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Carbohydrate Research 325 (2000) 183–191

CARBOHYDRATE RESEARCH

Distribution of methyl substituents in amylose and amylopectin from methylated potato starches

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Received 29 July 1999; accepted 2 December 1999

Abstract

Granular potato starches were methylated in aqueous suspension with dimethyl sulfate to molar substitution (MS) values up to 0.29. Fractions containing mainly amylose or amylopectin were obtained after aqueous leaching of the derivatised starch granules. Amylopectin in these fractions was precipitated with Concanavalin A to separate it from amylose. Amylose remained in solution and was enzymatically converted into D-glucose for quantification, thereby taking into account the decreased digestibility due to the presence of methyl substituents. It was found that the MS of amylose was 1.6–1.9 times higher than that of amylopectin in methylated starch granules. The distributions of methyl substituents in trimers and tetramers, prepared from amylose- or amylopectin-enriched fractions, were determined by FAB mass spectrometry and compared with the outcome of a statistically random distribution. It turned out that substituents in amylopectin were distributed heterogeneously, whereas substitution of amylose was almost random. The results are rationalised on the basis of an organised framework that is built up from amylopectin side chains. The crystalline lamellae are less accessible for substitution than amorphous branching points and amylose.

Keywords: Methylated starch; Concanavalin A; Amylose; Amylopectin

1. Introduction

Starch is the main storage polysaccharide of higher plants [1]. It is deposited as a mixture of amylose and amylopectin in cold-water-insoluble granules. Amylose, essentially a linear (1→4)-linked polymer of α-D-glucose, which can contain a few (1→6) branches linking the linear chains [2], is located mainly in the amorphous domains of the starch granule [3]. The degree of polymerisation (DP) is controversial and seems to be dependent on the botanical source and the stage of the growth of the plants. DP values up to 15,000 have been reported [4]. Amylopectin, a (1→6)-branched (1→4)-α-D-glucan, is one of the largest naturally occurring macromolecules. DP values up to 3×10^6 have been reported [5]. The cluster model for the structure of amylopectin [6–8] is now widely accepted. Amylopectin side chains make up the framework of the crystalline lamellae, whereas branching points are located in the disordered amorphous domains between the crystallites

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0008-6215/00/$ - see front matter
PII: S0008-6215(99)00329-8

[9–11]. Profiles of amylopectin side chains obtained after enzymatic debranching show a polymodal distribution that is characteristic of the botanical origin [12].

Chemical modification of starch is carried out to fulfil the various demands for functionality in different products [13]. So-called starch derivatives are used in both the food and non-food sectors with tailored application profiles. The number, location and distribution of the substituents are not expected to be random in view of the microscopic organisation within the starch granule [6,14–16]. The level of substitution in derivatised starch granules is expressed in terms of degree of substitution (DS) or molar substitution (MS). The relation between chemical structure and functional properties is largely unknown. Substitution of amylose is necessary to avoid retrogradation, to maintain viscosity and to improve clearness of starch gels. Long-term changes in retrogradation of gelatinised starches have been attributed to amylopectin [17], whereas short-term changes seem to result from gelation and crystallisation of amylose [18,19]. More detailed structural information on the location and distribution of substituents is indispensable to gain further insight into the behaviour of derivatised starches. Previously, we have reported a method to separate linear and branched parts from methylated starch granules and it was shown for amylopectin that methylation resulted in the preferential substitution of branched compared with linear regions [20]. Indirect evidence for a relatively high DS of amylose was obtained after comparison of fragments from methylated potato starch with those of methylated amylopectin potato starch. The present study is aimed at the isolation and molecular characterisation of amylose and amylopectin from methylated potato starch to define, in detail, the substitution patterns in starch granules.

2. Results and discussion

Determination of amylose contents and digestibilities of methylated starch granules.— All procedures used for the analysis of amylose and amylopectin from methylated potato starches are summarised in Scheme 1.

Methylated potato starches (**P10**, **P20** and **P30**) were prepared by methylation of starch granules (**P0**) in an aqueous suspension using dimethyl sulfate [20,21]. Their MS values are included in Table 1.

