proportion of agglomerates. A suspension of this product in methanol was sonicated overnight with stirring. The remaining aggregates were separated by several sedimentation cycles of 1 min.

Acid treatment. — A suspension of granular methylated starch (8 g) in 2.2M HCl in aqueous 65% ethanol (40 mL) was stored at 37° for 8 days. The product was collected by centrifugation, washed with aqueous 65% ethanol, neutralised with 0.1M NaOH in aqueous 65% ethanol, and washed with aqueous 80% ethanol, then acetone. In a similar experiment, 0.86M H_2SO_4 in aqueous 65% ethanol was used instead of HCl.

Fractionation into amylose and amylopectin. — Fractionation of methylated starch was achieved by a modification of the method of Muetgeert[29]. Several portions of methylated starch (1.6 g) were gelatinised in distilled water, then autoclaved (140°, 15 min). Sodium sulphite (antioxidant, 0.4 g) and appropriate amounts of $MgSO_4 \cdot 7H_2O$ and water were added to a final weight of 72 g. The mixtures were autoclaved (140°, 30 min), then stored overnight at 80–90°. The amylose was collected by centrifugation, washed twice with aqueous magnesium sulphate, dialysed, and freeze-dried. The supernatant solutions, which contained the amylopectin, were combined with the washings, dialysed, and freeze-dried.

Methylated starch of d.s. 0.06 was fractionated[12] with isopentyl alcohol. After storage for 6 months, the amylose–isopentyl alcohol complex was collected, washed with saturated aqueous isopentyl alcohol, methanol, and acetone, then dried. Isopentyl alcohol was removed from the supernatant solution by repeated evaporation under reduced pressure after the addition of water. Amylopectin was recovered by freeze drying.

Analyses. — Moisture content was determined by drying for 2 h at 130°. The d.s. was measured by the Zeisel method[30], as used for *O*-(2-hydroxyethyl)starch.

Methylated starches (10 mg) were subjected in sequence[31] to acid hydrolysis, borohydride reduction, and acetylation. Hydrolysis was achieved[32] with 2M trifluoroacetic acid at 125°. The partially methylated alditol acetates were analysed by g.l.c. on a capillary column of CP Sil-5CB (50 m) in a Carlo Erba capillary gas chromatograph (Type HRGC) equipped with an f.i.d. detector; the temperature program was 1 min at 50°, →205° at 10°/min, 20 min at 205°, →240° at 15°/min, and 7 min at 240°. Individual components were identified by comparison with authentic standards.

Permethylation of amylose and amylopectin, obtained from partially methylated starch, was performed by the Hakomori method[31]. The permethylated products were analysed by g.l.c. as described above.

Iodine-binding capacity (i.b.c.) of methylated starches was measured by amperometric titration with KIO_3 in an acidic medium[33]. Granular size was determined by a microphotographic method from the average of 100–200 granules.

Birefringence of granular methylated starches was estimated with a polarising microscope (Nikon L Ke) equipped with a rotating analyser and fitted with a Sénarmont compensator at 546 nm. Birefringence was calculated by dividing the difference in the optical path-length by 0.561 *d* (the granule diameter)[34]. An average of 25 readings was taken.

RESULTS AND DISCUSSION

Methylation of starches and iodine-binding capacity of the products. — Methylation was chosen as a model for slow, kinetically controlled etherification reactions[5]. The d.s. and i.b.c. of the methylated starches, prepared by different methods, are shown in Table I. The i.b.c. value reflects the amount and the properties of the amylose fraction. Methylation of only a minor proportion of the glucose residues strongly diminishes the

TABLE I

Method of preparation, degree of substitution (d.s.), and iodine-binding capacity (i.b.c.) of methylated starches

State of starch	*Method of preparation*	*Methylating agent*	*D.s.*	*I.b.c. (%)*
Granular	Suspension	Me_2SO_4	0.06	n.d.[a]
Granular	Suspension	Me_2SO_4	0.16	1.9
Granular	Semi-dry	MeCl	0.14	2.7
Dissolved	Solution	Me_2SO_4	0.15	2.6

[a] Not determined.

ability of amylose to form inclusion complexes. The i.b.c. of *O*-methylstarch prepared in suspension is appreciably lower than those of the other methylated starches with similar values of d.s. This finding points to a relatively high level of substitution of the amylose fraction.

