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# Internal unit chain composition in amylopectins

Eric Bertoft [a,*], Kuakoon Piyachomkwan [b], Pathama Chatakanonda [c], Klanarong Sriroth [d]

[a] Department of Biochemistry and Pharmacy, Åbo Akademi University, BioCity, Artillerigatan 6, FIN-20520, Turku, Finland
[b] Cassava and Starch Technology Research Unit, National Center for Genetic Engineering and Biotechnology, Pathumthani, Thailand
[c] Kasetsart Agricultural and Agro-Industrial Product Improvement Institute, Kasetsart University, Bangkok, Thailand
[d] Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, Bangkok, Thailand

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ABSTRACT

A series of 17 samples represented a diverse collection of different starch granules from different plants. The amylose content ranged from 0% to 28% and the crystallinity was established to represent A, B and C allomorphic types. The amylopectin components were isolated and their unit chain distributions analysed by anion-exchange chromatography. The internal unit chain profiles were obtained from the φ,β-limit dextrins. The internal profiles possessed two major fractions of short and long B-chains. The long B-chains were divided into two subgroups also found in the profiles of the whole amylopectins. The short, internal B-chains contained also two subgroups in all samples, of which one was a major group at DP 8–25, and the other was a minor group at DP 3–7. On the basis of the internal chain distribution profiles of (i) the long chains, reflecting the interconnection of clusters, and (ii) the short chains, reflecting the internal structure of the clusters, the samples were divided into four structural groups with typical characteristics. It was found that the unit chain profiles of the whole amylopectins could be divided into the same characteristic groups. The positions of the internal unit chains were theoretically traced into their original lengths in the profiles of the whole amylopectins. It was concluded that A-chains generally overlap with the shortest B-chains at DP 13–23, which coincided with fraction $fb_1$ of the periodic intervals described by Hanashiro et al. [Hanashiro, I., Abe, J.-i., & Hizukuri, S. (1996). *Carbohydrate Research, 283*, 151–159].


## 1. Introduction

Starch granules are composed almost exclusively of the two polymers amylose and amylopectin. In the vast majority of granules the amylopectin component predominates, and in some cases (in "waxy" starches) it is the sole component. As the latter granules have the same appearance as normal, amylose-containing granules, it is generally believed that it is the amylopectin that forms the skeleton of typical growth rings in the granules (Gallant, Bouchet, & Baldwin, 1997). These are alternating amorphous and semi-crystalline structures, of which the latter possess thin, alternating amorphous and crystalline lamellae of a universal thickness of approximately 9 nm (Jenkins, Cameron, & Donald, 1993). A great number of investigations of the unit chain distribution of amylopectins from different sources have shown that, generally, the macromolecule is composed of two major fractions of chains of α-(1 → 4)-linked D-glucosyl residues. Long chains (L-chains) of a degree of polymerisation (DP) of approximately >35 interconnect short (S) chains of DP 6–35 (Hizukuri, 1986). The proportion of S:L-chains is in the order of 5–12, depending on the source of the amylopectin (Manners, 1989). S-chains form clusters of α-(1 → 6)-branches inside the amorphous lamellae (Bertoft, 2007), and from these clusters external chains extend in the form of left-handed double helices (Imberty, Buléon, Tran, & Pérez, 1991). It is the double helices that build up the crystalline lamellae, approximately 4–6 nm thick (Kozlov, Noda, Bertoft, & Yuryev, 2006; Waigh et al., 2000). Two different allomorphs of the crystals exist and are designated A-type (more compact, less water-containing) and B-type (less compact, more water-containing), respectively (Imberty et al., 1991). Some starch granules are also known to contain both types of crystals and are called C-type (Buléon, Gérard, Riekel, Vuong, & Chanzy, 1998). The proportion of A- and B-crystals in C-type granules varies, and it is known that A-starches in general possess amylopectin with at average shorter chains than B-starches. The latter contain higher amounts of L-chains, and thus S:L is low (Hizukuri, 1985).

The chains of amylopectin are possible to distinguish as external and internal. The former build up the crystalline lamellae with comparatively well-known structures, and the latter are mainly found among the clusters of branches in the amorphous lamellae, of which the structure is far less known (Bertoft, 2007). The chains are also traditionally divided into A- and B-chains, of which the former are defined as chains not substituted with other chains, whereas the latter are substituted by either A-, B-, or both types of chains (Peat, Whelan, & Thomas, 1952). As a consequence, the entire A-chains

* Corresponding author. Tel.: +358 2 215 4272; fax: +358 2 215 4745.
*E-mail address:* eric.bertoft@abo.fi (E. Bertoft).

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are external, whereas the B-chains are composed of one external segment and one internal segment. The internal chain is divided into one or several smaller parts depending on the number of chains that are connected to them. The average internal chain length (ICL) between the branches is in the order of 5–8 residues, whereas the external chain length (ECL) is 11–16 (Manners, 1989).

The external chains can be largely removed using two exo-acting enzymes, namely β-amylase and phosphorylase. β-Amylase removes maltosyl residues from the non-reducing ends until it is blocked by the outermost branch points (Manners, 1989). A-chains remain as maltosyl or maltotriosyl stubs depending on whether the chains originally contained an even or odd number of residues, whereas the external segments of the B-chains remain as glucosyl or maltosyl stubs in the resistant β-limit dextrin (β-LD) (Summer & French, 1956). Phosphorylase removes glucosyl residues in the form of glucose 1-phosphate, and in the φ-LD the A-chains are reduced into maltotetraosyl stubs and external B-chains into maltotriosyl stubs (Bertoft, 1989). Hence, in the φ-LD all A-chains and external B-chain stubs are of equal lengths (DP 4 and 3, respectively) regardless the original constitution of residues. The external chains of the φ-LD are further reduced by two residues if they are treated with β-amylase to form a φ,β-LD. Thus, in this LD A-chains appear as maltosyl stubs and the external segments of the B-chains as glucosyl stubs (Bertoft, 1989). Because the exo-acting enzymes cannot by-pass the branches, the limit dextrins contain all original branches of the amylopectin and possess a profile of the B-chains that shows the distribution of the internal chains of the original amylopectin macromolecule.

In comparison with the extensive number of investigations that were undertaken to analyse the unit chain profiles of whole amylopectins from all kind of plants (comprehensive overviews are found in e.g., Fredriksson, Silverio, Andersson, Eliasson, & Åman, 1998; Hanashiro, Abe, & Hizukuri, 1996; Hizukuri, 1985; Sanderson, Daniels, Donald, Blennow, & Engelsen, 2006; Srichuwong, Sunarti, Mishima, Isono, & Misamatsu, 2005), the internal unit chain composition has only obtained scarce attention. In addition, only single or few amylopectin samples were compared in these works. Pioneering works showed the distribution of chains in β-LDs from legumes, cereals, and potato (Biliaderis, Grant, & Vose, 1981; MacGregor & Morgan, 1984; Robin, 1981). More recently Shi and Seib (1995) and Klucinec and Thompson (2002) analysed the unit chain composition of some β-LDs of mutant maize varieties. Yao, Thompson, and Guiltinan (2004) noted that the internal short B-chains in maize possessed a minor subgroup around DP 5, which was designated B1a-chains, and a major group, which they called B1b. Bertoft and Koch (2000) analysed the distribution of chains in the φ,β-LDs of waxy rice. Later waxy maize and amylose-free potato was investigated (Bertoft, 2004a). In these samples minor subgroups of short chains were also found and called "fingerprint" B-chains ($B_{fp}$), apparently identical to the group of B1a-chains in the work of Yao et al. (2004).

The aim of the present investigation was to obtain a general overview of the internal unit chain distribution in amylopectins. Starch samples were selected into a collection representing a broad range of different sources with regards to the type of plant and to the type of crystalline structure. The internal unit chain profiles of the φ,β-LDs were compared with the distributions of chains in the original, whole amylopectins in order to trace the contribution of the internal chains to the overall structure of amylopectin.

## 2. Materials and methods

### 2.1. Starch samples and enzymes

Starch granules were kind gifts provided as follows: waxy rice (WRS) and medium amylose-containing rice starch (MRS) were from Remy Industries n.v., Leuven-Wijgmaal, Belgium, waxy maize (WMS) and potato starch (PS) from Chiba Chemicals, Raisio, Finland, waxy barley (WBS), normal barley (NBS), and oat starch (OS) were from Altia Oy, Rajamäki, Finland, amylose-free potato starch (PAPS) from Lyckeby Stärkelsen, Kristianstad, Sweden, Andean yam bean starch (AYS) from Prof. Wolfgang Bergthaller, Detmold, Germany, and kudzu starch (KS) from Dr. Jean-Luc Puteaux, Grenoble, France. Tapioca (TS, grown in Thailand) and arrowroot starch (AS, S:t Vincent, West India) were purchased at markets in Finland. Yam (YS), canna (CS), mung bean (MBS), and sago starch (SS) were collected in Thailand. The latter starches were extracted by grinding peeled samples with water and passing through a 170-mesh sieve. Starch slurry was then collected and settled. After starch sedimentation, water was decanted and the starch cake was re-washed twice before drying. Starch purity was determined by an enzyme method (AACC, 1995). The content of protein, fat, fiber and ash were also quantified according to the AOAC methods (AOAC, 1990). Rye starch (RS) was extracted from Finnish rye flour, which had been softened in 0.01 M $HgCl_2$ solution, by passing through a 35-μm mesh sieve and repeated sedimentations. Starches from barley, oat and rye, known to be rich in lipids, were defatted by extraction with 85% methanol in a Soxhlet apparatus. Other starches were used directly as they were provided.

The following enzymes were from Megazyme (Wicklow, Ireland): Isoamylase (glycogen 6-glucanohydrolase; EC 3.2.1.68; specific activity 280 U/mg) from *Pseudomonas* sp., pullulanase (amylopectin 6-glucanohydrolase; EC 3.2.1.41; 42 U/mg) from *Klebsiella planticola*, and barley β-amylase [(1 → 4)-α-D-glucan maltohydrolase; EC 3.2.1.2; 1400 U/mg]. Phosphorylase *a* [(1 → 4)-α-D-glucan:ortophosphate α-D-glucosyltransferase; EC 2.4.1.1; 25 U/mg] from rabbit muscle was purchased from Sigma–Aldrich (Deisenhofen, Germany).

### 2.2. Wide-angle X-ray diffraction (WAXS)

X-ray diffraction patterns of granular starch samples were examined using a JDX 3530 diffractometer (Jeol, Tokyo, Japan) with Ni filter Cu-$K_\alpha$ radiation operated at 40 kV and 30 mA. The X-ray source had a wavelength of 1.542 Å and the diffraction patterns were recorded at $2\theta$ angles of 1–40 degrees with a step angle of 0.01 degree and a counting time of 1 s. The percentage of relative crystallinity of starches was calculated from the ratio of peak areas to total area of the diffractogram (Nara & Komiya, 1983).

### 2.3. Isolation of amylopectin from starch granules

The procedure followed basically that of Forsyth et al. (2002) but with several minor changes. Starch granules were dissolved in 90% dimethyl sulphoxide to a concentration of 40 mg/mL by stirring 3 days at room temperature. The starch in the solution (1 mL) was precipitated by addition of 5 mL ethanol, collected by centrifugation and re-dissolved in 8 mL water on a boiling water bath. Finally 16 μL 5 M NaOH was added. Not dissolved matter was removed by centrifugation from the non-granular starch preparation. An aliquot (4.45 mL, corresponding to 22.25 mg starch) was applied to a column (2.5 × 90 cm) of Sepharose CL 2B (Pharmacia, Uppsala, Sweden) and eluted with 0.01 M NaOH containing 0.02 M NaCl at 11 mL/h. Fractions (4.45 mL) were collected and small aliquots taken for carbohydrate measurements by the phenol-sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Recoveries from the column were >93% (without NaCl in the elution medium, however, they were much lower). Fractions containing amylopectin (no. 29–41) were pooled. The isolation of amylopec-

tin was repeated two times and the preparations were pooled into one single sample. Hydroxide and salt ions were removed by tangential flow filtration (TFF) in a Minimate TFF Capsule containing Omega 10K membrane (Pall Life Sciences, Ann Arbor, MI, USA). The amylopectin sample was finally concentrated to 3 mg/mL using the TFF technique.

### 2.4. Production of φ,β-limit dextrins

Purified amylopectin was treated successively with phosphorylase *a* and β-amylase as described elsewhere (Bertoft, 2004a) with the following changes: the β-amylolysis was repeated once, and the removal of low molecular weight products (glucose 1-phosphate and maltose) was done using the TFF technique instead of dialysis. The final φ,β-limit dextrins (φ,β-LDs) were then lyophilised.

