

The influence of amylose and amylopectin characteristics on gelatinization and retrogradation properties of different starches

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Physico-chemical properties of starch from wheat, rye, barley (waxy, high-amylose and normal-amylose), waxy maize, pea and potato (normal-amylose and high-amylopectin) were studied. Emphasis was given to the amylose (total, apparent and lipid-complexed) and amylopectin characteristics as well as to the gelatinization and retrogradation properties measured using differential scanning calorimetry. The total amylose content varied from *ca.* 1% for waxy maize to 37% for high-amylose barley. The amylopectin characteristics were determined by high-performance size-exclusion chromatography after debranching with isoamylase. The weight-average degree of polymerization (\overline{DP}_w) was 26, 33 and 27 for the A-, B-, and C-type starches, respectively. In general, the potato starches exhibited the highest retrogradation enthalpies and the cereal starches the lowest, while the pea starch showed an intermediate retrogradation enthalpy. The data were analysed by principal component analysis (PCA). The \overline{DP}_w showed positive correlation to the melting interval, the peak minimum, the offset temperatures of the retrogradation-related endotherm as well as to the gelatinization and retrogradation enthalpies. However, the high-amylose barley retrograded to a greater extent than the other cereal starches, despite low \overline{DP}_w (24). The amylose content was negatively correlated to the onset and the peak minimum temperatures of gelatinization. © 1998 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

Starch often contributes to the characteristic properties of foods, and is also added as a functional ingredient in many products. The demand for functionality may vary for different products and, in order to fulfil this demand, chemical modification of the starch is often needed. The botanical source is of great importance for the chemical and functional properties of a starch, but only in a few studies have starches originating from different starch-rich crops been compared using the same experimental methods (Orford et al., 1987; Kalichevsky et al., 1990; Shi and Seib, 1992; Ward et al., 1994). The relations between chemical composition and functional properties are therefore still, to a great degree, unknown.

The physical properties of starch are influenced by the amylose/amylopectin ratio. During gelatinization, the starch granules swell and form gel particles. In general, the swollen granules are enriched in amylopectin, while the linear amylose diffuses out of the swollen granules and makes up the continuous phase outside the granules (Hermansson and Svegmärk, 1996). Waxy starches usually swell to a greater extent than their normal-amylose counterparts (Tester and Morrison, 1990a), and amylose is proposed to act as a restraint to swelling (Hermansson and Svegmärk, 1996). Internal lipids in native cereal starches have been shown to have effects on the swelling and gelatinization properties of the granules (Morrison, 1995). The term retrogradation is used to describe the changes that occur upon cooling and storage of gelatinized starch. Short-term development of crystallinity in starch gels is attributed to the gelation and crystallization of the amylose fraction (Miles et al., 1985; Sievert and Würsch, 1993). Retrograded amylose or resis-

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Table 1. The different starch samples used, their X-ray diffraction pattern (A, B or C) and other characteristics

Starch type		X-ray diffraction pattern	Characteristics
Wheat	c.v. Holme	A ^a	Bread wheat
Rye	c.v. Motto	A ^a	Population rye
Barley	c.v. Golf	A ^b	Normal-amylose, covered
	Line 906129 waxy	A ^b	High-amylopectin, covered
	c.v. Glacier	A ^b	High-amylose, covered
Waxy maize	c.v. unknown	A ^b	High-amylopectin
Potato	c.v. Desiree	B ^b	Consumption potato
	cv. Prevalent	B ^b	Starch potato
	PAP	B ^c	High-amylopectin
Pea	c.v. Capella	C ^c	Smooth yellow pea

^aKatz and van Itallie (1930)^bZobel (1988)^cAnalysed

tant starch type III (Englyst et al., 1992) is a physiologically important indigestible starch fraction. This fraction is heat stable and melts above 120°C (Sievert and Pomeranz, 1989). The long-term changes that occur during storage of starch gels have been attributed to the amylopectin fraction (Eliasson, 1985). The retrogradation behaviour of amylopectin has been related to the starch source (Orford et al., 1987; Kalichevsky et al., 1990; Shi and Seib, 1992; Ward et al., 1994) and concentration (Orford et al., 1987; Slade and Levine, 1987). Other factors, such as the storage temperature (Slade and Levine, 1987; Eliasson and Ljunger, 1988), amylopectin structure and other components present, are also of importance. Lipids (Eliasson and Gudmundsson, 1996) and short amylopectin unit-chains with degree of polymerization (DP) 6–9 (Shi and Seib, 1992) have been shown to inhibit retrogradation. In high-amylose starches, the amylose fraction has been suggested to have synergetic effects on the amylopectin retrogradation (Russell, 1987).