Amylose contents were determined by a method that includes: (i) initial selective precipitation of amylopectin by Concanavalin A (Con A) [22–25]; (ii) exhaustive digestion with glucoamylase of the remaining amylose, and (iii) enzymatic estimation of the formed glucose. Con A specifically complexes with branched polysaccharides consisting of α-D-gluco- or α-D-mannopyranosyl residues at multiple non-reducing ends, leading to the formation of a precipitate [26,27]. For unmodified starches, the percentage of amylose follows from the ratio of glucose in the Con A supernatant to that in intact starch granules. In the case of the methylated starches **P10**, **P20** and **P30**, only very few non-reducing glucose residues of amylopectin are likely to contain methyl substituents that inhibit Con A complexation. In this context, it should be noted that iodine complexation is strongly affected by the presence of substituents [28]. In our approach, the incompleteness of digestion of amylose from **P10**, **P20** and **P30** using glucoamylase, due to the presence of methyl substituents, was taken into account by measuring the digestibilities of gelatinised granules of **P10**, **P20** and **P30**. Here, the digestibility is defined as the ratio of enzymatically obtained glucose to that of the real amount of glucose present in the sample (the total mass of the sample divided by $M_W = 162$ for a $(1\rightarrow4)$-linked glucose residue). If at a given MS value of the derivatised starch granules f_1(MS) and f_2(MS) represent the enzymatic digestibilities of amylopectin and amylose, respectively, then the digestibility of the gelatinised methylated potato starch granule (A) is given by:

$$A = [f_1(\mathrm{MS}) \times (\text{real percentage Ap}) + f_2(\mathrm{MS}) \times (\text{real percentage Al})]/100\% \quad (1)$$

where (real percentage Ap) and (real percentage Al) are the real percentages of amylopectin and amylose, respectively, present in the granule. The percentage of amylose that is

measured after digestion (B) is related to f_1(MS) and f_2(MS) by:

$$B = (f_2(\mathrm{MS}) \times \text{real percentage Al}) \times 100\%/[(f_1(\mathrm{MS}) \times \text{real percentage Ap} + f_2(\mathrm{MS}) \times \text{real percentage Al})] \quad (2)$$

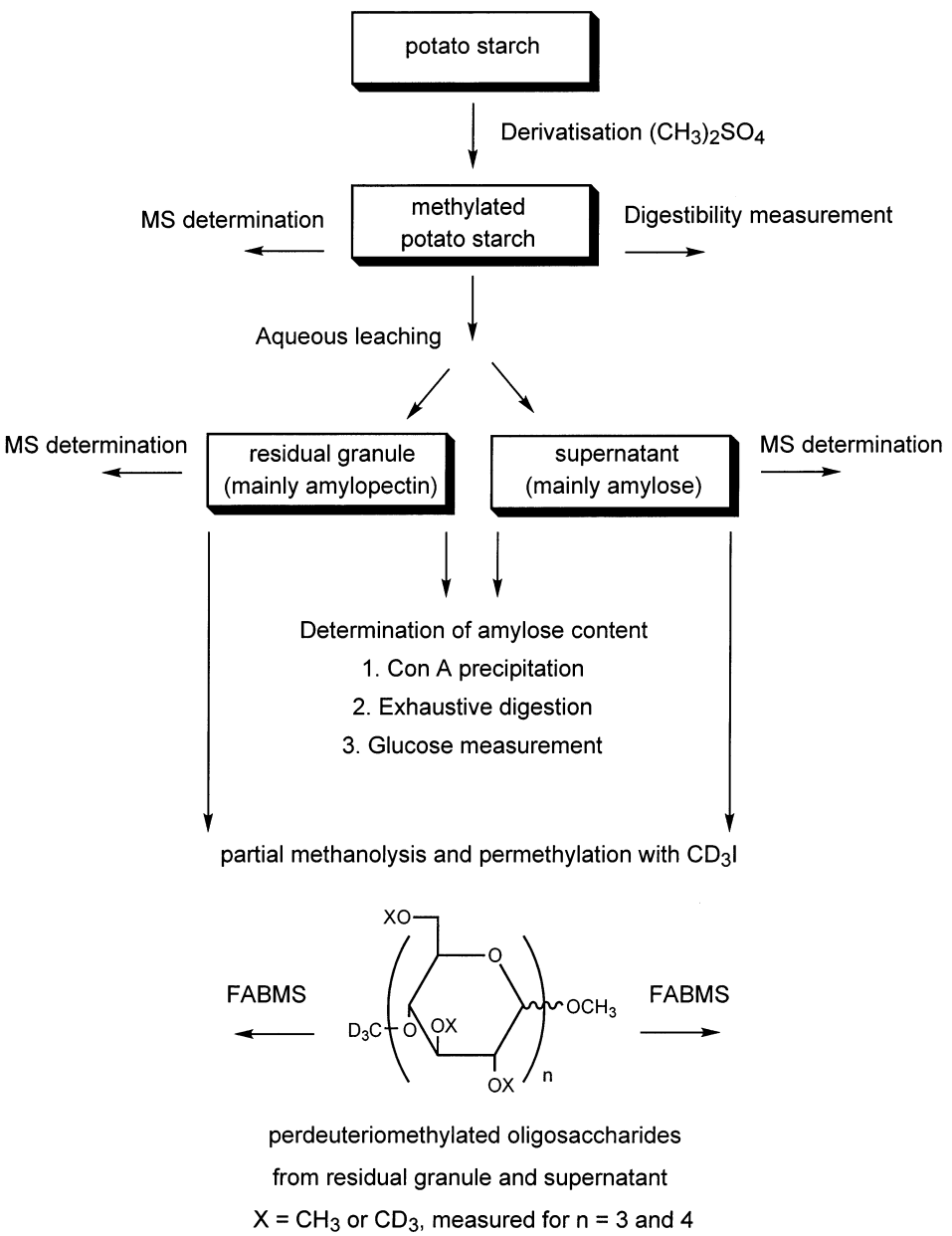
Note that this measured percentage B represents an *apparent* amount of amylose, because the parts of amylose that remain undigested are not determined as glucose. It is reasonable to assume that the real percentage of methylated amylose present in granules of starch derivatives is the same as the percentage of amylose present in native potato starch (that is the amounts of amylose and amylopectin that remain unchanged during derivatisation):