### 2.5. Debranching and chromatographic techniques

Non-granular starch preparations, amylopectins, or φ,β-LD:s were debranched as described previously (Bertoft, 2004a) but with the simultaneous addition of both pullulanase and isoamylase at pH 5.5, instead of successive addition of the enzymes. The debranched starch and amylopectin samples (~3 mg/mL) were analysed by gel-permeation chromatography on a column (1 × 90 cm) of Sepharose CL 6B (Pharmacia, Uppsala, Sweden). An aliquot of the sample (0.2 mL) was treated with 5 M KOH (0.02 mL), applied on the column and eluted with 0.5 M KOH at 0.5 mL/min. Fractions of 0.5 mL were collected and analysed for carbohydrates with the phenol-sulphuric acid reagent (Dubois et al., 1956). The recovery from the column was generally >95%.

Debranched amylopectins and φ,β-LDs were also analysed by high-performance anion-exchange chromatography (HPAEC). The samples were diluted to ~1 mg/mL and filtered (pore size 0.45 μm), prior to analysis on the HPLC system (series 4500i, Dionex, Sunnyvale, CA, USA) equipped with a BioLC gradient pump and pulsed amperometric detection (PAD). The column (Carbo-Pac PA-100, 4 × 250 mm, combined with a guard column) was eluted at 1 mL/min with a mixture of eluent A (0.15 M NaOH, 93%) and eluent B (0.15 M NaOH containing 1.0 M NaOAc, 7%) from 0 to 1 min, whereafter the sample was injected (25 μL). Samples were eluted with a gradient made by increasing the proportion of eluent B as follows: from 1 to 10 min from 7% to 18%; 10–19 min from 18% to 22%; 19–111 min from 22% to 50%; and 111–113 min from 50% to 7% (return to the start mixture). For samples containing a lot of maltose (debranched φ,β-LDs), the PAD signal was recorded at a 10 × less sensitive output range (3000 nA instead of 300 nA) for an initial period until the maltose peak had eluted completely.

### 2.6. Calculations

All chromatographic analyses were made in duplicates and the average values were used in subsequent calculations. Carbohydrate contents in fractions of GPC analyses were normalised and the relative weight amount of amylose or amylopectin was estimated by suitable division of the chromatograms as described in Section 3. Peak areas in HPAE-chromatograms were corrected to carbohydrate concentration using the method of Koch, Andersson, and Åman (1998) and normalised. Peaks of DP > 60, which were not resolved, were quantitatively approximated by continuous area divisions of the chromatograms (Bertoft, 2004a). Relative molar amounts, expressed as mol%, were calculated as $(c_i/\mathrm{DP}_i)/\sum(c_i/\mathrm{DP}_i) \times 100$ and average DP values were estimated as $\sum c_i/\sum(c_i/\mathrm{DP}_i)$, in which $c_i$ is the normalised carbohydrate concentration and $\mathrm{DP}_i$ is the DP of peak *i*.

## 3. Results and discussion

### 3.1. Characterisation of starch and amylopectin samples

The starch samples analysed in this investigation are all in commercial use either globally or in more restricted, local areas in different parts of the world. In addition to starches with normal amylose content, some waxy-types were included into the series. Cereal starches were represented by five different plants, of which barley, rye, oat, and rice possessed normal amylose content and maize was of the waxy type. In addition, two waxy varieties of barley and rice were included (Table 1). Mung bean starch is also produced in seeds and thus this plant tissue was also represented by one legume. Sago starch is accumulated in large quantities in the trunk of the sago palm. Starches from West Indian arrowroot and edible canna (or Queensland arrowroot), are collected from the rhizomes (underground trunks). Root starches were from three kinds of plants, namely tree (tapioca or cassava), legume (Andean yam bean), and a perennial vine (kudzu). Finally, tuber starches were represented by lesser yam and potato, of which the latter one amylose-free type was also included. The starch samples were thus selected to represent a broad range of plants and plant tissues, in order to obtain a general overview of the internal unit chain composition in amylopectins.

The granular starch samples were partly characterised with respect to their type of crystalline allomorphic form and content and to amylose content. The results of wide-angle X-ray diffraction studies are summarised in Table 1. All cereal starches possessed the A-type diffraction, which is typical for these types of plants if they have a normal or low amylose content. Starch from mung beans was characterised as C-type by Hoover, Li, Hynes, and Senanayake (1997), and many other bean starches were described as C-types in the literature (Biliaderis et al., 1981). In this work, the diffraction type was A, however, in agreement with reports by Jane et al. (1999) and Thitipraphunkul, Uttapap, Piyachomkwan, and Takeda (2003a). The starch from sago palm trunk was of the C-type and was in accordance with a recent work by Srichuwong et al. (2005), in which sago starch granules were classified as A-types with a small part of B-crystals present, resulting in $C_A$-pattern. Granules from the rhizomes of arrowroot and canna possessed C- and B-types of allomorphs, respectively. Canna was shown to be B-crystalline in several reports (Hanashiro et al., 1996; Jane et al., 1999; Thitipraphunkul et al., 2003a). Two of the root starches, tapioca and Andean yam bean, were A-crystalline (Table 1). Tapioca is known for this crystalline pattern (Thitipraphunkul et al., 2003a). However, different samples of Andean yam bean starch granules were found to be of either A- or C-types (the latter more precisely $C_A$-type) (Forsyth et al., 2002). The third root sample was kudzu. Van Hung and Morita (2007) found that the type of crystallinity in kudzu starch was either A, B, or C depending on the place of growth. Kudzu from Japan possessed C-type pattern, which was the same as in this work. (Indeed, the kudzu sample was grown in Japan.) Tuber starches were B-crystalline and represented by yam and potato, of which potato is one of the best-known examples of the B-type. However, yam was characterised as C-type by Srichuwong et al. (2005).

The estimated crystallinity content varied between the samples from 22.9% (rye) to 31.4% (waxy maize). Waxy samples (WBS, WRS and PAPS) possessed generally more crystallinity than their amylose-containing counterparts (NBS, MRS, and PS), but otherwise there was no correlation with amylose content, crystallinity allomorph or type of plant and tissue. Among the cereals the estimated crystallinity content of rye, barley, and oat starch granules was lower (22.9–23.3%) compared to rice (29.1%). Possibly, this partly reflected the lower amylose content in the rice starch (19.5%),

**Table 1**
Origin, crystallinity, and amylose content of granular starches

| Scientific name | Starch source | Abbr. | Plant tissue | Crystallinity | | Amylose content (%)[a] | | |
|---|---|---|---|---|---|---|---|---|
| | | | | Type | (%) | Total | LC[b] | SC[c] |
| *Avena sativa* | Oat | OS | Seed | A | 23.3 | 27.1 | 19.1 | 8.0 |
| *Secale cereale* | Rye | RS | Seed | A | 22.9 | 24.9 | 13.4 | 11.5 |
| *Hordeum vulgare* | Normal barley | NBS | Seed | A | 23.3 | 28.3 | 17.7 | 10.4 |
| *Hordeum vulgare* | Waxy barley | WBS | Seed | A | 28.1 | 4.5 | 3.0 | 1.5 |
| *Oryza sativa* | Medium rice | MRS | Seed | A | 29.1 | 19.5 | 10.8 | 8.7 |
| *Oryza sativa* | Waxy rice | WRS | Seed | A | 31.1 | 1.0 | 0.8 | 0.2 |
| *Zea mays* | Waxy maize[d] | WMS | Seed | A | 31.4 | 0 | – | – |
| *Vigna radiata* | Mung bean | MBS | Seed | A | 25.3 | 28.8 | 18.4 | 10.4 |
| *Metroxylon sagu* | Sago palm | SS | Trunk | C | 30.5 | 23.8 | 19.7 | 4.1 |
| *Maranta arundinacea* | Arrowroot[e] | AS | Rhizome | C | 26.3 | 20.3 | 15.5 | 4.8 |
| *Canna edulis* | Canna[f] | CS | Rhizome | B | 31.2 | 23.0 | 14.0 | 9.0 |
| *Manihot esculenta* | Tapioca | TS | Root | A | 29.9 | 17.6 | 14.2 | 3.4 |
| *Pachyrhizus ahipa* | Andean yam bean | AYS | Root | A | 23.5 | 16.6 | 12.8 | 3.8 |
| *Pueraria lobata* | Kudzu | KS | Root | C | 27.8 | 21.6 | 14.7 | 6.9 |
| *Solanum tuberosum* | Amylose-free potato[d] | PAPS | Tuber | B | 28.7 | 0 | – | – |
| *Solanum tuberosum* | Potato | PS | Tuber | B | 26.4 | 17.9 | 12.9 | 5.0 |
| *Dioscorea esculenta* | Yam[g] | YS | Tuber | B | 25.1 | 17.9 | 8.4 | 9.5 |

[a] Analysed by GPC of debranched starch.
[b] Long chains of amylose eluting at void volume on Sepharose CL 6B.
[c] Short chains of amylose eluting between amylose LC and amylopectin chains.
[d] From Bertoft (2004a).
[e] West Indian arrowroot.
[f] Also called Queensland arrowroot.
[g] Lesser yam.

but as waxy barley possessed a slightly lower crystallinity content than the amylose-containing rice, other structural factors were also involved. These factors were likely related to the amylopectin macromolecule, as it is this component that forms the crystals. Altogether, the relative crystallinities were comparable with that in the literature, but as the content is dependent on the water-saturated state of the granules (Nara & Komiya, 1983), closer comparisons were difficult to perform.

The starch samples were also analysed by GPC on a column of Sepharose CL 6B after debranching. In the chromatograms in Fig. 1, the short chains of amylopectin eluted after the long chains of amylose. The division between the two components was estimated as the lowest point between the two major fractions, which slightly differed between the samples. (WMS and PAPS were not included as they were analysed previously on a column of Superdex 75 and found to be amylose-free (Bertoft, 2004a). Generally, the estimated amylose content agreed with values found in the literature and ranged from 16.6% to 28.8% in the non-waxy starch granules (Table 1). The size-distribution of the amylose chains differed between samples. A division between long chains (LC), eluting at the void volume of the gel particles, and short chains (SC), eluting between amylose LC and the amylopectin chains, was made and their contribution to the amylose content is shown in Table 1. Most starches possessed clearly more of the amylose LC-fraction than amylose SC, but in some samples the proportion of SC was of the same order as LC (in rye, rice, and yam). It is well known that a fraction of the amylose component in most starches is slightly branched. This component has generally higher molecular weight than the corresponding linear component, but the chains are shorter (Takeda, Maruta, & Hizukuri, 1992; Thitipraphunkul, Uttapap, Piyachomkwan, & Takeda, 2003b). Therefore, a high content of amylose SC suggested a comparatively high content of branched amylose molecules.

The amylopectin component was isolated from the starch by GPC on Sepharose CL 2B (except from WRS, WMS, and PAPS). A typical run is shown in Fig. 2. Ideally, the gel would give a separation of the starch components, in practice however, a rather broad overlapping area was found. In order to avoid contamination of amylose, some fractions of the peak at the void volume were avoided, and the sample finally collected represented roughly 60–70% of the amylopectin component originally present in the starch granules. To characterise the isolated amylopectin samples, they were debranched and analysed on the Sepharose CL 6B column (Fig. 1). With the exception of MBS, the amylopectin content in the preparations was generally of the order $\geqslant$95% (Table 2) and the unit chain profile fitted into the profile possessed by the original, whole starch (when considering the amylopectin content of the starch). However, the chains of the amylopectin component from Andean yam bean and mung bean starches possessed somewhat different profiles. The reason is not known. Possibly, branched amylose components of the original starches contained very short chains of similar lengths as the chains of amylopectin. Such short chains were shown to exist in maize amylose (Takeda, Shitaozono, & Hizukuri, 1990) and in mung bean the branched amylose component consists of comparatively many chains (Thitipraphunkul et al., 2003b). It is also possible that the fraction of the amylopectin component that was isolated was not representative of the whole sample. The amylopectin preparation of mung bean starch contained rather high amounts (8%) of remaining amylose chains. Also in the normal barley and rye amylopectin samples comparatively high levels were found. It is known that "super long chains" exist in some amylopectins, notably in some rice starches (Koroteeva et al., 2007; Takeda, Hizukuri, & Juliano, 1987), but also in other types of plants (Hanashiro, Matsugasako, Egashira, & Takeda, 2005). If the long chains remaining in the amylopectin preparations were amylose or a true part of the amylopectin component was not investigated further in this work. It was noted, however, that the proportion of amylose SC chains in the amylopectin preparations was generally higher than that of amylose LC, i.e., it was different from that of the original starch samples (Table 1 and 2), and suggested that the material possibly was derived from branched amylose components of high molecular weights.