The botanical source of the starch will thus have an impact on the physico-chemical properties of starch. This influence might be attributed to the granule size distribution, to the crystallinity (degree and/or polymorphic form), to the organisation of the molecules within the granule or to the chemical nature of the starch polymers. The present investigation was undertaken in order to study in more detail the relation between physico-chemical properties of starch and the chemical nature of its constituents. Therefore, 10 different starches, representing all the polymorphic forms (A, B and C), were chosen to cover a broad range of chemical and thermal characteristics. The starch granule size distribution was measured and, in the chemical analysis, emphasis was given to the amylose content and the amylopectin characteristics. The physico-chemical properties studied were gelatinization and retrogradation measured using differential scanning calorimetry (DSC). The gelatinization was studied at limited water content, and the retrogradation was studied under conditions to ensure extensive retrogradation (storage at 6°C). The results were evaluated by principal component analysis (PCA).

MATERIALS AND METHODS

Six cereal starches, three potato starches and one pea starch were used in this study, as outlined in Table 1. The pea and the cereal samples, except for the waxy maize, were obtained from Svalöf-Weibull AB (Landskrona, Sweden), and the starch was isolated according to Meredith et al. (1978). The three barleys were pearled prior to starch isolation in a Strong-Scott Seedburo Mill. The waxy maize starch and the three potato starches were supplied by Lyckeby-Stärkelsen (Kristianstad, Sweden).

Chemical characterization

The starch content was determined enzymatically according to Åman et al. (1994), and the dry matter by oven drying at 105°C for 16 h. The size distribution of the starch granules was determined using a Coulter Counter with a 100-channel analyser (Morrison and Scott, 1986). The channel diameters ranged from 1.98 to 60.74 µm and were converted to a linear size scale. Smoothing was applied on the distribution profiles using cubic spline interpolation, and the volumes calculated assuming spherical granules. The X-ray diffraction patterns of the pea and high-amylopectin potato starches were determined according to Svensson and Eliasson (1995). The total and apparent amylose contents were analysed by iodine staining (Morrison and Laignelet, 1983). The lipid-complexed amylose (LAM) content was calculated as the difference between the total and the apparent amylose (FAM) contents. For the three barley starches, the amylose content was calculated by the equation suggested by Tester and Morrison (1992). The amylose content was also determined by gel permeation chromatography (GPC). The samples (1.5 mg ml⁻¹) were prepared essentially as described by Torneport et al. (1990). For debranching, however, 5 µl isoamylase from *Pseudomonas amyloclavata* (EC 3.2.1.68, 71 000 U mg⁻¹ protein, obtained from Hyashibara, Biochemical Labs, Inc., Okayama, Japan) was used, and the enzyme was inactivated in a boiling water-bath for 5 min. The samples were injected on a Sepharose CL 6B column (1.6 × 70 cm) using 0.25 M KOH as eluent

at a flow rate of 13 ml h⁻¹. Two-ml fractions were collected, and the elution profile was detected by the phenol-sulphuric acid method of Dubois et al. (1956). The amylose, consisting of the long-chain α -1,4-glucans after debranching of the starch, comprised the first fraction eluted, including the void peak and the intermediate material between the amylose and the amylopectin unit-chains.