$$\begin{aligned} \text{real percentage Ap} &= 80\% \\ \text{real percentage Al} &= 20\% \end{aligned} \quad (3)$$

From Eqs. (1), (2) and (3) it follows that

$$f_1(\mathrm{MS}) = A \times (100 - B)/80 \quad (4)$$

and



Scheme 1. Methodology for the analysis of amylose and amylopectin from **P10**, **P20** and **P30**.

Table 1
Digestibility of amylose and amylopectin from (methylated) potato starch

Samples	Code	MS [a] of intact granule (GLC [b])	Digestibility [c] of gelatinised granules *A*	Measured percentage of amylose *B*	Digestibility [c] of amylopectin f_1(MS)	Digestibility [c] of amylose f_2(MS)
Native potato starch	**P0**	0	100	20	100	100
Methylated potato starch	**P10**	0.103	66	18.2	67	60
Methylated potato starch	**P20**	0.211	56	15.0	59	42
Methylated potato starch	**P30**	0.296	43	12.5	47	27

[a] The molar substitution (MS) is defined as mol of substituents/mol of glucose monomer. The MS varies from 0 (native starch) to 3 (permethylated linear starch).

[b] The MS is measured via monosaccharide analysis [20]. All monomers are characterised as trimethylsilylated methyl glucopyranosides ($\alpha+\beta$) and are given in molar percentages.

[c] The digestibility is defined as the amount of glucose that is obtained after exhaustive digestion with glucoamylase divided by the real amount of glucose present in the sample (the total mass of the sample divided by $M_W = 162$ for a (1→4)-linked glucose residue). The digestibility is given as a percentage.

$$f_2(\mathrm{MS}) = (A \times B)/20 \quad (5)$$

In Table 1 the measured digestibilities of **P0**, **P10**, **P20** and **P30** are given (*A*), as well as the measured percentages of amylose in these samples (*B*). From these two quantities, f_1(MS) and f_2(MS), were calculated according to Eqs. (4) and (5). The digestibility of the derivative decreases with increasing MS. Apparently, hydrolysis is hindered due to the presence of methyl substituents. Comparing f_1(MS) and f_2(MS) shows that the digestibility of amylose decreases more with increasing MS than that of amylopectin. This may be due to a relatively higher substitution level of amylose, a more blockwise substitution at amylopectin or a combination of both.