### 3.2. Internal unit chain profiles of limit dextrins

The external chains in the amylopectin preparations were removed by successive treatment with phosphorylase and β-amylase, thus producing φ,β-limit dextrins (φ,β-LDs). The unit chain

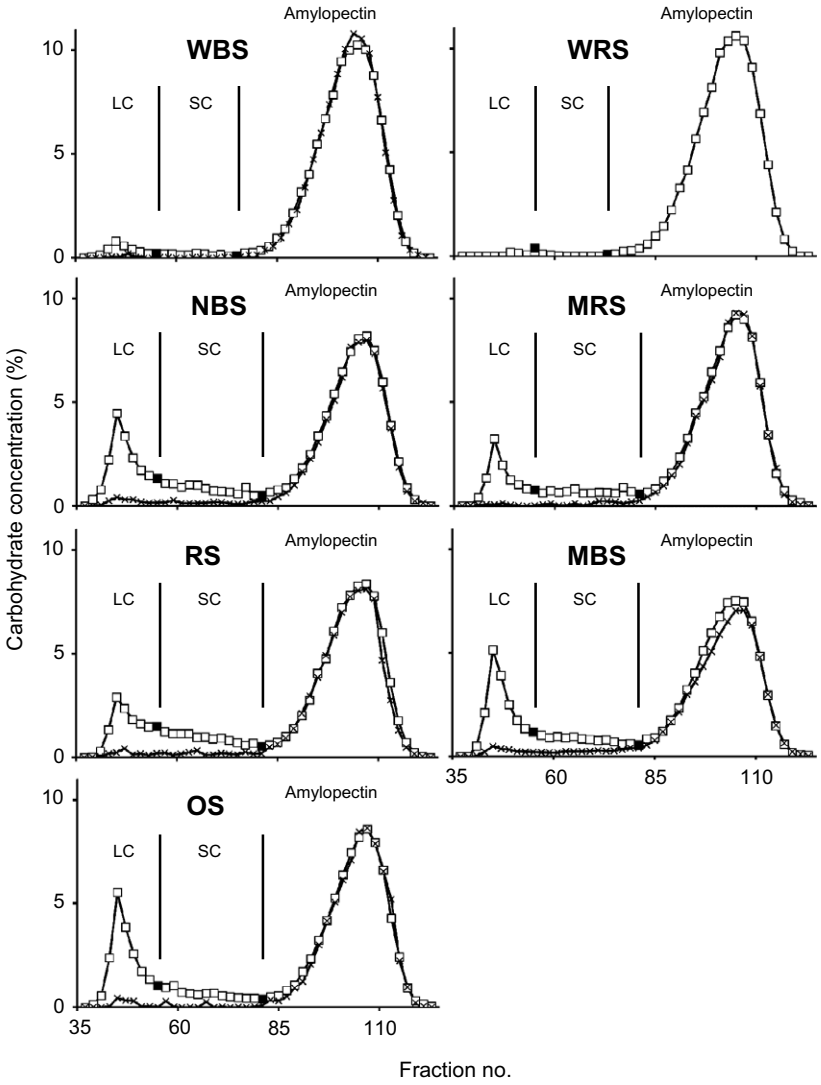


**Fig. 1a.** Fractionation on Sepharose CL 6B of debranched whole starches from seeds (□) and the respective isolated amylopectins (×). The unit chain distribution of amylopectin is drawn in proportion to its content in the starch. The chromatograms were divided into long chains (LC at void volume) and short chains (SC) of amylose and the chains of amylopectin as indicated. The abbreviations of starch samples are given in Table 1.

profile of the φ,β-LDs involved all the A-chains, now reduced into maltosyl chain stubs, and the internal segments with a single external glucosyl residue of the B-chains. The chromatograms of the latter chains thus reflected the composition of internal chains of the amylopectins and are shown in Fig. 3. Generally, all internal unit chain profiles possessed the two major groups of short and long chains, which are also found in the parent, whole amylopectins. However, when the short chains (S-chains) of amylopectin are mixtures of short A- and short B-chains (BS- or B1-chains), the S-chains of the φ,β-LDs represent only BS-chains. (The A-chains in the form of maltose were removed from the chromatograms in Fig. 3). A division between the groups was made at the lowest position (molar based) possessed in the chromatograms. This position depended on the individual samples and ranged between DP 22 (in MRS) and 31 (in AYS) and it was not related to starch crystallinity (Tables 1 and 3).

The CL-ranges of the short and long B-chain categories in the limit dextrins are given in Table 3. It was noted that the shortest B-chain in all samples was DP 3. Because this type of chain contained one external residue, the total internal structure included only 2 residues. As the outermost of these was involved in a branch linkage, it can be concluded that the ICL of this chain was 1 residue, which is the shortest possible internal segment found in amylopectin (Umeki & Yamamoto, 1975).

In all samples the long B-chains (BL-chains) could be subdivided into a major group with DP values covering the range 23–58 depending on the individual samples, and a minor group at DP over 47–55 (Table 3). The two groups were apparently similar to B2- and B3-chains characteristically found in the unit chain profiles of whole amylopectins (Hizukuri, 1986) and thus named similarly. An exact division between B2- and B3-chains was not possible because the two groups were not separated clearly. B3-chains were extending from the B2-chains as a continuous distribution rather than forming a distinct separate population of chains. Possibly therefore, the division of B2- and B3-chains was more or less superficial. Nevertheless, B3-chains were roughly estimated to be larger than DP 50–60 in most samples. The B-crystalline samples (PAPS, PS, YS, and CS) possessed generally more of the long chains, especially of B3-chains, compared to the other samples. This is also typical for the unit chains of the whole amylopectins of B-starches (Hizukuri, 1985). On the contrary, the content of long chains was very low in some of the cereal samples, especially in OS, RS, NBS, and WBS. Also in AYS, a root sample, the content was apparently low. In fact, in these samples the long B2-chains were detected

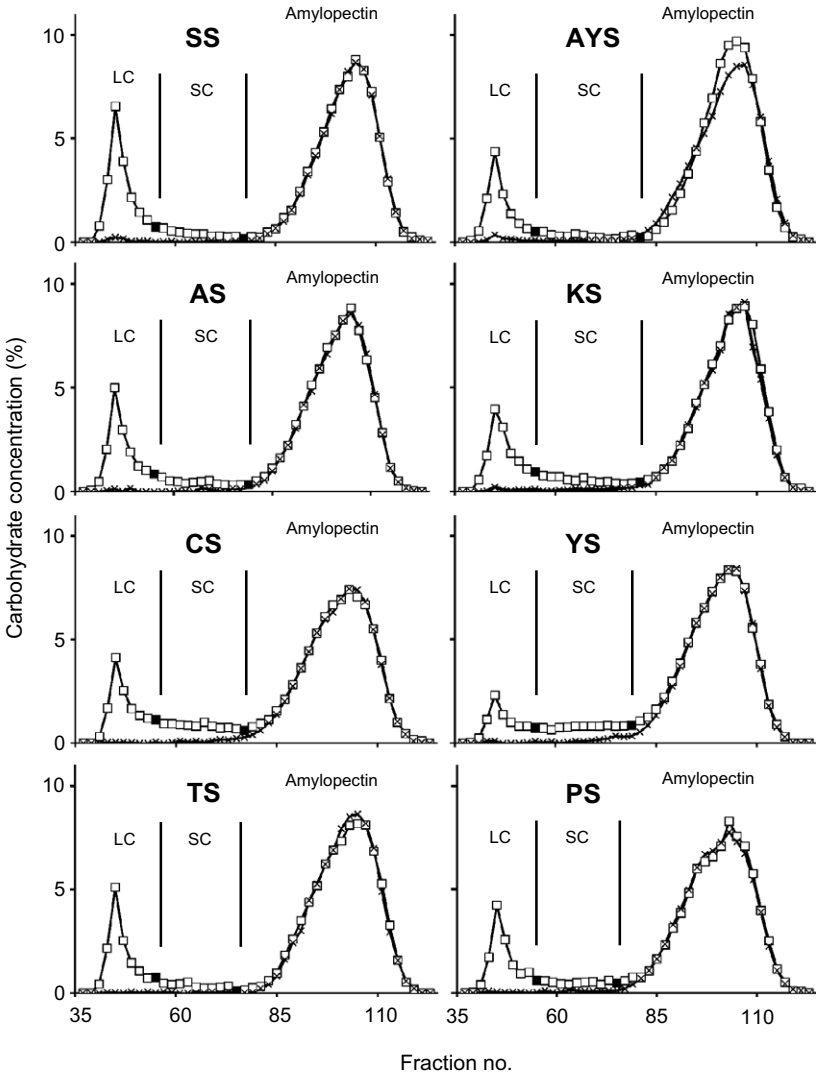


**Fig. 1b.** Fractionation on Sepharose CL 6B of debranched whole starches from trunk, rhizomes, roots, and tubers (□) and the isolated amylopectins (×). The unit chain distribution of amylopectin is drawn in proportion to its content in the starch. The chromatograms were divided into long chains (LC at void volume) and short chains (SC) of amylose and the chains of amylopectin as indicated. The abbreviations of starch samples are given in Table 1.

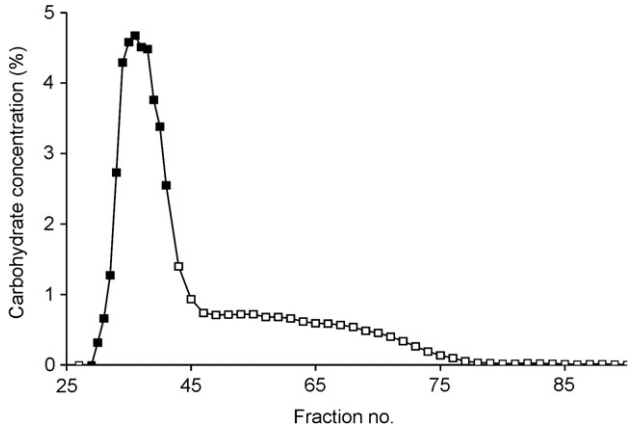


**Fig. 2.** Example of the preparative fractionation of starch (sago starch) on Sepharose CL 2B. Black symbols show fractions pooled to collect the amylopectin component.

only as a plateau, rather than a group clearly separate from the short chains.

The internal unit chain profiles of the short B-chains suggested also a division into two distinct subgroups. A major group constituted chains with DP 8 up to the higher range of the BS-chains. This group is here abbreviated $BS_{major}$ to distinguish it from the minor group found at DP 3–7 in all LDs. The latter group was also described in a few samples earlier (Bertoft, 2004a; Bertoft & Koch, 2000) and was named "fingerprint" B-chains ($B_{fp}$) because of its characteristic profile in different samples. $B_{fp}$-chains were found as parts of large, branched building blocks inside clusters of amylopectin (Bertoft, 2007; Bertoft & Koch, 2000). Thus, the profile of the internal, short B-chains is a reflection of the internal structure of the clusters and it was interesting to notice the distinct differences between the samples.

Peak-DP values for the different categories of chains found in the internal amylopectin structure (in the form of φ,β-LDs) are collected in Table 3. As a summary of these values, it was concluded that they were similar in all samples, regardless the origin or type of crystallinity. (Values for B3-chains are not shown because there were no peaks present in the chromatograms.)