Distribution of substituents in granules of different size. — Surface effects in the modification of starch have been studied by optical and electron microscopy of thin sections of modified granules after staining[9,11,20] or by specific degradation of parts of crushed granules[35]. Modified regions may be stained by reactive dyes[36] or metal salts[20]. Scanning electron microscopy with X-ray microanalysis may be applied to measure local concentrations of metal[10]. These methods probe the distribution of substituents on a rather coarse (μm) scale. Unfortunately, their use is limited to ionic derivatives of starch or to modified starches that contain "heavy" elements. Hence, the use of an indirect method was necessary. For a surface reaction, the probability of reaction is assumed to be equal for all the elements in the surface, and zero for the interior. As the ratio of area to volume for a spherical granule of diameter d is $6/d$, the d.s. of a fraction (S_d) with diameter d will be proportional to $6/d$. For a granule with average diameter d_m, the level of substitution S_m is equal to the d.s. of the whole methylated starch. It follows that $S_d/S_m = d_m/d$. Hence, for a surface reaction, a plot of S_d/S_m *vs.* $1/d$ should be linear with a slope d_m.

The granular methylated starches were classified on the basis of size. Fractionation into various sizes was achieved by repeated sedimentation in ethanol, in which the modified starches do not swell. Potato starches are well suited to this approach because of their broad distribution of particle sizes. The results are shown in Table II. The d.s. of individual granules was independent of their size. Hence, it is likely that the methyl groups were distributed uniformly throughout the interior of the granules.

A surface effect may be expected if the rate of reaction is much faster than those of the diffusion of reagent and catalyst into the granule. The rates of reaction of granular starch in suspension with methyl sulphate, or in the semi-dry state with methyl chloride, were appreciably lower than the rates of penetration of these reagents and the catalyst into the granule. Apparently, the moisture content of the starch in the semi-dry reaction was sufficiently high to allow solid sodium hydroxide to be transferred into the interior of the granule.

TABLE II

Distribution of substituents over granules of different size in methylated starches

Method of preparation	*Sedimentation time (min)*	*Yield (%)*	*Size of granule (μm)*	*D.s.*
Suspension	2	19.4	67	0.16
(d.s. 0.16)	5	39.7	53	0.15
	12	27.9	35	0.16
	20	6.4	26	0.16
	>20	4.4	17	0.16
	loss	2.2		
Semi-dry	2	10.1	72	0.13
(d.s. 0.14)	5	43.3	54	0.14
	12	24.3	39	0.13
	20	0.8	25	0.13[a]
	>20	0.8	21	0.13[a]
	lumps	15.3	80	0.18
	loss	5.4		

[a] Determined by g.l.c.

Reaction in the semi-dry state afforded a product that contained an appreciable proportion of lumps. These could not be disrupted and had a d.s. which was higher than that of the methylated starch as a whole. This finding probably reflects an uneven distribution of sodium hydroxide within the mass of starch. A high local concentration of alkali may result in a higher d.s. The formation of aggregates might be caused by local gelatinisation at the surface of the granule due to a combination of a high local concentration of alkali, moisture, and elevated temperature.

Distribution of substituents in the amorphous and crystalline regions. — Starch is a semi-crystalline polymer. According to their X-ray diffraction pattern, starches of types A, B, and C may be distinguished[1]. Estimates of the degree of crystallinity of starch of type B, such as potato starch, range from 28% measured by X-ray diffraction[37] to 50% determined by c.p.-m.a.s.–^{13}C-n.m.r. spectroscopy[24]. In normal types of starch, the crystalline regions consist of clusters of the short side-chains of amylopectin[1,23], and the layering of crystalline and amorphous domains is tangential[21,22] with a periodicity of ~ 10 nm (refs. 22, 25). The most promising technique for investigating the distribution of substituents in the crystalline and amorphous zones appears to be c.p.-m.a.s.–^{13}C-n.m.r. spectroscopy. In principle, it is possible to separate the ^{13}C resonances that originate from the crystalline and amorphous domains[38]. Recently, some significant advances have been made in the field of cellulosics[39,40].

As an alternative approach, we have used acid hydrolysis of starch granules. On treatment of granular starches with dilute acid at moderate temperature, the amorphous regions are removed preferentially, whereas the overall structure of the granule is preserved. The characteristic concentric ring pattern is accentuated and the sharpness of

the X-ray diffraction pattern is increased[37,41]. Treatment with dilute acid is a simple method for determining the reactivity of crystalline and amorphous domains in granular starch on the basis of the d.s. of the acid-resistant and/or soluble material. However, acid hydrolysis is not selective but merely proceeds at different rates in the amorphous and crystalline zones[23].