Aqueous leaching of (methylated) potato starch.—The different methods available for the isolation of amylose and amylopectin on a preparative scale from native starch granules give rise to problems when applied to starch derivatives. (Partially) methylated amylose does not form complexes with butanol by using the Schoch fractionation (data not shown) and (partially) methylated amylopectin disturbs the crystallisation of (partially) methylated amylose. Separation of amylose from amylopectin using $MgSO_4$ has been used for low-substituted starches, but cross-contamination increases with MS [29]. The aforementioned precipitation with Con A is suitable on an analytical scale only. For these reasons aqueous leaching of starch has been adopted here [30]. The leaching procedure was carried out at temperatures below the gelatinisation temperature in order to isolate amylose and amylopectin from **P10**, **P20** and **P30**. The percentages of amylose (*B* in Eq. (2)) in residual granules and supernatants thus obtained were measured after removal of amylopectin by Con A. The real percentage of amylose can be expressed as (from Eq. (2), and using percentage amylopectin + percentage amylose = 100):

$$\text{real percentage Al} = [B \times f_1(\mathrm{MS})] \times 100\% / [f_2(\mathrm{MS}) \times (100 - B)] + [B \times f_1(\mathrm{MS})] \quad (6)$$

In this way real percentages of amylose present in the supernatants and the residual granules of **P10**, **P20** and **P30** were calculated from measured percentages of amylose (*B*), using their corresponding values of f_1(MS) and f_2(MS) (Table 2). As is evident from Table 2, the amount of amylose leached and the purity thereof depend on the temperature.

Above 60 °C a substantial amount of carbohydrate leaches from native potato starch included as blank (**P0**), and up to 80 °C this material mainly consists of amylose. Further

Table 2
Aqueous leaching of potato starch (**P0**) and methylated potato starch (**P10**, **P20** and **P30**)

Sample	T (°C)	Starch in supernatant [a,b] (of total starch content)	Real amylose in supernatant [a,c]	Real amylose in residual granule [a,c]	Amylose in supernatant [a,d] (of total amylose content)
P0	60	4	n.d.	n.d.	n.d.
	65	10.7	n.d.	n.d.	n.d.
	70	13.0	89	10	58
	75	14.3	93	8	66
	80	16.1	91	6	73
	85	20.9	72	5	75
P10 [e]	55	15.9	92	7	73
	60	17.6	83	5	73
P20 [e]	55	19.8	87	7	86
	60	20.5	84	9	86
P30 [e]	45	14.0	90	12	63
	50	17.1	92	8	79

[a] As a percentage.
[b] Determined by mass measurement of the granules before and after leaching.
[c] Calculated using Eq. (6).
[d] Calculated according to (potato starch contains 20% of amylose): (real percentage of amylose in supernatant × percentage of starch in supernatant)/20%.
[e] Preparative leaching of **P10**, **P20** and **P30** was carried out at temperatures that are underlined.

increases in temperature result in additional leaching of amylose, however, becoming more contaminated with amylopectin. Methylated potato starches (**P10**, **P20** and **P30**) leach at lower temperatures, which is in agreement with the lower gelatinisation temperature of these derivatives. **P10**, **P20** and **P30** were incubated on a preparative scale at the temperatures that are underlined in Table 2. At these temperatures over 70% of amylose could be leached with a purity of over 87%. Fractions enriched in amylose or amylopectin, that is supernatants or residual granules, respectively, were obtained using this leaching procedure.

Molar substitutions of amylose and amylopectin from methylated potato starches.—All supernatants and residual granules obtained after leaching of **P10**, **P20** and **P30** contain both amylose and amylopectin (Table 2). Their MS values can be expressed in the following way:

$$\mathrm{MS}_{(\mathrm{supernatant})} = \mathrm{Al\ percentage}_{(s)} \times \mathrm{MS(Al)} + \mathrm{Ap\ percentage}_{(s)} \times \mathrm{MS(Ap)} \quad (7)$$

$$\mathrm{MS}_{(\mathrm{residual\ granule})} = \mathrm{Al\ percentage}_{(r)} \times \mathrm{MS(Al)} + \mathrm{Ap\ percentage}_{(r)} \times \mathrm{MS(Ap)} \quad (8)$$

where Al = amylose and Ap = amylopectin.