For characterization of the chain length distribution of the amylopectin, the starch was dissolved in 0.1 M NaOH. The amylopectin was isolated by GPC (Lloyd et al., 1996) and freeze-dried, prior to debranching by isoamylase and further analysis by high-performance size-exclusion chromatography (HPSEC). Amylopectin unit-chain fractions with defined average DP were used for calibration of the HPSEC system (Fredriksson et al., 1997). This calibration was also used for calculation of the weight average DP (\overline{DP}_w). The distribution profiles of the amylopectin unit-chains were divided into four parts, defining sub-fractions (F1–F4) with decreasing chain lengths. The limits between the different sub-fractions were determined by the inflection points at DP 82.3 (F1/F2) and DP 17.8 (F3/F4) and the minimum at DP 37.4 (F2/F3), estimated from the first-derivative curve of an average chromatogram calculated from the 10 amylopectins examined. All the chemical analyses, except the determination of the starch granule size distribution, were performed in duplicates at the least.

DSC measurements

The thermal transitions of starch were examined with a Perkin-Elmer DSC 2c (starch from wheat, rye, barley, potato Desiree and pea) or with a DSC 6200 from Seiko Instruments Inc. (starch from waxy maize, high-amylopectin potato and potato Prevalent). Coated sample pans of aluminium from TA Instruments were used with an empty sample container as reference. Starch (5–10 mg) was transferred into weighed sample containers, and the appropriate amount of water was added. The sample containers were then sealed and reweighed. The samples were allowed to equilibrate for at least 2 h before d.s.c. analysis to attain an even distribution of water. The exact water content of each sample was determined by drying the punctured container in an oven at 105°C for 24 h after the DSC scan. The starch:water ratio for all samples studied was approximately 1:1 (0.49–0.53). In the starch gelatinization studies, each sample was examined by DSC after the equilibration period. In the starch retrogradation studies, the sample containers were heated in an oven for 15 min at 105°C and then stored at 6°C for 2 or 4 days before DSC analysis. All the samples were analysed at 17–127°C, with a heating rate of 10°C min⁻¹.

Each DSC endotherm of gelatinization was characterized by the onset temperature ($T_{o,gel}$), the temperature at peak minimum ($T_{m,gel}$), the offset temperature ($T_{f,gel}$), the melting interval ($\Delta T_{gel} = T_{f,gel} - T_{o,gel}$) and the melting enthalpy (ΔH_{gel}). The DSC endotherms related to starch retrogradation were evaluated by determining the onset temperature of

melting of recrystallized amylopectin ($T_{o,2}$ or $T_{o,4}$), the temperature at peak minimum ($T_{m,2}$ or $T_{m,4}$), the offset temperature ($T_{f,2}$ or $T_{f,4}$), the interval of melting ($\Delta T_2 = T_{f,2} - T_{o,2}$ and $\Delta T_4 = T_{f,4} - T_{o,4}$) and the melting enthalpy (ΔH_2 or ΔH_4). The dissociation of the amylose-lipid complex was assessed by determining the peak minimum temperature of melting of the complex (T_{cx}) and the melting enthalpy (ΔH_{cx}) of the complex. Except for ΔH_{cx} , the enthalpy values were calculated on an amylopectin basis, using the amylopectin contents obtained by the GPC procedure, and all enthalpies were expressed on a dry matter basis. All DSC results are the means of three measurements. The measured enthalpies and transition temperatures were generally within 5% of the given values.

Statistical analysis

The variations observed in the chemical and physical properties of the 10 different starches were examined by principal component analysis (PCA) with the computer software SIRIUS (Pattern Rec System A/S, N-5015, Bergen, Norway). To determine the significance of the principal components (PC), the procedure of cross-validation was used (Wold, 1978).

RESULTS AND DISCUSSION

Origin of samples and starch granule distribution

Ten starches of different botanical origin were chosen to cover a broad variation of chemical and thermal characteristics. The studied material included commonly grown starch-rich crops, such as wheat, rye, barley, waxy maize, pea and potato, as well as different genotypes from conventional breeding programs (high-amylose and waxy barley) and genetically modified starch (high-amylopectin potato).