Granular methylated starches were treated with 2.2M hydrochloric acid in aqueous 65% ethanol for 8 days at 37°. In this medium, the swelling of the granules was prevented. The results are shown in Table III. The d.s. of the dissolved material (S_s) is given by $(S - w_r S_r)(1 - w_r)$, where S and S_r are the d.s. of the parent *O*-methylstarch and the acid-resistant fraction, respectively, and w_r is the weight fraction of the acid-resistant material recovered.

The heterogeneity of reaction was estimated from the ratio S_s/S_r; if this ratio is equal to unity, then the reaction is uniform. The results pointed to preferential reaction in the amorphous zones. A more quantitative assessment would require measurement of the crystallinity of the parent methylated starch and the acid-degraded residue. Komiya and Nara[42] have monitored the crystallinity of native potato starch during hydrolysis and, by using these data, we performed a tentative calculation of the d.s. of the amorphous and crystalline regions. For the crystalline zones, values of d.s. close to zero were obtained, so that large differences in the levels of substitution of the crystalline and amorphous domains are feasible. The assumption that the reaction proceeds exclusively in the amorphous regions is reasonable, regardless of the method of preparation. Hood and Mercier[43] also postulated a preferential reactivity of the amorphous regions on the basis of studies of the enzymic degradation of lightly cross-linked hydroxypropyl manioc starch.

The crystallinity of starch is a variable property. The crystallinity of starch acetates decreases gradually[44] as the d.s. increases from 0 to 1. It is suggested that reactions with starch take place exclusively in the amorphous zones, which then grow in size at the expense of the crystalline domains.

Methylation of starch in a semi-dry reaction leads to a more uniform distribution of methyl groups than methylation in suspension, probably because the structure of the granule is affected more strongly by the more rigorous conditions of the former

TABLE III

Acid degradation[a] of granular methylated starches

Method of preparation	*Acid*	*Acid-resistant residue (%)*	S_s[b]	S_r[c]	S_s/S_r
Suspension (d.s. 0.16)	2.2M HCl	44	0.20	0.12	1.7
	0.86M H_2SO_4	59	0.19	0.13	1.5
Semi-dry	2.2M HCl	32	0.15	0.11	1.4

[a] 2.2M HCl or 0.86M H_2SO_4 in aqueous 65% EtOH for 8 days at 37°. [b] D.s. of dissolved material. [c] D.s. of acid-resistant material.

TABLE IV

Birefringence of methylated starches in paraffin and water

Method of preparation	*Birefringence*	
	In paraffin	*In water*
Native potato starch	0.0096	0.0077
Suspension (d.s. 0.16)	0.0086	0.0073
Semi-dry (d.s. 0.14)	0.0077	0.0027

reaction. This inference followed from the lower resistance of this product towards acid. The birefringence of both methylated starches was measured in paraffin and in water (Table IV). Because of the large variations in size between individual granules, the results are regarded as qualitative only. The measurements in paraffin point to a slight decrease in the ordering of the starch molecules due to the reaction. The product from the semi-dry reaction was somewhat more affected than that from the reaction in suspension. Immersion of native starch in water leads to a minor decrease in birefringence which can be explained by an increase in volume of the granules due to sorption of water. A similar effect was observed for starch methylated in suspension. On the other hand, immersion of the methylated starch, prepared by semi-dry reaction, led to a sharp decrease in birefringence. This finding suggested that the organisation of the granule had been affected strongly under the conditions of reaction, which led to a more uniform pattern of substitution. However, traces of alkali that remained in the product might also be responsible for some loss of birefringence.

Distribution of substituents in amylose and amylopectin. — The discovery[45] that amylose complexes selectively with higher alcohols forms the basis of the favoured method for the separation of amylose and amylopectin. The formation of a crystalline inclusion complex requires regularity of the polymer chain, as in amylose. This is affected by the presence of substituents. Therefore, the fractionation of starch becomes increasingly difficult as the d.s. rises. Doane *et al.*[12] described the fractionation of methylated starch of d.s. 0.13 but, in our hand, fractionation could be achieved only up to d.s. 0.06. This procedure necessitated storage of the starch–alcohol mixture for several weeks. For cationic starch[13], the limit was as low as d.s. 0.01. Muetgeert[29] discovered that starch can be fractionated from aqueous solution by using such inorganic salts as $MgSO_4$. When starch is heated up to 160° in the presence of aqueous $MgSO_4$ and the mixture is cooled to a temperature that is below the solubility temperature of amylose and above that of amylopectin, amylose separates as small droplets which solidify, become water-insoluble on storage, and can be isolated after dilution of the mixture. Dilution prevents precipitation of amylopectin. This method is suitable for the industrial manufacture of amylose and amylopectin[46].