The $\mathrm{MS}_{(\mathrm{supernatant})}$ and $\mathrm{MS}_{(\mathrm{residual\ granule})}$ values of **P10**, **P20** and **P30** were measured using monosaccharide analysis (Table 3) [20]. Percentages of amylose present in supernatants and residual granules are given in Table 2. Combining Eqs. (7) and (8), the MS values of amylose [MS(Al)] and amylopectin [MS(Ap)] in **P10**, **P20** and **P30** were derived (Table 3).

It is clear from Table 3 that the MS of amylose is 1.6–1.9 times higher than that of amylopectin. In a previous study, indirect evidence of these differences in substitution levels was obtained by comparing methylated potato starch and amylopectin potato starch [20]. Also, differences between f_1(MS) and f_2(MS) values indicate a higher DS of amylose (Table 1). The preferred substitution of amylose is in agreement with the current model of a starch granule, in which amylose is present in the amorphous domains. These domains are better accessible than crystalline lamellae that consist of amylopectin side chains [21]. The calculated MS value for the 'granule' (virtual granule reconstituted from MS values of amylose and amylopectin, present in 20 and 80%

Table 3
MS values of amylose (Al) and amylopectin (Ap)

Samples	MS [a] (granule)	MS [a] supernatant	MS [a] residual granule	MS [b] (Al)	MS [b] (Ap)	MS [c] ('granule')
P10	0.103	0.154	0.101	0.159	0.097	0.109
P20	0.211	0.301	0.177	0.321	0.166	0.197
P30	0.296	0.399	0.272	0.411	0.260	0.290

[a] MS values measured via monosaccharide analysis [20].
[b] Calculated using Eqs. (7) and (8).
[c] Calculated according to (potato starch contains 20% amylose): MS ('granule') = 20% × MS(Al) + 80% × MS(Ap).

respectively) deviates only slightly from the MS value, as measured directly from the granule, indicating that no material is lost during aqueous leaching. The monomer compositions of the supernatants (amylose-enriched) and residual granules (amylopectin-enriched) obtained from **P10**, **P20** and **P30**, given in Table 4, show that the HO-2 group has by far the highest positional DS. The ratios between all substituted monomers are the same as those reported in earlier studies [20,21]. Therefore, no topochemical effects can be demonstrated on the level of monosaccharides.

Distribution of substituents in amylose and amylopectin.—The distribution of substituents in oligomer fragments represents the substitution pattern in the corresponding polymer backbone [31,32]. In order to prepare suitable derivatives for FAB mass spectrometric analysis, supernatants (87–92% amylose) and residual granules (92–93% amylopectin) obtained after aqueous leaching of **P10**, **P20** and **P30** (Table 2) were permethylated with CD_3I [33] after partial methanolysis (Scheme 1). It can be anticipated that the thus obtained per(deuterio)methylated oligomers with the same DP, only varying in number of CH_3- and CD_3-substituents, show the same ionisation behaviour because of their chemical equivalency [20,21]. Therefore, quantification of all FAB intensities in a pseudo-molecular-ion region ($[M + Na^+]$) of oligomers with the same DP is valid. In this respect trimers and tetramers are of specific interest for FAB mass spectrometric analysis, because resolution decreases using larger oligomers. The FAB mass spectrometric data of oligomers prepared from amylose- or amylopectin-enriched fractions were compared with the expected distributions according to binomial statistics. In the calculation, CH_3-substituents are distributed randomly over equal and independent glucose monomers. The differences between expected and observed distributions are expressed in the quantitative parameter H_n, indicating the degree of heterogeneity where n corresponds to the DP of the per(deuterio)methylated oligomers. All heterogeneity values in Table 5

Table 4
Monomer composition of supernatants (s) and residual granules (r) from leached methylated potato starch (**P10**, **P20** and **P30**)