The starch content (glucose residues) was high in all samples and ranged from 92.9 to 96.6% of dry matter. Wheat, barley and rye starches have a bimodal granule distribution (Karlsson et al., 1983; Lineback, 1984), with larger lens-shaped A-granules and smaller spherical B-granules (Fig. 1a). The starch isolation was carried out with the aim of mimicking commercial conditions, which resulted in some losses of material. Small starch granules tend to associate with the protein fraction and may be lost during the isolation procedure (McDonald and Stark, 1988), and the results also indicated such losses for the cereal starches, especially for the three barley starches (Oscarsson et al., 1997). The rye starch differed from the wheat starch through the broader distribution profile and the larger diameter of the A-granules. The starch granules of normal-amylose and waxy barley had similar distribution profiles, both with only traces of the B-granule fraction, and with slightly smaller A-granules than the wheat starch. The high-amylose barley had smaller A-granules than the other two barleys which was in agreement with the findings of Oscarsson et al.

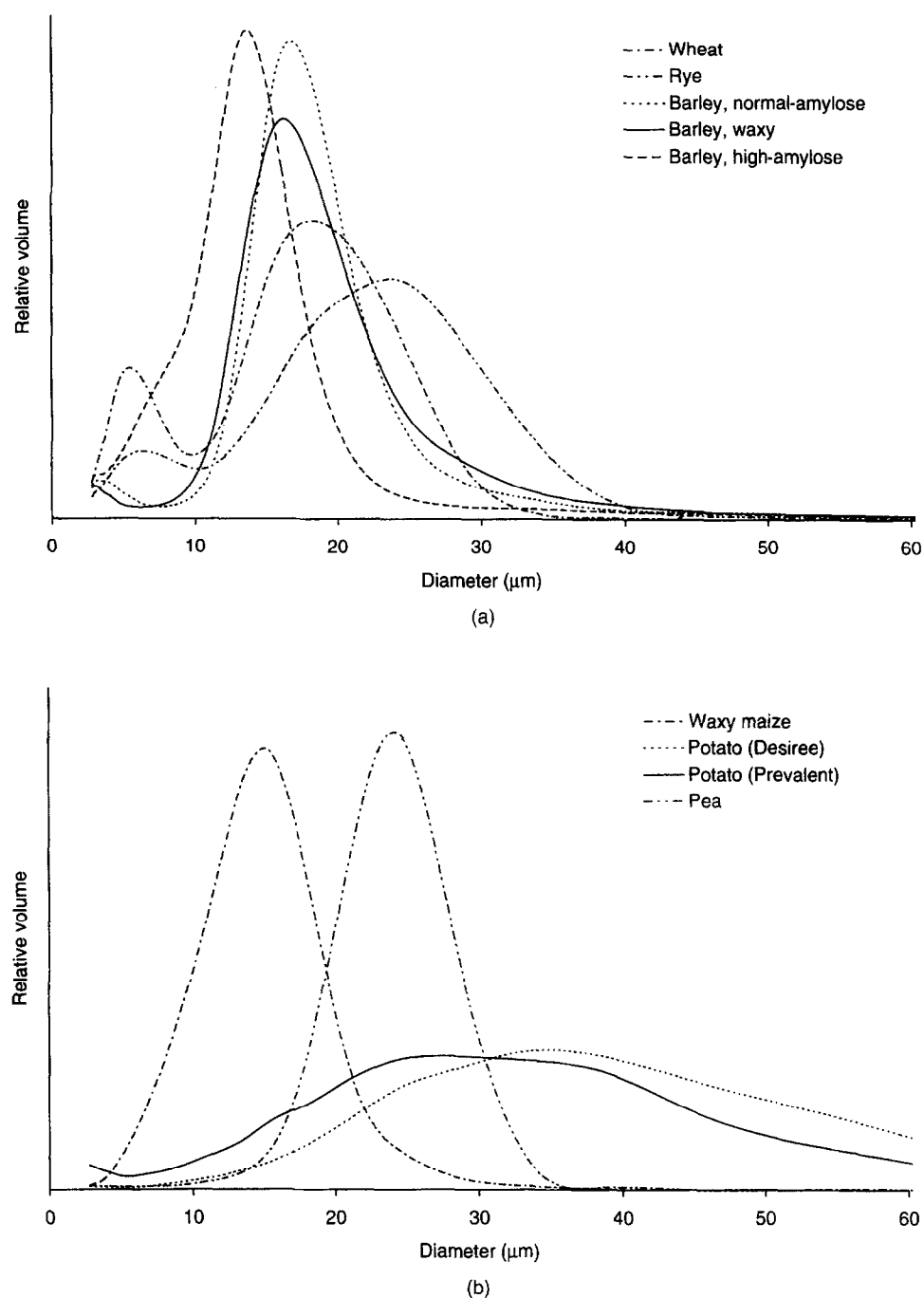


Fig. 1. Granule size distribution of starches from (a) wheat, rye and barley, (b) waxy maize, potato and pea

(1997). Waxy maize, potato (Lineback, 1984; Snyder, 1984) and pea starches (Eliasson, 1988) have monomodal starch granule distributions (Fig. 1b). In waxy maize, the peak maximum of the starch granule distribution profile appeared between those shown by normal- and high-amylose barley. The size of the pea starch granules was similar to that of the A-granules in rye starch, but the distribution profile was narrower. The starches from potato cultivars Desiree and Prevalent had the broadest starch granule distribution and the largest granules of the starches studied. The starch granule distribution of the high-amylopectin potato starch could

not be analysed, since the instrument was blocked by aggregating or possibly cold swelling granules.

Amylose content

When determined by iodine staining, the total amylose content was at a similar level (25.0–27.4%) in the wheat, rye and normal-amylose barley starches (Table 2). The LAM content was close to 5% in both the wheat and normal-amylose barley starches but lower in the rye starch. There was a linear relationship between the total amylose and