The average chain length of the LDs ($CL_{LD}$) ranged from 7.4 to 8.4 in A- and C-crystalline samples (Table 4). It was longer in B-type samples (9.0–10.5). The average CL of the short B-chains in

**Table 2**
Characterisation of samples of purified amylopectin[a]

| Amylopectin source | Abbrev. | Amylopectin (%)[b] | Amylose content (%)[b] | | |
|---|---|---|---|---|---|
| | | | Total | LC[c] | SC[d] |
| Oat | OS | 97.4 | 2.6 | 1.6 | 1.0 |
| Rye | RS | 94.7 | 5.3 | 2.0 | 3.3 |
| Normal barley | NBS | 94.7 | 5.3 | 2.4 | 2.9 |
| Waxy barley | WBS | 99.5 | 0.5 | 0.3 | 0.2 |
| Medium rice | MRS | 97.6 | 2.4 | 0.5 | 1.9 |
| Mung bean | MBS | 92.0 | 8.0 | 3.0 | 5.0 |
| Sago palm | SS | 98.8 | 1.2 | 0.9 | 0.3 |
| Arrowroot | AS | 98.7 | 1.3 | 0.8 | 0.5 |
| Canna | CS | 98.0 | 2.0 | 0.3 | 1.7 |
| Tapioca | TS | 99.4 | 0.6 | 0.3 | 0.3 |
| Andean yam bean | AYS | 97.0 | 3.0 | 1.3 | 1.8 |
| Kudzu | KS | 97.2 | 2.8 | 0.8 | 2.0 |
| Potato | PS | 98.5 | 1.5 | 0.4 | 1.1 |
| Yam | YS | 97.4 | 2.6 | 0.4 | 2.2 |

[a] WRS, WMS, and PAPS were not analysed as they were obtained from more or less amylose-free starches.
[b] Analysed by GPC of debranched starch.
[c] Long chains of amylose eluting at void volume on Sepharose CL 6B.
[d] Short chains of amylose eluting between amylose LC and amylopectin chains.

the φ,β-LDs (BS-$CL_{LD}$) was 8.6–11.4. The cereals OS, RS, NBS, and WBS, together with AYS and MBS, possessed the highest average values (10.3–11.4), whereas all other A-crystalline, as well as the C- and B-crystalline samples, had shorter chains at average (8.6–10.2). Thus, BS-$CL_{LD}$ did not correlate with the type of crystallinity. With the exception of PAPS, the length of the long B-chains (BL-$CL_{LD}$), however, correlated with crystallinity, as the B-type starches possessed slightly higher values (43.1–45.5) than the other samples (36.4–42.4).

### 3.3. Division of amylopectins on the basis of their internal structure

A closer look on the internal unit chain profiles in Fig. 3 suggested that different samples had certain features in common. Thus, all cereal samples possessed a clearly distinguished group of $B_{fp}$-chains with a peak position at DP 5–6. On a relative molar basis, the chains constituted between 12.8% and 20.2% of all chains in the amylopectins (Table 5). Another common feature was an almost negligible amount of B3-chains (⩽1%). As already noted above, the cereals OS, RS, NBS, and WBS, together with the root starch AYS, contained also low amounts of B2-chains (3.5–4.7%) that were only distinguished as a shoulder in the chromatograms. Furthermore, these particular samples possessed a rather broad distribution of the BS-chains from DP 3 up to between 27 and 31 (Table 3). This was in contrast to the rice and maize samples that possessed sharper distributions up to DP 22–25, which was similar to all the other amylopectin samples. In rice and maize the amount of B2-chains was significantly higher than in the other cereals (Table 5), and this was reflected as a clear group of chains in the chromatograms (Fig. 3). With regards to the B2- and B3-chains, the two C-crystalline samples KS and SS were similar to MRS, WRS, and WMS. These features suggested that the samples could be divided into two distinct groups (Fig. 3) including OS, RS, NBS, WBS, and AYS in one group, that here is called group 1, and MRS, WRS, WMS, KS, and SS in the other group (group 2).

In a similar fashion, samples AS, MBS, and TS possessed common features (Fig. 3). The $B_{fp}$-chains were only distinguished as shoulders on the peaks of the $BS_{major}$-chains in these samples and, with the exception of TS, they contained somewhat more B3-chains than in groups 1 and 2 (Table 5). They were therefore characterised as a third group. Altogether, the A- and C-crystalline samples were thus divided into three distinct groups on the basis of the features of their internal unit chain distributions. The B-crystalline samples comprised a forth group, mainly characterised by its high content of long B-chains and, therefore, low content of short chains.

To highlight the characteristic features of the four groups, their average internal unit chain distributions were theoretically constructed for each group (Fig. 4) and an overview of the molar distributions of the chains is given in Table 6. Major criteria for the division into groups were based on the internal unit chain distribution profiles of (i) the long chains, participating in the interconnection of clusters, and (ii) the subgroups of the short chains, participating in the fine structure of the clusters. In group 1 the internal structure was characterised by a very low relative molar content of B3-chains (practically no such chains), as well as a low content of B2-chains. Altogether, the long chains constituted only around 5 mol% of the chains in the LD:s (Table 6). As a consequence, the ratio of short B-chains to long B-chains (BS:BL) was high in these samples (7.3–9.4). As a result of the comparatively broad distribution of BS-chains, the relative amount of chains at DP 8–27 ($BS_{major}$) was high, which resulted in a low ratio of $B_{fp}$:$BS_{major}$ in the clusters.

As for group 1, B3-chains were only found in trace amounts in group 2, but B2-chains were typically present in higher amounts from 5.8 to 7.3 mol% (Table 6). The ratio of BS:BL was therefore lower. Further, this group was characterised by comparatively high amounts of $B_{fp}$-chains in their clusters. In WMS not less than 20.2% of all chains (including the A-chains) were $B_{fp}$, and MRS possessed the highest ratio of $B_{fp}$:$BS_{major}$ (1.0) of all samples investigated.

Group 3 contained similar amounts of B2-chains as group 2 (Fig. 4), but possessed also intermediate amounts of B3-chains (0.7–2.2 mol%, Table 6). The amount of $BS_{major}$ was similar to group 2, but $B_{fp}$-chains were found in much smaller amounts resulting in an intermediate ratio of $B_{fp}$:$BS_{major}$. The $B_{fp}$-chains were typically only distinguished as a weak shoulder in the unit chain profiles.

Group 4, finally, characteristically contained the highest amounts of both B3- and B2-chains, which resulted in the lowest ratio of BS:BL (2.3–3.1, Table 6). Though the molar amounts of BS-chains were low, the relative distribution of $B_{fp}$-chains to $BS_{major}$ was similar to that of group 3, suggesting a similar type of cluster architecture (Fig. 4).

The classic division of starches is based on their crystalline structure, to which the amylopectin component contributes. As the samples analysed in this investigation represented a broad range of different starch samples, the results suggested that starches in general also can be classified according to the chemical

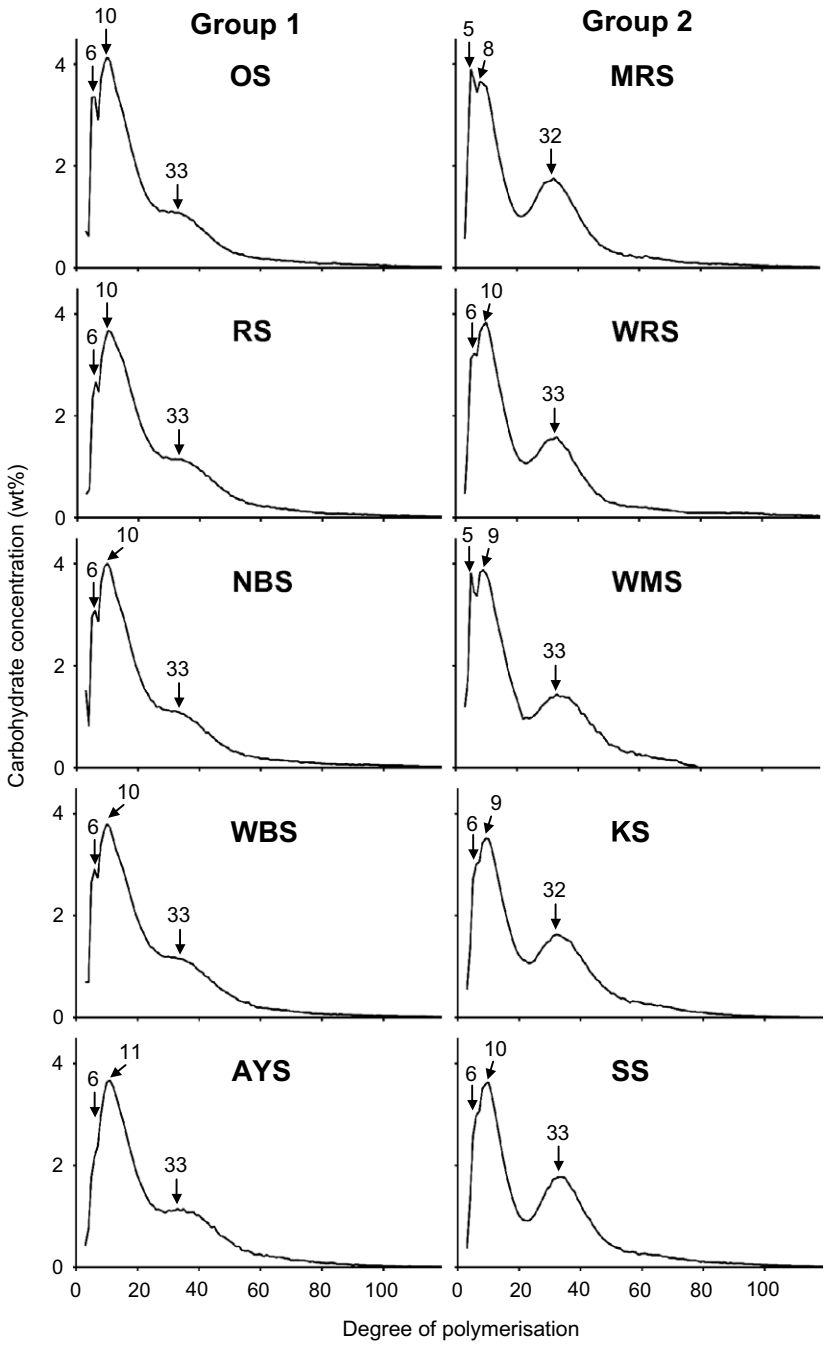


**Fig. 3a.** Fractionation by HPAEC of internal unit chains obtained from debranched φ,β-limit dextrins of groups 1 and 2. Special features in the profiles are discussed in the text and highlighted by arrows with numbers indicating DP. The abbreviations of starch samples are given in Table 1.

structure of the amylopectin component as outlined above. In Table 5 (and in Table 4) the structure types of the samples are given both according to their crystalline and molecular structure as A:1, A:2, C:2, etc. (i.e., physical:chemical structure). With the exception of waxy barley, it was interesting to note that the starches with normal amylose content of group A:1 (OS, RS, NBS, and AYS) all possessed the lowest degree of crystallinity (Table 1). The most distinguished characteristic with regards to the internal molecular structure was the broad distribution of BS-chains and low content of B2-chains, resulting in the poor separation of long and short chains (Fig. 4). Possibly, this suggested that some of the longer types of the $BS_{major}$-chains had a structural role similar to the B2-chains, i.e., in effect being "short B2-chains" involved in cluster interconnections, rather than taking part of the internal cluster structure. As a result, this somehow could affect the granular structure resulting in the low degree of crystallinity in the granules.