Samples	Glc [a]	Glc2Me	Glc3Me	Glc6Me	Glc2,3Me_2	Glc2,6Me_2	Glc3,6Me_2
P10 [b]	90.1	6.3	1.9	1.3	0.2	0.2	0
P10 (s)	82.0	11.3	3.2	1.9	0.4	1.0	0.2
P10 (r)	90.4	6.1	1.8	1.2	0.2	0.2	0.1
P20 [b]	80.7	12.2	3.3	2.0	0.9	0.7	0.2
P20 (s)	73.2	15.9	4.6	3.0	1.3	1.6	0.4
P20 (r)	84.0	9.6	2.8	1.9	0.7	0.8	0.2
P30 [b]	73.7	16.4	4.2	2.4	1.7	1.3	0.3
P30 (s)	65.4	20.1	5.5	3.7	2.2	2.6	0.5
P30 (r)	75.7	15.1	4.0	2.2	1.5	1.2	0.3

[a] All monomers are characterised as trimethylsilylated methyl glucopyranosides (α + β) and are given in molar percentages.
[b] Monomer composition of methylated starch granules [20].

Table 5
Heterogeneity of substituent distribution in methylated potato starch

Sample	P10	P20	P30
H_3/MS(trimers, Al) [a,b]	0.211 [c]	0.071	0.046
H_3/MS(trimers, Ap) [d]	0.444	0.354	0.322
H_4/MS(tetramers, Al) [a]	0.299	0.160	0.096
H_4/MS(tetramers, Ap)	0.640	0.413	0.417

[a] Trimers, Al = trimers obtained from partially methanolysed supernatants (mainly containing amylose). Al, amylose; Ap, amylopectin.

[b] Calculation of H_3 and H_4 is described in Ref. [31].

[c] MS values determined from FAB mass spectrometric intensities (for example in the case of trimers: [mole fraction DS(0) × 0 + mole fraction DS(1) × 1 + … + mole fraction DS(9) × 9]/3) differ by up to 25% from MS values of the corresponding supernatants or residual granules. These differences are indicative of non-random substituent distributions for reasons that are discussed previously [21].

[d] Trimers, Ap = trimers obtained from partially methanolysed residual granules (mainly containing amylopectin).

are normalised by dividing by the MS value of the corresponding trimers or tetramers. In this way heterogeneities of samples with different degrees of substitution can conveniently be compared. If there are no differences between observed and calculated mole fractions, the CH_3-substituent distribution is homogeneous ($H_n = 0$).

It is clear from Table 5 that CH_3-substituents in amylose from **P20** and **P30** are almost randomly distributed. In contrast, the H_n values of amylopectin are much higher, indicating different substitution levels within one amylopectin molecule. Amylose from **P10** shows a somewhat higher degree of heterogeneity compared with **P20** and **P30**, but it is still significantly lower than that of amylopectin from **P10**.

3. Conclusions

In this study it is shown that methylation of starch granules in an aqueous suspension takes place preferably at amylose compared with amylopectin. This observation corroborates our previous studies on differences in substitution behaviour between amorphous and crystalline domains, wherein it was found that the former domains contained most substituents [21]. Amylose is located in amorphous domains and is therefore more sensitive to derivatisation. According to the current model of a starch granule, amylopectin side chains appear as crystalline lamellae that are less accessible for chemical substituents [6,7,14–16]. However, the branching points of amylopectin are relatively highly substituted, because they are located in amorphous subdomains. Studies on heterogeneity have shown that areas containing less substituents in the starch granule are derivatised in a non-random way. Amorphous domains comprising branching points and amylose are substituted more randomly during methylation of potato starch granules in an aqueous suspension. Recently, the relationship between the length of amylopectin side chains and starch crystallinity has been studied by using molecular modelling [34]. In this respect, it would be interesting to obtain information on the precise location of methyl substituents in amylopectin and their influence on the crystallinity of the starch granule.

4. Materials and methods

Aqueous leaching of (methylated) potato starch.—Potato starch **P0** and methylated potato starches **P10**, **P20** and **P30** (3.0 g, prepared by methylation of starch granules in an aqueous suspension by using dimethyl sulfate [20,21]) were suspended in 500 mL Millipore water at the temperatures listed in Table 2. After 1 h of incubation in a shaking bath the supernatants were separated from the residual swollen granules by centrifugation. The residual granules were incubated for a further 1 h after suspension in 500 mL Millipore water at the temperatures listed in Table 2, again followed by separation of the supernatants from the residues using centrifugation. This procedure was repeated once more. The supernatants of each sample were collected, concentrated, and dried at 100 °C. The residual granules were washed with 4.5 and 1 L EtOH with intermediate decanting, then dried at 60 °C.