Table 2. Amylose contents of the starch samples, as determined by iodine staining (% of dry matter) and GPC (% of sugar residues), as well as gelatinization enthalpies (J g^{-1} dry starch) and temperatures ($^{\circ}\text{C}$) of the amylose/lipid complex

	[FAM ^a]	[LAM ^b]	[Total]	[Amylose content by GPC]	ΔH_{cx}	T_{cx}
Wheat	20.4	4.6	25.0	28.4	1.4	110.1
Rye	22.7	3.3	26.0	28.6	0.8	107.8
Barley						
Normal-amylose	21.9	5.5	27.4	29.3	1.8	110.3
Waxy	3.2	2.4	5.6	7.1	0.8	112.8
High-amylose	29.6	7.5	37.1	39.0	2.8	110.8
Waxy maize	0.3	0.5	0.8	1.6	ND ^c	ND ^c
Potato						
Normal-amylose (Desiree)	28.2	-0.9	27.3	23.0	ND ^c	ND ^c
Normal-amylose (Prevalent)	24.7	-0.1	24.6	20.4	ND ^c	ND ^c
High-amylopectin	3.8	0.4	4.2	1.0	ND ^c	ND ^c
Pea	33.9	-0.5	33.4	33.7	ND ^c	ND ^c

^aApparent amylose^bLipid-complexed amylose^cNot detected

LAM contents for the three barleys. One interesting characteristic of the waxy barley seems to be a high proportion of LAM in relation to the total amylose (Morrison, 1995). The amylose content of starch from potato Prevalent (24.6%) was significantly lower than that of starch from potato Desiree (27.3%). The negative LAM values obtained for Prevalent, Desiree and for pea were probably due to the uncertainty of the method. This may become more pronounced in samples with little or no native starch lipids. The amylose contents reported in this study were similar to those obtained previously using the same methodology (Morrison and Laignelet, 1983; Morrison et al., 1984; Doublier, 1987; Radosta et al., 1991).

The total amylose contents determined by GPC were comparable with those obtained by the iodine staining (Table 2). The main differences may be explained by variations in the amylopectin or amylose structure. The amylose contents obtained by GPC for the potato starches were probably more accurate than those obtained by iodine staining. In general, potato starch has longer amylopectin unit-chains than, e.g. cereal and pea starches (Kalichevsky et al., 1990). Therefore, the potato amylopectin may have contributed to the iodine staining to a larger extent than the other starches studied, resulting in somewhat overestimated amylose contents.