Methylated starch was gelatinised in water at 140°, the mixture was cooled, and the required amount of $MgSO_4$ and a small amount of Na_2SO_3 (an antioxidant) were added. The mixture was heated to 140°, then stored overnight at 80–90°. The amylose

TABLE V

Fractionation of methylated starches into amylose and amylopectin

Method of preparation	*Suspension (d.s. 0.06)*	*Suspension (d.s. 0.16)*	*Semi-dry (d.s. 0.14)*	*Solution (d.s. 0.15)*
Fractionating agent (%, w/w)	IA[a] 7.3	$MgSO_4$ 9	$MgSO_4$ 9	$MgSO_4$ 9
Yield (%)				
Amylose	16[b]	16	16	14
Amylopectin	77[b]	66	52	55
Loss	7[b]	18	32	30
I.b.c. (%)				
Amylose	17.4	8.0	11.1	12.4
Amylopectin	0.2	0	0.6	0.5
Branching degree (%)				
Amylose	n.d.[c]	1.7[d]	0.8	1.4
Amylopectin	n.d.[c]	3.9	4.0	3.7

[a] Isopentyl alcohol. [b] Approximate value. [c] Not determined. [d] Upper limit (undermethylated sample).

was precipitated only on cooling to room temperature, and separated as tiny globules, the size and proportion of which varied considerably with the amount of salt used. In fact, fractionation of *O*-methylstarch in this manner is more convenient than that of unmodified starch; methylated amylopectin does not precipitate from aqueous solutions of salt at room temperature. In contrast to unmodified amylose, the globules of methylated amylose were soluble in water. The *O*-methylamylose was purified by washing with aqueous $MgSO_4$ and dialysis. The efficiency of the fractionation at different concentrations of salt was judged from the yields and i.b.c. of the fractions. A 9% solution of $MgSO_4$ was optimal for *O*-methylstarch of d.s. 0.15.

The results of the fractionations are shown in Table V. Methylation analysis on the fractions revealed that the separation was not complete. On the other hand, the i.b.c. values of the methylated amyloses and amylopectins differed by a factor of at least ten and the yields were as expected. Hence, the efficiency of the fractionation was satisfactory. The i.b.c. values of methylated amyloses are low in comparison with that (19.5%) of unmodified amylose[2] due to the interference of the substituents.

The distributions of methyl groups in the amylose and the amylopectin are given in Table VI. In reactions with granular starch, the amylose was methylated preferentially, in agreement with experiments on cationic starch[13], but the reaction in solution proceeded uniformly with both fractions. Apparently, amylose is packed more loosely within the starch granule than is amylopectin.

The short side-chains of amylopectin are responsible for the crystallinity of starch. The most convincing evidence comes from studies of acid degradation[23,47]. Gel-permeation chromatography of acid-degraded starch revealed two populations of

TABLE VI

Distribution of substituents over amylose and amylopectin in methylated starches

Method of preparation	S_{Am} [a]	S_{Ap} [b]	S_{Am}/S_{Ap}
Suspension (d.s. 0.06)	0.07[c]	0.04[c]	1.8
Suspension (d.s. 0.16)	0.21	0.14	1.5
Semi-dry (d.s. 0.14)	0.14	0.13	1.1
Solution (d.s. 0.15)	0.15	0.14	1.1

[a] D.s. of amylose. [b] D.s. of amylopectin. [c] Determined by g.l.c.

fragments with d.p. of 15 and 25 that corresponded to linear and singly branched chains, respectively. The former population grew at the expense of the latter as the hydrolysis proceeded[23]. The branch point of the population of d.p. 25 is located near the reducing end[47] and these fragments are believed to originate from the short side-chains of amylopectin, which are organised in a double-helical fashion[48]. The presumed role of amylopectin in the crystallinity of starch accords with the preferential leaching of amylose from the granule with hot water[2,45] and with the fact that waxy starches exhibit an X-ray diffraction pattern.