Determination of amylose contents.—Amylose contents in supernatants and in residual

granules obtained after aqueous leaching of **P10**, **P20** and **P30** were determined after selective precipitation of amylopectin by Con A [22]. A testkit for the measurement of amylose contents in starches is supplied by Megazyme (Sydney, Australia). Aliquots, taken from each supernatant and residual granule fraction, were stirred with a Con A solution, whereby amylopectin precipitated as a complex with Con A and amylose remained in solution. After removal of the precipitate, the carbohydrate content in the solution was determined as glucose by the glucose oxidase/peroxidase method after exhaustive conversion into glucose using glucoamylase. The incompleteness of digestion of all carbohydrates, due to the presence of methyl substituents, was taken into account by measuring the digestibilities of gelatinised granules from **P10**, **P20** and **P30**. From these measurements amylose- and amylopectin contents were calculated using Eq. (6) (Section 2).

Analytical procedures.—Monosaccharide analysis was carried out by subjecting partially methylated oligo- or polyglucan samples to methanolysis (methanolic 1 M HCl, 18 h, 85 °C). The resulting mixtures of methyl glucoside derivatives were trimethylsilylated (1:1:5 hexamethyldisilazane – trimethylchlorosilane–pyridine), identified by GLC–mass spectrometry, and quantified by GLC using empirical molar response factors [20]. GLC analyses were performed on a WCOT CP-SIL 5CB fused-silica capillary column (25 m × 0.32 mm) using a temperature program of 110–230 °C at 4 °C min^{-1}. GLC–mass spectrometry of (O-methylated) glucose derivatives, measured as trimethylsilylated methyl glucosides, was carried out on an MD800/8060 system (Fisons Instruments, electron energy, 70 eV), equipped with a DB-1 fused-silica capillary column (30 m × 0.32 mm, J&W Scientific), using a temperature program of 110–230 °C at 4 °C min^{-1}. Partially methylated oligoglucans from supernatants and residual granules, obtained after aqueous leaching of **P10**, **P20** and **P30** and subsequent partial methanolysis (methanolic 0.5 M HCl, 2 h, 60 °C), were permethylated with CD_3I as described previously (Me_2SO–NaOH) [33], and analysed by using FAB mass spectrometry.

FAB mass spectrometry was performed on a Jeol JMS SX/SX 102A four-sector mass spectrometer, operated at 10 kV accelerating voltage, equipped with a JEOL MS-FAB 10 D FAB gun operated at a 10 mA emission current, producing a beam of 6 keV xenon atoms. The per(deuterio)methylated oligosaccharide samples were measured over a mass range of m/z 10–1200 in a matrix of *m*-nitrobenzyl alcohol saturated with NaI, using the standard resolution of 1500. The intensities of the sodium-cationised oligosaccharide ions were determined from their FAB spectra and compared with the statistically expected ones. The statistically expected ratios between non-, di-, tri-, tetra- and penta-substituted oligomers with specific DP were calculated according to a random distribution of CH_3 substituents at a given overall MS value. The heterogeneity parameter H_n is then calculated from the sum of all absolute differences between determined and expected intensities of each oligomer.

Acknowledgements

This study was supported by the PBTS Research Program with financial aid from the Ministry of Economic Affairs and the Integral Structure Plan for the Northern Netherlands from the Dutch Development Company.

References

[1] S.G. Ring, M.J. Miles, V.J. Morris, R. Turner, P. Colonna, *Int. J. Biol. Macromol.*, 9 (1987) 158–160.
[2] Y. Takeda, S. Tomooka, S. Hizukuri, *Carbohydr. Res.*, 246 (1993) 267–272.
[3] P.A.M. Steeneken, A.J.J. Woortman, *Carbohydr. Res.*, 258 (1994) 207–221.
[4] Th. Aberle, W. Burchard, W. Vorwerg, S. Radosta, *Starch/Stärke*, 46 (1994) 329–335.
[5] W. Banks, C.T. Greenwood, *Starch and its Components*, Edinburgh University Press, Edinburgh, 1975, p. 45.
[6] D. French, in R.L. Whistler, J.N. BeMiller, E.F. Paschall (Eds.), *Starch: Chemistry and Technology*, second ed., Academic Press, London, 1984, pp. 183–247.
[7] J.P. Robin, C. Mercier, F. Duprat, R. Charbonnière, A. Guilbot, *Cereal Chem.*, 51 (1974) 389–406.
[8] D.J. Manners, N.K. Matheson, *Carbohydr. Res.*, 90 (1981) 99–110.
[9] J. Jane, K. Wong, A.E. McPherson, *Carbohydr. Res.*, 300 (1997) 219–227.
[10] P.J. Jenkins, R.E. Cameron, A.M. Donald, W. Bras, G.E. Derbyshire, G.R. Mant, A.J. Ryan, *J. Polym. Sci. Polym. Phys. Ed.*, 32 (1994) 1579–1583.