In high-amylose rice and maize, atypical starch materials have been detected (Asaoka et al., 1986; Inouchi et al., 1987). Therefore, the possibility of finding anomalous types of starch has been discussed also for high-amylose barley. In this study, the GPC analyses of normal- and high-amylose barley resulted in almost identical cumulative curves, when including the void peak and the intermediate material preceding the amylopectin unit-chains in the amylose fraction. This result showed that the intermediate material constituted the same proportion of the amylose in both samples. This observation is also in agreement with previous findings reported by Tester et al. (1991).

Characterization of amylopectin

The distribution of the amylopectin unit-chains generally appears to be genetically controlled and characteristic of a species (Hizukuri, 1985; Kalichevsky et al., 1990). However, some genotypes with a somewhat different amylopectin structure do exist, for example, high-amylose genotypes of maize and rice (Hizukuri, 1985; Asaoka et al., 1986; Inouchi et al., 1987). Smaller variations observed in wheat and barley amylopectin have been assigned to the growing temperature (Tester et al., 1991; Shi et al., 1994). The amylopectin chain distribution profiles for the 10 starches were examined by HPSEC, after debranching with isoamylase. All the cereal starches showed a polymodal distribution, with local peak maxima or shoulders at DP 11–12, 18–19 and 46–48 (Fig. 2). The amylopectins from potato Desiree and Prevalent also exhibited a polymodal distribution profile with peak maxima or shoulders at DP 13, 17, 50 and 79–80, whereas the profile for the high-amylopectin potato was slightly different, with peak maxima at 15, 18, 52 and 82. The amylopectin profile for pea was similar to that of the waxy maize with DP maxima of 14 and 47–49 and a shoulder at DP 19–20. Of the cereal amylopectins studied, that of the high-amylose barley had the lowest $\overline{\text{DP}}_{\text{w}}$ value (24.0) (Table 3), whereas the waxy maize amylopectin had the highest (28.0). The $\overline{\text{DP}}_{\text{w}}$ value for the pea amylopectin was 26.6. The overall highest values were obtained for the three potato amylopectins (31.4–34.8). These $\overline{\text{DP}}_{\text{w}}$ values were in the same range as those found in previous studies. Hizukuri (1985) investigated the relationship between the chain profiles of amylopectin and the crystalline structure of starch granules for 20 samples, including wheat, waxy maize and potato. The A-type amylopectins had $\overline{\text{DP}}_{\text{w}}$ of 26 (23–29), the B-type amylopectins 36 (30–44) and the C-type amylopectins around 28. Similar results were reported for barley (MacGregor and Fincher, 1993), wheat and potato (Hizukuri, 1986). Some smaller deviations between results from different studies may, at