### 3.4. Chain lengths in amylopectins

Samples of the whole amylopectins were also debranched and from the size-distributions measured by HPAEC the CL and other data were obtained (Table 4). As expected, A- and C-crystalline samples possessed amylopectin with shorter CL than the B-types (Hizukuri, 1985; Jane et al., 1999). The CL of the short chains (SCL, which in amylopectin represent a mixture of A- and BS-

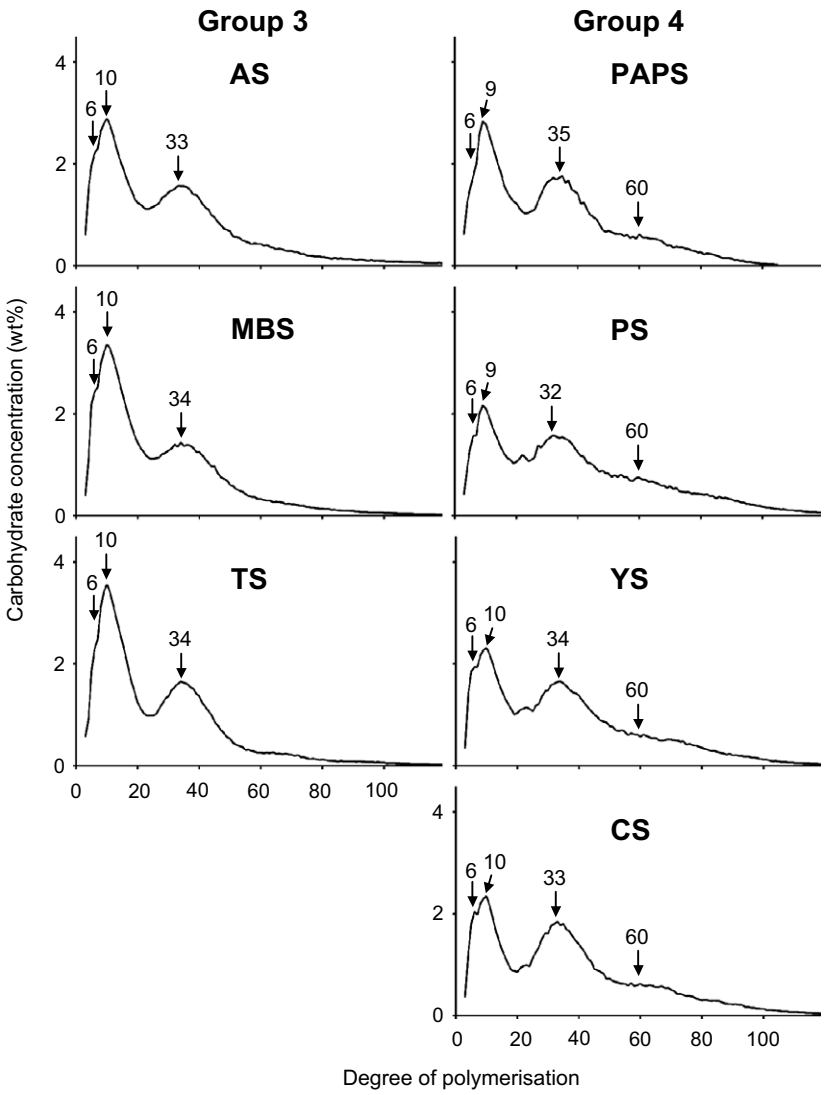


**Fig. 3b.** Fractionation by HPAEC of internal unit chains obtained from debranched φ,β-limit dextrins of groups 3 and 4. Special features in the profiles are discussed in the text and highlighted by arrows with numbers indicating DP. The abbreviations of starch samples are given in Table 1.

chains) of the B-crystalline samples was only slightly longer or similar to that of A-samples (15.8–18.0 and 15.0–16.4, respectively). The major source of the difference in the average CL was found in the CL of the long chains (LCL). This value ranged from 54.7 to 59.6 in B-type amylopectins, whereas LCL in A- and C-types were 48.1–54.4. However, two exceptions were MBS with LCL similar to the B-types (58.4) and AYS that possessed the highest LCL (60.8) of all samples.

The average external CL (ECL, which was calculated from the difference of the CL values of amylopectin and limit dextrin) was higher

**Table 3**
DP-ranges and peak-DP values of subgroups of B-chains in φ,β-LDs and of short ($S_{Ap}$) and long chains of amylopectin ($L_{Ap}$) obtained by HPAEC

| Starch sample[a] | OS | RS | NBS | WBS | AYS | MRS | WRS | WMS | KS | SS | AS | MBS | TS | PAPS | PS | YS | CS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *DP-ranges* | | | | | | | | | | | | | | | | | |
| BS (=B1) | 3–26 | 3–27 | 3–27 | 3–27 | 3–31 | 3–22 | 3–24 | 3–25 | 3–25 | 3–23 | 3–24 | 3–25 | 3–25 | 3–23 | 3–24 | 3–24 | 3–23 |
| B2 | 27–50 | 28–50 | 28–50 | 28–49 | 32–54 | 23–47 | 25–53 | 26–49 | 26–57 | 24–48 | 25–46 | 26–47 | 26–58 | 24–48 | 25–51 | 25–49 | 24–50 |
| B3 | ⩾51 | ⩾51 | ⩾51 | ⩾50 | ⩾55 | ⩾48 | ⩾54 | ⩾50 | ⩾58 | ⩾49 | ⩾47 | ⩾48 | ⩾59 | ⩾49 | ⩾52 | ⩾50 | ⩾51 |
| $S_{Ap}$ | 6–38 | 6–38 | 6–37 | 6–37 | 6–41 | 6–34 | 6–35 | 6–35 | 6–37 | 6–36 | 6–36 | 6–38 | 6–36 | 6–35 | 6–38 | 6–39 | 6–37 |
| $L_{Ap}$ | ⩾39 | ⩾39 | ⩾38 | ⩾38 | ⩾42 | ⩾35 | ⩾36 | ⩾36 | ⩾38 | ⩾37 | ⩾37 | ⩾39 | ⩾37 | ⩾36 | ⩾39 | ⩾40 | ⩾38 |
| *Peak-DP values*[b] | | | | | | | | | | | | | | | | | |
| $B_{fp}$ | 6 (5) | 6 (5) | 6 (5) | 6 (5) | – (6) | 5 (5) | 6 (5) | 5 (5) | – (5) | – (5) | – (5) | – (5) | – (6) | – (4) | – (5) | – (5) | 6 (5) |
| $BS_{major}$ | 10 (8) | 10 (8) | 10 (8) | 10 (8) | 11 (9) | 8 (–) | 10 (8) | 9 (8) | 9 (–) | 10 (8) | 10 (8) | 10 (8) | 10 (8) | 9 (8) | 9 (8) | 10 (–) | 10 (–) |
| B2 | – (–) | – (–) | – (–) | – (–) | 33 (–) | 32 (29) | 33 (30) | 33 (31) | 32 (31) | 33 (31) | 33 (30) | 34 (32) | 34 (33) | 35 (32) | 32 (30) | 34 (31) | 33 (31) |
| $S_{Ap}$ | 12 (11) | 12 (11) | 12 (11) | 12 (11) | 12 (11) | 12 (12) | 12 (11) | 14 (12) | 12 (12) | 13 (12) | 13 (12) | 13 (12) | 12 (11) | 13 (13) | 14 (13) | 15 (13) | 14 (13) |
| $L_{Ap}$ | 43 (–) | – (–) | – (–) | – (–) | 46 (–) | 43 (40) | 44 (41) | 45 (43) | 44 (44) | 46 (43) | 45 (43) | – (–) | 47 (44) | 47 (47) | 47 (44) | 48 (46) | 47 (45) |

[a] Abbreviations of starch samples are given in Table 1.
[b] Weight based peak-values in HPAE-chromatograms shown in Figs. 3 and 5 (molar based values are shown in parenthesis). No number indicates that a shoulder or plateau was present instead of a peak.

**Table 4**
Average chain lengths of different chain categories in φ,β-LDs and their parent amylopectins and the φ,β-limit values of amylopectins obtained by HPAEC

| Starch sample[a] | OS | RS | NBS | WBS | AYS | MRS | WRS | WMS | KS | SS | AS | MBS | TS | PAPS | PS | YS | CS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Structure type[b] | A:1 | A:1 | A:1 | A:1 | A:1 | A:2 | A:2 | A:2 | C:2 | C:2 | C:3 | A:3 | A:3 | B:4 | B:4 | B:4 | B:4 |
| *φ,β-limit dextrin*[c] | | | | | | | | | | | | | | | | | |
| $CL_{LD}$ | 7.8 | 8.2 | 7.8 | 8.0 | 7.4 | 7.4 | 7.5 | 7.6 | 7.7 | 7.8 | 8.4 | 8.2 | 7.8 | 9.0 | 10.5 | 9.4 | 9.4 |
| B-$CL_{LD}$ | 13.6 | 14.9 | 13.3 | 14.3 | 15.2 | 13.3 | 13.9 | 13.0 | 14.5 | 14.8 | 16.5 | 15.7 | 15.6 | 17.6 | 20.9 | 18.9 | 19.1 |
| BS-$CL_{LD}$ | 10.5 | 11.3 | 10.3 | 10.9 | 11.4 | 8.6 | 9.6 | 9.1 | 9.6 | 9.4 | 9.6 | 10.7 | 10.0 | 10.0 | 10.2 | 10.0 | 9.6 |
| BL-$CL_{LD}$ | 40.7 | 41.9 | 41.5 | 41.1 | 42.4 | 36.4 | 38.8 | 37.6 | 38.2 | 38.3 | 40.7 | 42.4 | 38.8 | 41.3 | 45.5 | 44.2 | 43.1 |
| Bfp-$CL_{LD}$ | 5.4 | 5.4 | 5.1 | 5.3 | 5.4 | 5.2 | 5.3 | 5.1 | 5.3 | 5.3 | 5.2 | 5.4 | 5.3 | 5.1 | 5.2 | 5.2 | 5.3 |
| *Amylopectin*[d] | | | | | | | | | | | | | | | | | |
| CL | 17.0 | 17.4 | 17.5 | 18.4 | 17.8 | 17.8 | 17.7 | 18.1 | 18.3 | 18.5 | 20.3 | 19.7 | 18.8 | 21.1 | 23.1 | 23.0 | 21.9 |
| SCL | 15.4 | 15.6 | 15.7 | 16.0 | 15.1 | 15.3 | 15.0 | 15.7 | 15.7 | 15.8 | 16.6 | 16.4 | 15.6 | 15.8 | 17.0 | 18.0 | 16.6 |
| LCL | 52.5 | 53.8 | 53.2 | 53.5 | 60.8 | 48.1 | 51.2 | 48.6 | 52.0 | 52.9 | 54.4 | 58.4 | 52.1 | 54.7 | 59.6 | 58.7 | 57.9 |
| ECL | 10.7 | 10.7 | 11.2 | 11.9 | 12.2 | 11.9 | 11.7 | 11.9 | 12.1 | 12.2 | 13.4 | 13.0 | 12.4 | 13.7 | 14.1 | 15.0 | 14.0 |
| ICL | 5.3 | 5.7 | 5.3 | 5.5 | 4.6 | 4.9 | 5.0 | 5.1 | 5.2 | 5.3 | 5.9 | 5.7 | 5.3 | 6.5 | 8.0 | 6.9 | 6.9 |
| TICL | 12.6 | 13.9 | 12.3 | 13.3 | 14.3 | 12.3 | 12.9 | 12.0 | 13.5 | 13.8 | 15.5 | 14.7 | 14.6 | 16.6 | 19.9 | 17.9 | 18.1 |
| NC:B | 2.0 | 2.1 | 2.0 | 2.0 | 2.6 | 2.1 | 2.1 | 1.9 | 2.2 | 2.2 | 2.3 | 2.2 | 2.3 | 2.2 | 2.2 | 2.2 | 2.3 |
| φ,β-limit value (%) | 54.0 | 52.9 | 55.6 | 56.4 | 58.3 | 58.4 | 57.4 | 57.7 | 58.0 | 57.7 | 58.6 | 58.2 | 58.3 | 57.7 | 54.5 | 58.9 | 57.1 |

[a] Abbreviations of starch samples are given in Table 1.
[b] Structure according to crystallinity:internal molecular structure (i.e., physical:chemical structure).
[c] $CL_{LD}$ = average chain length of whole sample (including A-chains); B-$CL_{LD}$ = CL of B-chains; BS-$CL_{LD}$ = CL of short B-chains; BL-$CL_{LD}$ = CL of long B-chains; $B_{fp}$-$CL_{LD}$ = CL of "fingerprint" B-chains.
[d] CL = average chain length of whole sample; SCL = CL of short chains; LCL = CL of long chains; ECL (external chain length) = CL × (φ,β-limit value/100) + 1.5; ICL (internal chain length) = CL − ECL − 1; TICL (total internal chain length) = B-$CL_{LD}$ − 1; NC:B (number of chains per B-chain) = TICL/(ICL + 1); φ,β-limit value was calculated from the difference in CL of amylopectin and its φ,β-LD.