[11] S. Pérez, A. Imberty, *Carbohydr. Eur.*, 15 (1996) 17–21.
[12] S. Hizukuri, *Carbohydr. Res.*, 147 (1986) 342–347.
[13] O.B. Wurzburg (Ed.), *Modified Starches: Properties and Uses*, CRC Press, Boca Raton, FL, 1986.
[14] A. Imberty, S. Pérez, *Biopolymers*, 27 (1988) 1205–1221.
[15] G.T. Oostergetel, E.F.J. van Bruggen, *Carbohydr. Polym.*, 21 (1993) 7–12.
[16] A. Buléon, P. Colonna, V. Planchot, S. Ball, *Int. J. Biol. Macromol.*, 236 (1998) 85–112.
[17] A.-C. Eliasson, in R.D. Hill, L. Munck (Eds.), *New Approaches to Research in Cereal Carbohydrates*, Elsevier, Amsterdam, 1985, pp. 93–98.
[18] M.J. Miles, V.J. Morris, P.D. Orford, S.G. Ring, *Carbohydr. Res.*, 135 (1985) 271–281.
[19] D. Sievert, P. Würsch, *J. Food Sci.*, 58 (1986) 1332–1345.
[20] Y.E.M. van der Burgt, J. Bergsma, I.P. Bleeker, P.J.H.C. Mijland, A. van der Kerk-van Hoof, J.P. Kamerling, J.F.G. Vliegenthart, *Carbohydr. Res.*, 312 (1998) 201–208.
[21] Y.E.M. van der Burgt, J. Bergsma, I.P. Bleeker, P.J.H.C. Mijland, A. van der Kerk-van Hoof, J.P. Kamerling, J.F.G. Vliegenthart, *Carbohydr. Res.*, 320 (1999) 100–107.
[22] T.S. Gibson, V.A. Solah, B.V. McCleary, *J. Cereal Sci.*, 25 (1997) 111–119.
[23] N.K. Matheson, L.A. Welsh, *Carbohydr. Res.*, 180 (1988) 301–313.
[24] N.K. Matheson, *Carbohydr. Res.*, 199 (1990) 195–205.
[25] S.-H. Yun, N.K. Matheson, *Starch/Stärke*, 42 (1990) 302–305.
[26] I.J. Goldstein, C.E. Hollerman, J.M. Merrick, *Biochim. Biophys. Acta*, 97 (1965) 68–76.
[27] P. Colonna, V. Biton, C. Mercier, *Carbohydr. Res.*, 137 (1985) 151–166.
[28] G. Wulff, A. Steinert, O. Höller, *Carbohydr. Res.*, 307 (1998) 19–31.
[29] P.A.M. Steeneken, E. Smith, *Carbohydr. Res.*, 209 (1991) 239–249.
[30] A.H. Young, in R.L. Whistler, J.N. BeMiller, E.F. Paschall (Eds.), *Starch: Chemistry and Technology*, second ed., Academic Press, London, 1984, pp. 259–260.
[31] P.W. Arisz, H.J.J. Kauw, J.J. Boon, *Carbohydr. Res.*, 271 (1995) 1–14.
[32] P. Mischnick, G. Kühn, *Carbohydr. Res.*, 290 (1996) 199–207.
[33] I. Ciucanu, F. Kerek, *Carbohydr. Res.*, 131 (1984) 209–217.
[34] A.C. O'Sullivan, S. Pérez, *Biopolymers*, 50 (1999) 381–390.