Our results support the above-mentioned hypothesis on the crystallinity of starch. The higher reactivity of amylose in granular starch may be merely a consequence of the inaccessibility of the crystalline domains. In a previous study of cationic starch, the relative reactivity of the amylose was found to be affected strongly by the physical state of the starch[13]. Thus, the more uniform pattern of substitution in the product of the semi-dry reaction may result from a partial disruption of the granule and parallels the results of the study of acid degradation.

TABLE VII

Percentage of methyl groups located at HO-2,3,6 in methylated starches and fractions obtained therefrom

Method of preparation	*Suspension (d.s. 0.06)*	*Suspension (d.s. 0.16)*	*Semi-dry (d.s. 0.14)*	*Solution (d.s. 0.15)*
Whole starch		72:19:9	72:17:11	72:17:10
Granule size				
25 μm			72:18:10	
21 μm			72:18:10	
Acid-resistant residue (HCl)		78:14:8	77:13:9	
Amylose	70:21:9[a]	77:12:11	72:15:12	72:17:11
Amylopectin	70:23:7	74:17:9	73:15:11	73:17:10

[a] Ratios for HO-2,3,6.

Monomer composition. — The methylated starches were subjected in sequence[31] to hydrolysis with trifluoroacetic acid[32], borohydride reduction, and acetylation. The resulting partially methylated alditol acetates were analysed by capillary g.l.c. Di- and tri-*O*-methylated derivatives were present only in traces, so that only the relative reactivities of HO-2,3,6 need to be considered. The results in Table VII show that methylation occurred mainly at HO-2. This selectivity seems to be a common feature of etherification reactions at moderate concentrations of alkali[14,15,44]. According to Rowland[49], the principal factor which determines the regioselectivity is the composition of the reaction mixture. The physical state of the polysaccharide chain can influence the distribution of substituents, since the various hydroxyl groups may not be equally accessible. A significant effect of the crystallinity on the substitution pattern was demonstrated with cationic cellulose. The relative accessibility of the different hydroxyl groups could be related to the hydrogen-bonded structure of crystalline cellulose[26]. In contrast, the results in Table VII show that the physical state of the starch in the reaction has no influence on the monomer composition and indicates that each accessible glucose residue had the same micro-environment.

The results of this work suggest that the methylation of starch takes place exclusively in the amorphous regions. The pattern of substitution is not affected by the physical state of the starch on length scales much smaller and much larger than the size of the crystalline and amorphous domains.

Methylation is a model reaction for slow, kinetically controlled etherification reactions[5] and it is probable that the results do not apply to equilibrium-controlled or fast reactions.

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REFERENCES

1 R. L. Whistler, J. N. BeMiller, and E. F. Paschall (Eds.), *Starch: Chemistry and Technology*, 2nd. edn., Academic Press, Orlando, 1984.
2 W. Banks and C. T. Greenwood, *Starch and its Components*, Edinburgh University Press, 1975.
3 A. H. A. de Willigen, in J. A. Radley (Ed.), *Examination and Analysis of Starch and Starch Products*, Applied Science Publishers, London, 1976.
4 I. D. Evans and D. R. Haisman, *J. Texture Stud.*, 10 (1979) 347–370.
5 H. J. Roberts, in R. L. Whistler and E. F. Paschall (Eds.), *Starch: Chemistry and Technology*, Vol. I, Academic Press, New York, 1965, pp. 439–493.
6 H. J. Roberts, in R. L. Whistler and E. F. Paschall (Eds.), *Starch: Chemistry and Technology*, Vol. II, Academic Press, New York, 1967, pp. 293–350.
7 J. A. Radley (Ed.), *Starch Production Technology*, Applied Science Publishers, London, 1976.
8 K. J. Gardenier and W. Heimbürger, Ger. Pat. 2900073 (1980); *Chem. Abstr.*, 94 (1981) 17391s.
9 R. L. Whistler and W. W. Spencer, *Arch. Biochem. Biophys.*, 87 (1960) 137–139.
10 E. Berghofer and H. Klaushofer, *Staerke*, 29 (1977) 296–298.