**Table 5**
Relative molar amount (%) of chain categories in φ,β-LDs and A-chain categories in reconstructed amylopectin samples

| Starch sample[a] | OS | RS | NBS | WBS | AYS | MRS | WRS | WMS | KS | SS | AS | MBS | TS | PAPS | PS | YS | CS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Structure type[b] | A:1 | A:1 | A:1 | A:1 | A:1 | A:2 | A:2 | A:2 | C:2 | C:2 | C:3 | A:3 | A:3 | B:4 | B:4 | B:4 | B:4 |
| Reconstruction mode[c] | ±0 | ±0 | ±0 | ±0 | −1 | ±0 | ±0 | ±0 | +1 | +1 | +1 | +1 | +1 | −1 | +1 | +1 | +1 |
| S ( = A + BS) | 94.8 | 94.2 | 95.1 | 94.5 | 95.0 | 92.0 | 93.1 | 93.1 | 92.2 | 91.5 | 90.3 | 92.8 | 91.7 | 89.1 | 86.3 | 88.5 | 87.6 |
| A-chains | 49.8 | 52.0 | 49.0 | 51.0 | 58.9 | 52.1 | 53.4 | 48.5 | 54.6 | 54.5 | 55.9 | 54.6 | 57.1 | 55.4 | 55.1 | 55.9 | 56.7 |
| $A_{fp}$[d] | 5.5 | 5.6 | 4.1 | 3.9 | 14.1 | 5.0 | 8.0 | 4.6 | 8.8 | 7.9 | 3.9 | 6.3 | 7.4 | 9.0 | 6.9 | 3.5 | 6.5 |
| $A_{clustered}$[e] | 44.3 | 46.4 | 44.9 | 47.1 | 43.2 | 47.1 | 45.4 | 43.9 | 45.8 | 46.6 | 52.0 | 48.3 | 48.9 | 43.9 | 48.2 | 52.4 | 50.2 |
| Long A[f] | – | – | – | – | 1.6 | – | – | – | – | – | – | – | 0.8 | 2.5 | – | – | – |
| B-chains | 50.2 | 48.0 | 51.0 | 49.0 | 41.1 | 47.9 | 46.6 | 51.5 | 45.4 | 45.5 | 44.1 | 45.4 | 42.9 | 44.6 | 44.9 | 44.1 | 43.3 |
| BS ( = B1) | 45.0 | 42.2 | 46.1 | 43.5 | 36.9 | 39.8 | 39.7 | 44.9 | 38.0 | 37.0 | 34.7 | 37.5 | 35.2 | 33.4 | 31.2 | 32.3 | 30.6 |
| $B_{fp}$ | 15.9 | 12.8 | 17.3 | 14.5 | 10.3 | 19.7 | 16.3 | 20.2 | 15.5 | 15.1 | 14.0 | 13.2 | 12.0 | 12.5 | 11.7 | 13.5 | 13.2 |
| $BS_{major}$ | 29.1 | 29.4 | 28.8 | 29.0 | 26.6 | 20.2 | 23.4 | 24.6 | 22.5 | 21.9 | 20.7 | 24.3 | 23.3 | 20.8 | 19.5 | 18.8 | 17.3 |
| BL | 5.2 | 5.8 | 4.9 | 5.5 | 4.2 | 8.0 | 6.9 | 6.6 | 7.4 | 8.5 | 9.4 | 7.9 | 7.7 | 11.2 | 13.7 | 11.9 | 12.8 |
| B2 | 4.3 | 4.7 | 4.1 | 4.6 | 3.5 | 7.0 | 6.1 | 5.8 | 6.8 | 7.3 | 7.1 | 6.2 | 7.0 | 8.7 | 9.8 | 8.8 | 9.8 |
| B3 | 0.8 | 1.0 | 0.8 | 0.9 | 0.7 | 1.0 | 0.8 | 0.8 | 0.6 | 1.2 | 2.3 | 1.7 | 0.7 | 2.5 | 3.9 | 3.1 | 3.0 |

[a] Abbreviation of starch samples are given in Table 1.
[b] Structure according to crystallinity:internal molecular structure (i.e., physical:chemical structure).
[c] Position of B-chains reconstructed by addition of the external segment (ECL − 1) ± *x* residues.
[d] "Fingerprint" A-chains at DP 6–8 in original amylopectin sample.
[e] A-chains in clusters = all A-chains − $A_{fp}$ − long A.
[f] Long A-chains calculated from reconstructed unit chain profiles of amylopectins. Values ⩽0.5 mol% were within experimental errors and are not reported.

in all B-type samples (13.7–15.0, Table 4). As it is this part of the amylopectin that participates in the crystalline lamellae, it would suggest thicker lamellae in B-starch granules compared to A- and C-types, in which ECL was 10.7–13.4. When it is known that the pitch of the double helices is 2.1 nm and involves 6 glucosyl residues, the values suggested a thickness of the B-crystalline lamellae from 4.8 to 5.3 nm and of the A-lamellae from 3.7 to 4.7 nm. The former agrees with experimentally obtained values (Jenkins & Donald, 1995; Kozlov et al., 2006), but the latter is somewhat low, especially for the cereals OS, RS, and NBS. Very low ECL-values compared to lamellae thickness eventually suggest a high degree of branches scattered into the crystalline lamellae. A-starch granules were shown to have more such scattered branches than B-granules (Jane, Wong, & McPherson, 1997). The average internal CL (ICL) was also generally longer in the B-samples compared to A- and C-samples (6.5–8.0 and 4.6–5.9, respectively). The internal chains are to a large extent expected to participate in the amorphous lamellae, but their impact on this structure is unclear.

From the difference in CL of the amylopectin and limit dextrin, the φ,β-limit value was calculated. The value ranged from 52.9 (RS) to 58.9 (YS), but there was no correlation with type of crystallinity. This showed that, generally, the proportion of the external chains in all amylopectins is more or less constant. Indeed, as seen in Table 4, the longer CL of B-starches automatically resulted in both longer ECL and longer ICL, because the proportion between the external and internal parts was almost similar in all samples. If the single external glucosyl residue is subtracted from the B-chains in the limit dextrins, the total internal chain length (TICL) is obtained (i.e., the actual internal structure of amylopectin) (Bertoft, 1991). (TICL should not be mixed with the core chain length (CCL), that was defined similarly as TICL, but without the outermost branch point (Yun & Matheson, 1993), i.e., CCL = TICL − 1). This value shifted over a rather broad range from 12.0 to 15.5 in A- and C-crystalline samples and 16.6–19.9 in the B-samples. The TICL and the ICL values can be used to estimate the average number of chains carried by a B-chain (NC:B). Because ICL describes the

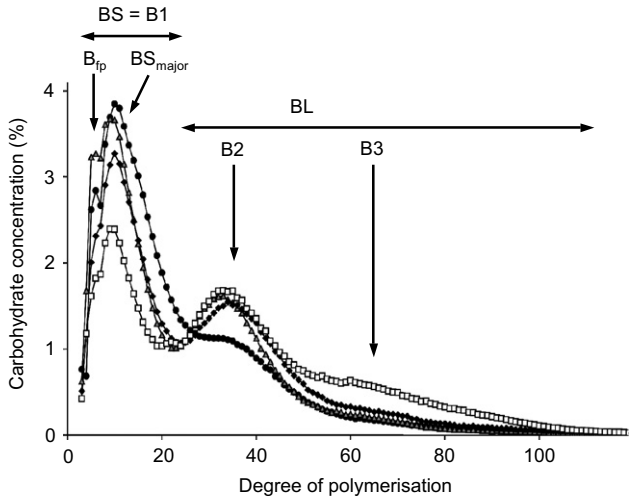


**Fig. 4.** Reconstructed "average" internal unit chain profiles by HPAEC of amylopectins from group 1 (●), group 2 (△), group 3 (♦), and group 4 (□), comparing typical distributions of short (BS or B1) and long B-chains (BL). "Fingerprint" B-chains ($B_{fp}$) and the major fraction of short B-chains ($BS_{major}$) are subgroups of BS-chains, whereas B2- and B3-chains are subgroups of BL-chains. Each curve is the simple average of all samples in the respective group.

**Table 6**
Summary of characteristic relative molar amounts and ratios of chain categories of different groups of amylopectins distinguished on the basis of their internal unit chain profiles obtained by HPAEC

| Structure group | Group 1 | Group 2 | Group 3 | Group 4 |
|---|---|---|---|---|
| *Relative molar amount (%)* | | | | |
| B3 | ⩽1.0 | ⩽1.2 | 0.7–2.3 | 2.5–3.9 |
| B2 | 3.5–4.7 | 5.8–7.3 | 6.2–7.1 | 8.7–9.8 |
| BS (= B1) | 36.9–46.1 | 37.0–44.9 | 34.7–37.5 | 30.6–33.4 |
| $BS_{major}$ | 26.6–29.4 | 20.2–24.6 | 20.7–24.3 | 17.3–20.8 |
| $B_{fp}$ | 10.3–17.3 | 15.1–20.2 | 12.0–14.0 | 11.7–13.5 |
| *Ratios* | | | | |
| S:L | 14.6–22.2 | 12.3–13.3 | 9.4–11.9 | 6.1–7.2 |
| BS:BL | 7.3–9.4 | 4.4–6.8 | 3.7–4.7 | 2.3–3.0 |
| $A_{clustered}$:BS | 1.0–1.2 | 1.0–1.3 | 1.4–1.5 | 1.3–1.6 |
| $A_{clustered}$:B | 0.9–1.1 | 1.0 | 1.1–1.2 | 1.0–1.2 |
| $B_{fp}$:$BS_{major}$ | 0.4–0.6 | 0.7–1.0 | 0.5–0.7 | 0.6–0.8 |

average number of residues between those involved in the branches, and TICL is the length of the whole internal chain (including all residues involved in branches), NC:B = TICL/(ICL + 1). In cereal amylopectins the average number of chains substituted to a B-chain was 1.9–2.1, and in the other samples slightly higher (2.2–2.3), regardless the type of crystallinity. In AYS, however, the number was clearly higher (2.6) than in any other sample. This particular amylopectin possessed the lowest ICL value, suggesting a comparatively high branching density.

### 3.5. Unit chain profiles of amylopectins

The unit chain profiles of the amylopectins are shown in Fig. 5. The profiles largely agreed with those described in the literature (Fredriksson et al., 1998; Hanashiro et al., 1996; Jane et al., 1999; Srichuwong et al., 2005). Very interestingly, however, the profiles also largely agreed with the profiles of the internal unit chains, and it was possible to divide the samples into the very same groups as discussed above. Thus, the typical groups of short, clustered chains and long B-chains were distinguished, and the relative amount of the long chains (B2- and B3-chains) followed that of the long chains in the respective φ,β-LD. The B-crystalline samples (PAPS, PS, YS, and CS) possessed generally more of the long chains, especially at DP > 60 (B3-chains), compared to the other samples. This is typical for B-starches (Hizukuri, 1985). The cereal starches possessed the lowest amounts of long chains. It was noticed that in OS, RS, NBS, and WBS, as well as AYS, the long chains were only seen as a plateau rather than a clearly distinguished group of chains, i.e., just as in the corresponding φ,β-LDs. The divisions between short and long chains, which depended on the individual samples and ranged between DP 34 (in MRS) and 41 (in AYS), and the peak-DP values are given in Table 3.

The short chains of amylopectins of group 1 (OS, RS, NBS, WBS, and AYS) possessed apparently several subgroups (Fig. 5). Besides the major peak at DP 12 (DP 11 on molar basis), there was a shoulder at DP 14 (somewhat broader in AYS), and a clearly marked shoulder or peak at DP 18–21. The latter was described in barley samples earlier (Fredriksson et al., 1998; Frigård, Andersson, & Åhman, 2002; MacGregor & Morgan, 1984) and recently it was noticed in oat (Stevenson, Jane, & Inglett, 2007). The two rice samples in group 2 also possessed similar traits, though not that pronounced. In MRS there was no shoulder at DP 20, whereas WMS possessed a single peak at DP 13–14 and no subgroups. Both samples were very similar to those described by Jane et al. (1999). The short chain profiles of the C-type samples in group 2 (SS and KS) showed an additional peak at DP 17, besides the major peak at 12–13, suggesting a similar division of the short chain population, but at slightly longer chain length (CL). It is known that C-type starches possess CL-values intermediate to those of A- and B-types (Hizukuri, 1985). There was, however, no shoulder corresponding to that at DP 20 in the cereals. AS (C-type), MBS and TS (A-types) of group 3 all possessed a major peak at DP 13 and a shoulder at DP 18–20 or 21. These features in MBS and TS were also noticed by Jane et al. (1999). B-crystalline samples (group 4) possessed a single distribution of short chains with a broad peak around DP 14.

The short chains of amylopectin also contained a distinct subgroup of the shortest chains at DP 6–8. This group has a profile that is characteristic to the source of plant (Koizumi, Fukuda, & Hizukuri, 1991) and was therefore named "fingerprint" A-chains ($A_{fp}$-chains) (Bertoft, 2004a). Indeed, the profiles of these chains were different in different samples (Fig. 5) and were generally in agreement with those published by others (Hanashiro et al. 1996; Jane et al., 1999; Srichuwong et al. 2005). (It is noticed, and should be underlined, that when $A_{fp}$-chains deal with the external parts of the amylopectin molecule, $B_{fp}$-chains describe features of the internal structure.)