11 E. Gruber, S. Alloush, K. John, and J. Schurz, *Staerke*, 24 (1972) 251–258.
12 W. M. Doane, C. R. Russell, and C. E. Rist, *Staerke*, 17 (1965) 77–81.
13 P. A. M. Steeneken, *Staerke*, 36 (1984) 13–18.
14 J. W. Mourits, H. G. Merkus, and L. de Galan, *Anal. Chem.*, 48 (1976) 1557–1561.
15 O. Larm, K. Larsson, and O. Theander, *Staerke*, 33 (1981) 240–244.
16 E. J. Roberts and S. P. Rowland, *Carbohydr. Res.*, 5 (1967) 1–12.
17 P. Mischnick-Lübbecke, W. A. König, and M. Radeloff, *Staerke*, 39 (1987) 425–431.
18 M. M. Kaminsky, Ph. D. Thesis, Rutgers University, 1978.
19 C. E. Weill, M. Kaminsky, and J. Hardenbergh, *Carbohydr. Res.*, 84 (1980) 307–313.
20 D. Gallant and A. Guilbot, *Staerke*, 21 (1969) 156–163.
21 M. Yamaguchi, K. Kainuma, and D. French, *J. Ultrastruct. Res.*, 69 (1979) 249–261.
22 G. T. Oostergetel and E. F. J. van Bruggen, *Staerke*, 41 (1989) 331–335.
23 J. P. Robin, C. Mercier, R. Charbonniere, and A. Guilbot, *Cereal Chem.*, 51 (1974) 389–406.
24 M. J. Gidley and S. M. Bociek, *J. Am. Chem. Soc.*, 107 (1985) 7040–7044.
25 J. M. V. Blanshard, D. R. Bates, A. H. Muhr, D. L. Worcester, and J. S. Higgins, *Carbohydr. Polym.*, 4 (1984) 427–442.
26 S. P. Rowland, E. J. Roberts, and J. L. Bose, *J. Polym. Sci., Part A-1*, 9 (1971) 1431–1440.
27 E. T. Hjermstad and C. C. Kesler, U. S. Pat. 2773057 (1956); *Chem. Abstr.*, 51 (1957) 4746c.
28 P. Decker and H. Höller, *J. Chromatogr.*, 7 (1962) 392–399.
29 J. Muetgeert, *Adv. Carbohydr. Chem.*, 16 (1961) 299–333.
30 H. J. Lortz, *Anal. Chem.*, 28 (1956) 892–895.
31 P.-E. Jansson, L. Kenne, H. Liedgren, B. Lindberg, and J. Lönngren, *Chem. Commun. Univ. Stockholm*, 8 (1976).
32 P. Albersheim, D. J. Nevins, P. D. English, and A. Karr, *Carbohydr. Res.*, 5 (1967) 340–345.
33 Y. Takeda, S. Hizukuri, and B. O. Juliano, *Carbohydr. Res.* 168 (1987) 79–88.
34 H. Speich, *Ber. Schweiz. Bot. Ges.*, 52 (1942) 175–214.
35 G. F. Fanta, F. L. Baker, R. C. Burr, W. M. Doane, and C. R. Russell, *Staerke*, 25 (1973) 157–161.
36 T. J. Schoch and E. C. Maywald, *Anal. Chem.*, 28 (1956) 382–387.
37 C. Sterling, *Staerke*, 12 (1960) 182–185.
38 D. L. Vanderhart and E. Perez, *Macromolecules*, 19 (1986) 1902–1909.
39 P. M. Patterson, D. J. Patterson, J. Blackwell, J. L. Koenig, A. M. Jamieson, Y. P. Carignan, and E. V. Turngren, *J. Polym. Sci., Polym. Phys. Ed.*, 23 (1985) 483–492.
40 D. Gagnaire, J. Saint-Germain, and M. Vincendon, *Polym. Bull.*, 13 (1985) 365–371.
41 R. Cleven, C. van den Berg, and L. van der Plas, *Staerke*, 30 (1978) 223–228.
42 T. Komiya and S. Nara, *Staerke*, 38 (1986) 9–13.
43 L. F. Hood and C. Mercier, *Carbohydr. Res.*, 61 (1978) 53–66.
44 P. A. M. Steeneken, unpublished data.
45 T. J. Schoch, *Adv. Carbohydr. Chem.*, 1 (1945) 247–277.
46 W. C. Bus, J. Muetgeert, and P. Hiemstra, U.S. Pat. 2822305 (1958); *Chem. Abstr.*, 52 (1958) 9635c.
47 K. Umeki and K. Kainuma, *Carbohydr. Res.*, 96 (1981) 143–159.
48 H. C. H. Wu and A. Sarko, *Carbohydr. Res.*, 61 (1978) 7–25.
49 S. P. Rowland, *Cellul. Chem. Technol.*, 14 (1980) 423–439.