### 3.6. The relation of the internal structure with the unit chains of the whole amylopectin

The fact that the unit chain distribution profiles of the original, whole amylopectin samples could be divided into the very same groups that were based on the internal chain distributions, strongly suggested that it is a reflection of the internal structure. It was therefore of interest to trace the original position of the internal chains, thereby tracing their contribution to the distribution of the chains in the whole amylopectin. Because the external chains are removed when producing the φ,β-LDs, they must theoretically be added back to the B-chains in order to reconstruct their original lengths. As the exact external length for each chain is unknown, it is necessary to use the average ECL-value (minus the single residue that is left on the B-chains in the φ,β-LDs) for this purpose. Similar reconstructions were made earlier with a few waxy samples, and it was found that the theoretically constructed curves of the B-chains matched well with the experimentally measured profiles. The difference between the curves traces the positions of the A-chains of the amylopectin (Bertoft, 2004a; Bertoft & Koch, 2000).

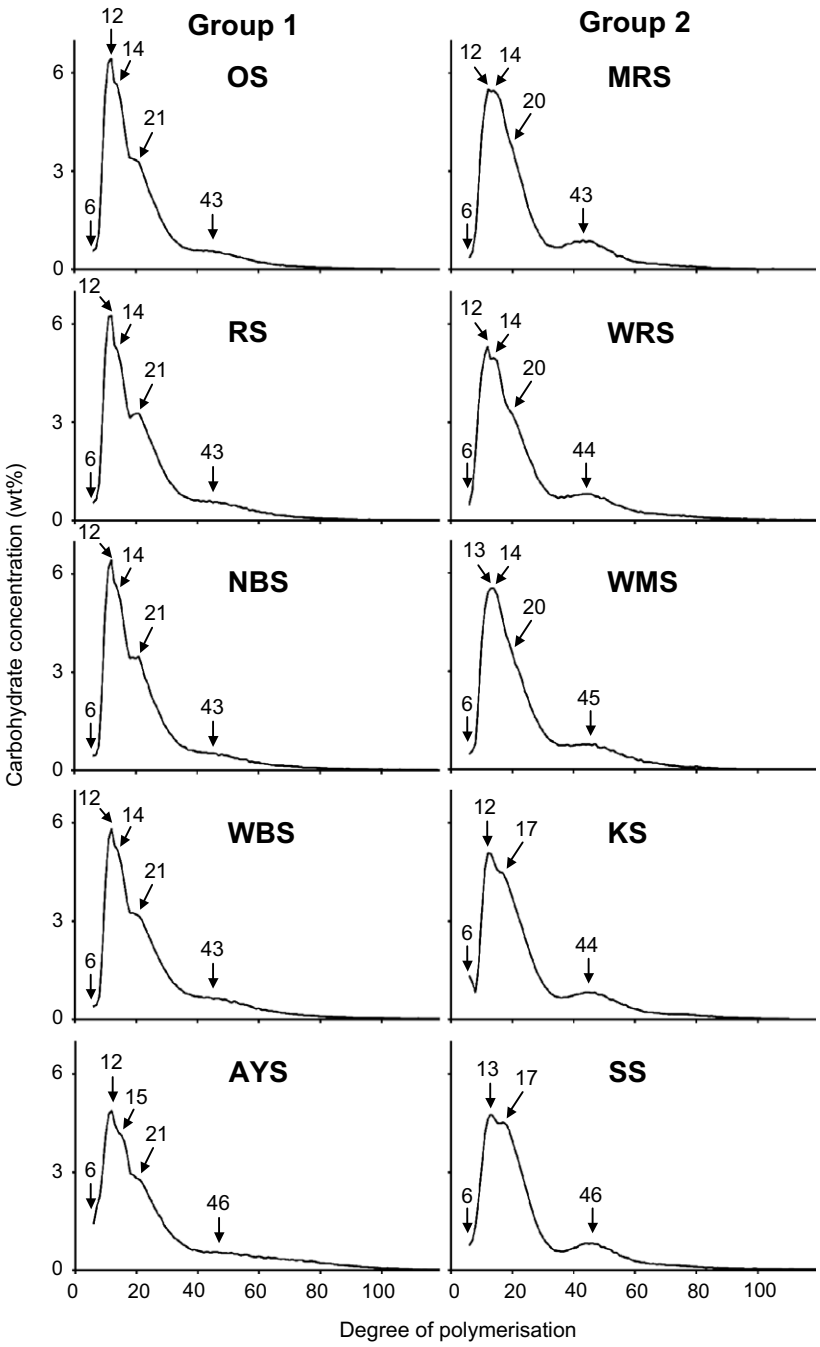


**Fig. 5a.** Fractionation by HPAEC of unit chains of amylopectins of groups 1 and 2. Special features in the profiles are discussed in the text and highlighted by arrows with numbers indicating DP. The abbreviations of starch samples are given in Table 1.

In Fig. 6 the reconstructions of the molar distributions of the B-chains are shown and compared with the original unit chains of the amylopectins. The cereal samples of group 1 (OS, RS, NBS, and WBS) possessed good match with the experimentally derived curves at DP $\geqslant$ 22. This suggested that the B-chains of these sizes generally carried external chains that closely corresponded to the average ECL-values of 11–12 (Table 4) and the "reconstruction mode" to obtain best general fit was ±0 (Table 5). With AYS, however, the best fitting curve was obtained with "reconstruction mode" – 1, i.e., one residue less than suggested by the average ECL. The samples of group 1 possessed generally less good fit at DP 18–21, resulting in a slight surplus of reconstructed B-chains. (This is best seen in Fig. 6 as an apparent negative amount of reconstructed A-chains.) Some of these chains, therefore, may carry somewhat shorter external segments. The chains, which corresponded to the DP-peak of the $BS_{major}$-chains, coincided with the clearly marked shoulder at DP 18–20 that the amylopectins of group 1 characteristically possessed (Fig. 6). At lower DP from 13 to 17 the $B_{fp}$-chains were found together with longer types of A-chains (Fig. 6). This corresponded to the area between the shoulder at DP 14 and the plateau at 18–20 (Fig. 5). In this area, thus, the A- and B-chains overlapped and the $B_{fp}$-chains involved in the building blocks of the clusters were apparently of this length originally.

As for the cereals in group 1, the "reconstruction mode" of rice and maize samples in group 2 was 0. The peak of $B_{fp}$-chains was very sharp (especially on the molar scale in Fig. 6) and tended to

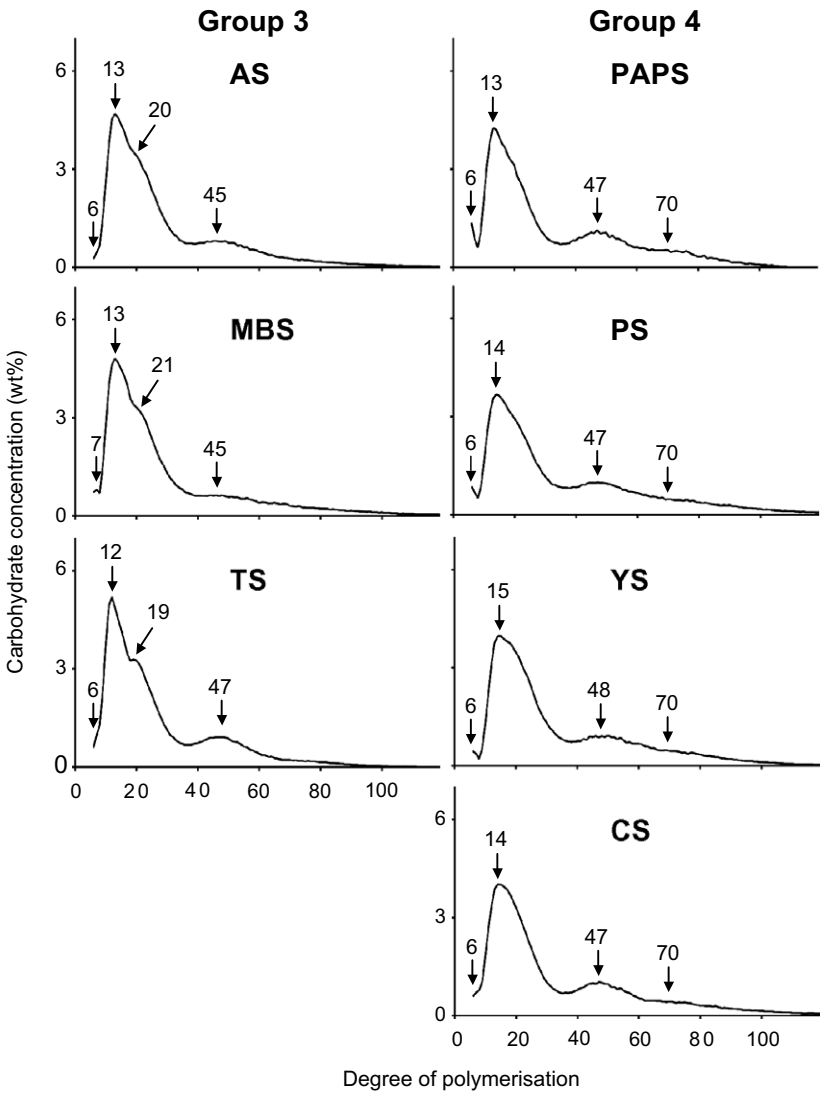


**Fig. 5b.** Fractionation by HPAEC of unit chains of amylopectins of groups 3 and 4. Special features in the profiles are discussed in the text and highlighted by arrows with numbers indicating DP. The abbreviations of starch samples are given in Table 1.

result in a smoother curve abolishing the peak of $BS_{major}$-chains. As a result, the shoulder or peak at DP 14 was as high as the major peak at DP 12 and the plateau at DP 18–20 was not distinguished in the amylopectins (Fig. 5), albeit similar subgroups of short, clustered chains as in group 1 were present. In the reconstruction of KS and SS, having C-type crystallinity but classified as molecular structure of group 2, it was necessary to add an additional residue to the ECL-value in order to obtain the best matching ("reconstruction mode" + 1), even though ECL was similar to the other samples of the same group. Apparently, the $BS_{major}$-chains carried somewhat longer external segments than the average ECL-value suggested. The $B_{fp}$-chains and overlapping A-chains corresponded to the peak at DP 17 in Fig. 5.

With the exception of the amylose-free potato (PAPS), the "reconstruction mode" of samples of groups 3 and 4 was similar to that of KS and SS (i.e., +1). The somewhat broader overlapping area between A- and B-chains compared to the cereals, and the relative distributions of $B_{fp}$- and $BS_{major}$-chains tended to hide the subgroups of chains in the profiles of the whole amylopectins, especially in group 4.

It was earlier reported that PAPS apparently contains a minor group of long A-chains (Bertoft, 2004a). (It should be noted that these chains also may be short B-chains with extra long ECL. Experimentally such chains cannot be distinguished from long A-chains.) These long A-chains are highlighted in the inset in Fig. 6 as the difference between the experimentally derived curve and the reconstructed B-chains at DP > 45, and they amounted 2.5 mol% of the chains in PAPS (Table 5). A small group of similar chains (1.6 mol%) was found in AYS, and trace amounts appeared present in TS. In MBS and CS a small group of long A-chains appeared to be present at DP> 60. However, as there also seemed to be a surplus of reconstructed B2-chains at DP 35–60 of equal small amounts, it was concluded that the samples probably contained very small amounts of B2-chains with extra long ECL. In SS there was also a surplus of reconstructed B2-chains. However, as the matching of the reconstructed curve otherwise was good with the chains at longer DP (>60), it seemed that the surplus was due to a minor population of chains possessing shorter ECL than normally (i.e., short chains to which a too long external segment was added theoretically). Altogether, the conclusion was that unusual types of chains, having either extra long or extra short external segments, may exist in amylopectins. They appear, however, to be found in only few samples and in trace amounts. Their contribution to the structure of amylopectin may therefore be of minor importance.

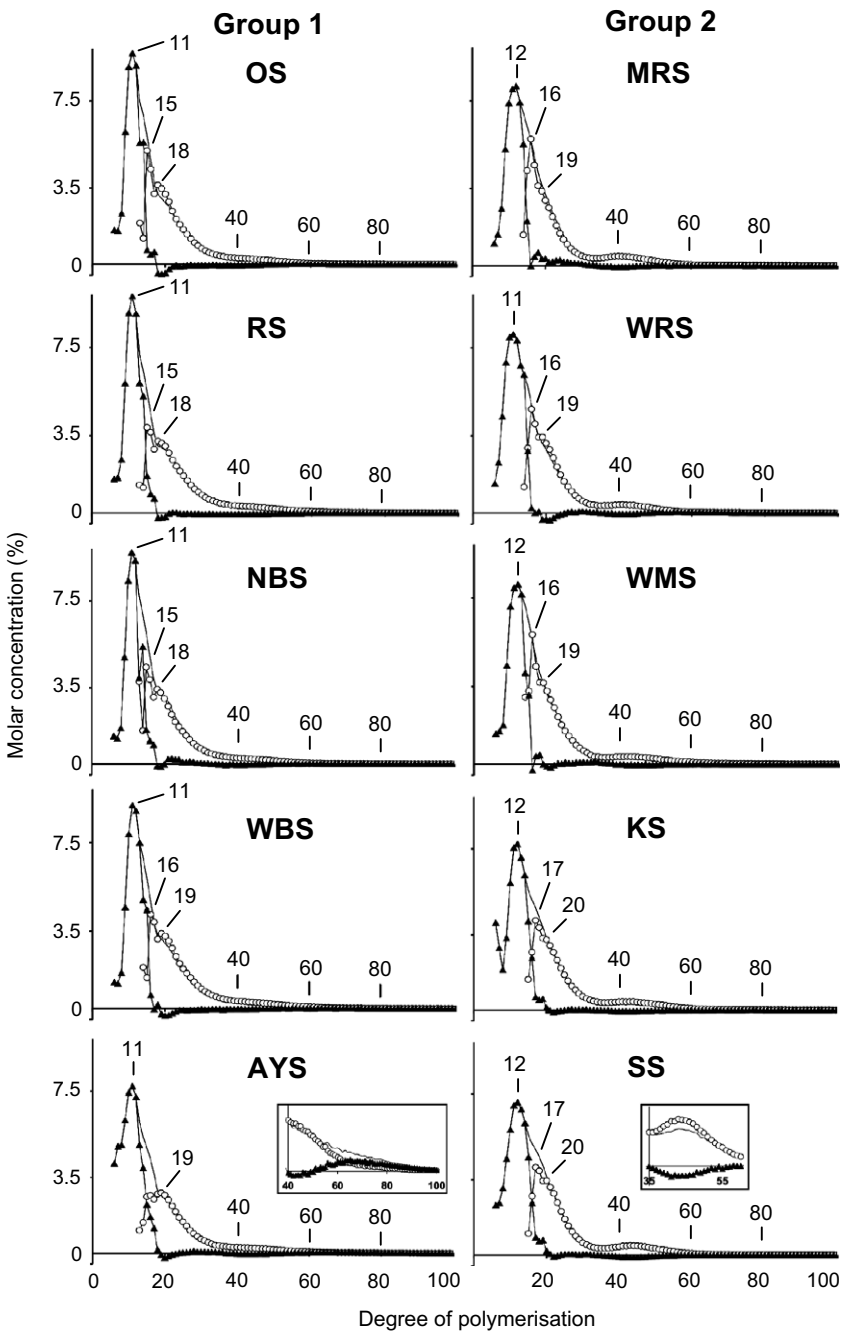


**Fig. 6a.** Reconstructions of the molar distribution of A- (▲) and B-chains (○) from the distribution in φ,β-LDs of group 1 and 2. The profiles are compared with the experimentally obtained distributions of chains in the amylopectins (line without symbols). Insets show enlargements of the profiles with poor matching. The abbreviations of starch samples are given in Table 1.

The A-chains are also traced in Fig. 6. As already discussed, the shortest chains at DP 6–8 comprised $A_{fp}$-chains with a characteristic profile in each sample. The molar amounts of $A_{fp}$ were also considerably different between the samples. In YS there was only 3.5 mol% of these chains, whereas in AYS the amount was as high as 14.1 mol% (Table 5). The role of these chains in the structure of amylopectin is not clear. As they are shorter than expected for chains to participate in the crystallites of the granules (Gidley & Bulpin, 1987), and disappear after acid treatment of the granules, they were suggested to generally be found outside the clusters (Bertoft, 2004b). One would therefore suppose that it is the rest of the (short) A-chains that participate in the clusters. The A-chains in the clusters ranged from 43.2 to 52.4 mol% without any obvious correlation to structural type. The ratio of $A_{clustered}$ to the short BS-chains was 1.0–1.2 in the amylopectins of group 1, and similar in group 2, whereas the ratio was higher (1.3–1.6) in groups 3 and 4 (Table 6). However, if $A_{clustered}$ was compared to all B-chains (i.e., BL are also considered as a part of the clusters) the ratio was more close to 1.0 in all samples. If considering the double helices of the clusters as being composed of one A- and one B-chain, a ratio of $A_{clustered}$:B close to 1.0 is expected.

From the unit chain profiles of the φ,β-LDs, it was also possible to calculate the ratio of A:B-chains. Experimentally, the amount of A-chains was detected as a large peak of maltose (not shown in Fig. 3), and as shown in Fig. 6 they were generally distributed from DP 6 to 17–22, depending on the sample. The ratio of A:B was close

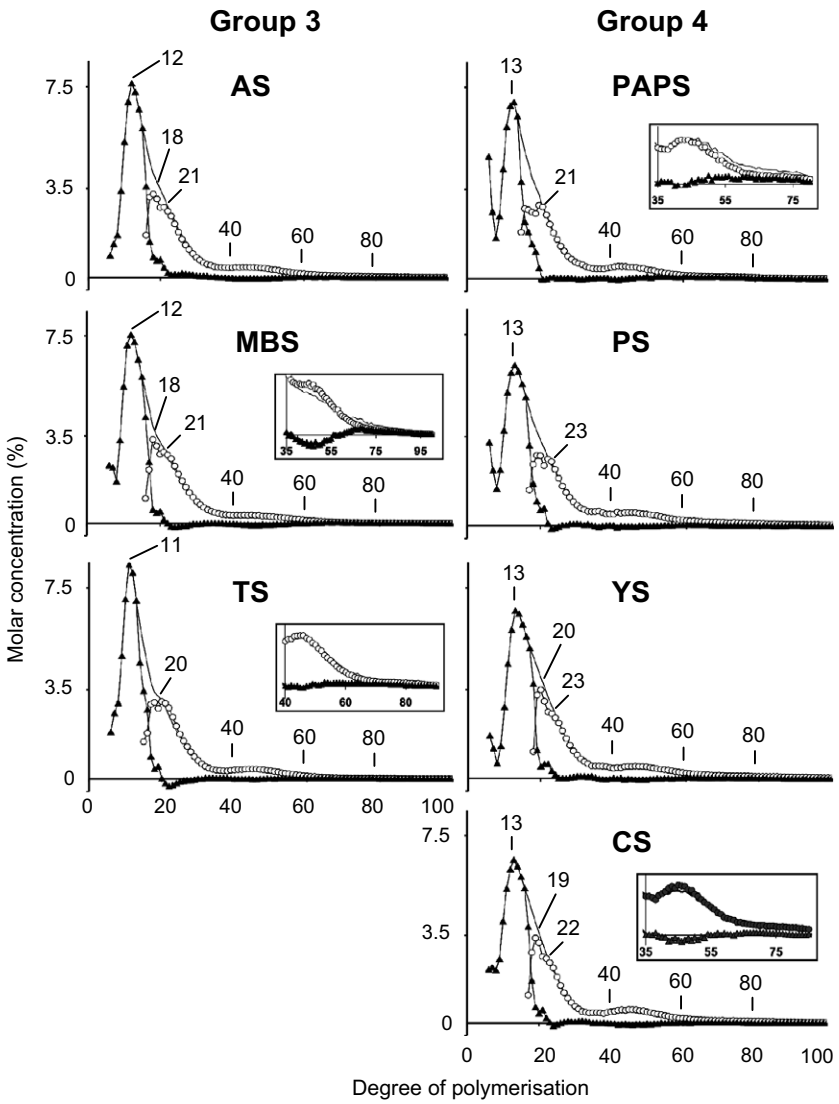


**Fig. 6b.** Reconstructions of the molar distribution of A- (▲) and B-chains (○) from the distribution in φ,β-LDs of group 3 and 4. The profiles are compared with the experimentally obtained distributions of chains in the amylopectins (line without symbols). Insets show enlargements of the profiles with poor matching. The abbreviations of starch samples are given in Table 1.

to 1.0 in cereal starches and slightly higher (1.2–1.3) in other samples (Table 7). Only in AYS it was 1.4, which was due to its very high content of $A_{fp}$-chains. It was suggested by Hanashiro et al. (1996) that the A-chains in amylopectin are the shortest chains and that in most cases their DP range from 6 to 12. This range was called fraction fa, whereas other fractions were identified in periods of 12. Fraction $fb_1$ covered the DP range 13–24, $fb_2$ 25–36, and $fb_3 > 36$, and they were suggested to correspond with B1-, B2- and B3-chains, respectively. The ratio of fa:fb was, however, considerably lower than the ratio of A:B and varied largely between samples from 0.2 to 0.7 (Table 7). In fact, fraction fa represented only a fraction of the A-chains (from 0.3 to 0.8) in all investigated samples. It was therefore concluded that the ratio of A:B-chains cannot be established from the unit chain profile of the whole amylopectin, because A-chains are overlapping with the shortest B-chains and are therefore not distinguished clearly. The amount of A-chains is necessary to measure from limit dextrins (Bertoft, 2004a).

From the discussion above, it follows that fraction $fb_1$ contains a mixture of A-chains and $B_{fp}$-chains (Fig. 6) in the approximate range DP 13–17/22, depending on the sample. The upper range of $fb_1$ includes also the peak of $BS_{major}$-chains. The latter chains were suggested to be involved in the interconnection of two branched, building block units inside clusters (Bertoft, 2007). Fraction $fb_2$ includes the longer chains of the group here called $BS_{major}$ and corresponds approximately to clustered chains suggested to be involved in interconnection of 2–3 building blocks (Bertoft, 2007). Thus, it appears that the periodic chain length found by Hanashiro et al. (1996) is connected to the building block structure inside clusters of amylopectin, rather than the traditional division of A- and B-chains.

### 3.7. Conclusions

The internal unit B-chains of amylopectins are in general divided into two major groups of long and short chains. The latter form two subgroups of which one is a major group and the other, minor group has DP 3–7 when measured in φ,β-LDs. The TICL of these "fingerprint" $B_{fp}$-chains is 2–6. On the basis of the internal B-chain distribution profiles of (i) the long chains, participating in the interconnection of clusters, and (ii) the subgroups of the short chains, participating in the fine structure of

**Table 7**
Ratio of A:B-chains in φ,β-limit dextrins and of fractions fa:fb in amylopectins obtained by HPAEC

| Starch sample[a] | A:B[b] | fa:fb[c] | fa:A[d] |
|---|---|---|---|
| OS | 1.0 | 0.7 | 0.8 |
| RS | 1.1 | 0.7 | 0.8 |
| NBS | 1.0 | 0.6 | 0.8 |
| WBS | 1.0 | 0.5 | 0.7 |
| AYS | 1.4 | 0.7 | 0.7 |
| MRS | 1.1 | 0.5 | 0.7 |
| WRS | 1.1 | 0.6 | 0.7 |
| WMS | 0.9 | 0.5 | 0.7 |
| KS | 1.2 | 0.5 | 0.6 |
| SS | 1.2 | 0.5 | 0.6 |
| AS | 1.3 | 0.4 | 0.5 |
| MBS | 1.2 | 0.4 | 0.6 |
| TS | 1.3 | 0.6 | 0.6 |
| PAPS | 1.2 | 0.4 | 0.5 |
| PS | 1.2 | 0.3 | 0.4 |
| YS | 1.3 | 0.2 | 0.3 |
| CS | 1.3 | 0.3 | 0.4 |

[a] Abbreviations of starch samples are given in Table 1.
[b] A = maltose, B = chains of CL ⩾ 3 after debranching in φ,β-limit dextrins of amylopectins.
[c] fa = CL 6–12 and fb = CL ⩾ 13 in amylopectins (Hanashiro et al., 1996).
[d] The fraction of fa to all A-chains.

the clusters, it was possible to distinguish four major groups of amylopectin samples. A- and C-crystalline samples formed three groups, whereas B-crystalline samples formed a single group. Amylopectin from cereals possessed notably high amounts of $B_{fp}$-chains, suggesting structural differences in the clusters compared to other samples. The internal chain profile was largely reflected in the unit chain distribution of the whole amylopectin. The external chain segments of the B-chains were generally of uniform length that closely equalled the experimentally obtained average ECL-value. A-chains were found in the DP-range 6–22. The longer A-chains were of the same length as $B_{fp}$-chains in the original amylopectins.

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