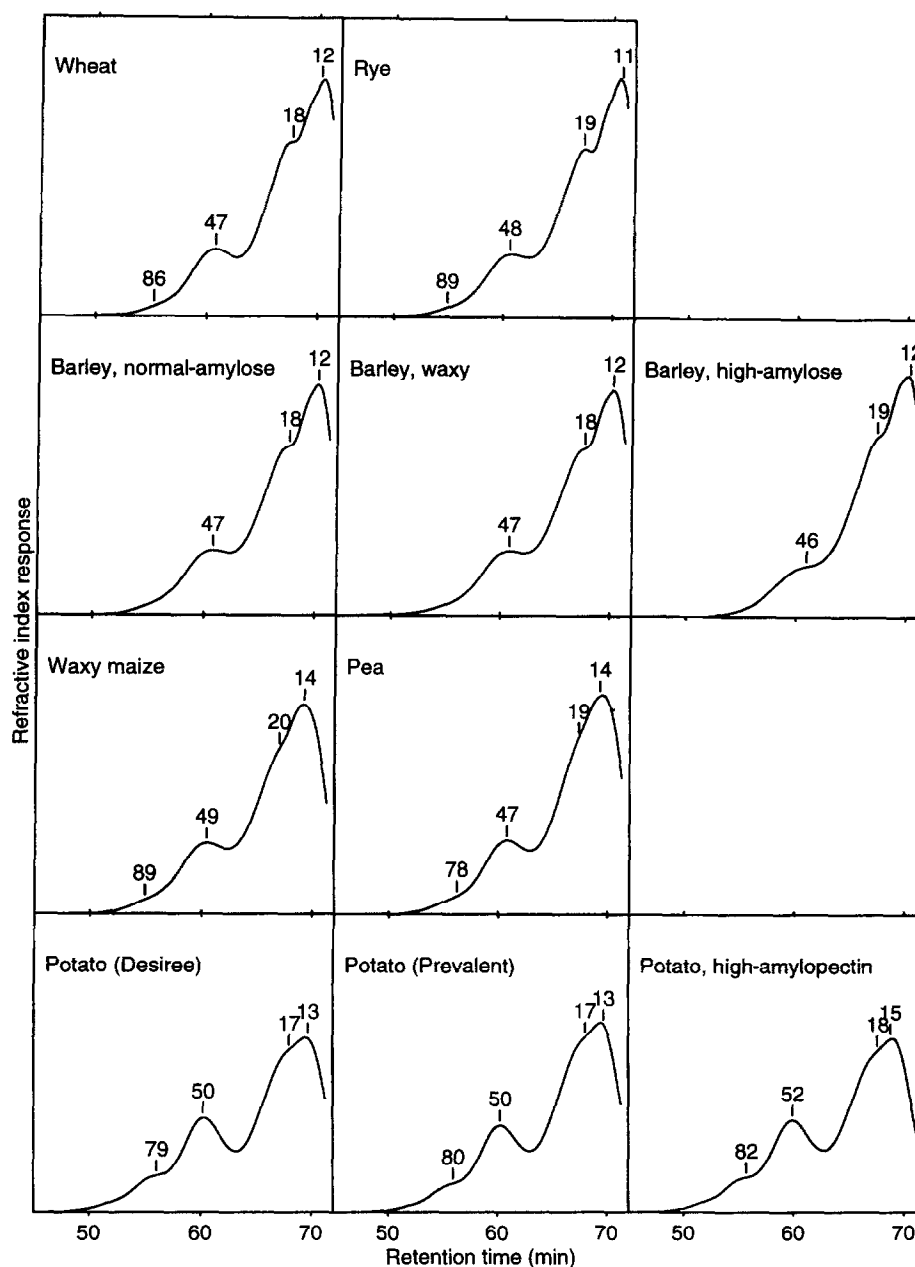


Fig. 2. Amylopectin unit-chain length distribution after debranching of the 10 starches. Arrows indicate DP at local peak maximum or shoulder

least in part, be of experimental character due to differences in the methods used but may also be of genetic or environmental origin.

The amylopectin chain length distribution profiles of the wheat, rye, normal-amylose barley and waxy barley were all very similar, with a relative chain length distribution between 20.1 and 21.3% for fractions F1 + F2 and between 78.7 and 79.8% for fractions F3 + F4, respectively (Table 3). The amylopectin from high-amylose barley contained a considerably lower proportion of fractions F1 + F2 than any of the other samples analysed. This is in line with the results by Tester et al. (1991), that also indicated smaller proportions of long amylopectin chains in high-amylose barley than in normal and waxy barley. Salomonsson and

Sundberg (1994), on the other hand, obtained a higher proportion of certain longer amylopectin unit-chains (DP 30–49) in the high-amylose barley than in the normal-amylose and waxy barley. These results imply that the amylopectin fraction of high-amylose barleys to some extent may differ in structure. The three potato amylopectins had the largest proportions of fractions F1 + F2 containing long amylopectin unit-chains, but the proportion was slightly smaller in potato Prevalent than in potato Desiree and the high-amylopectin potato. However, this result indicates that the amylopectin structure is similar in all three potato starches. The amylopectin distribution profiles of the waxy maize and pea were intermediate between those of the aforementioned starches. Hizukuri (1986) observed a

Table 3. Amylopectin content of the isolated starch samples, as determined by GPC (% of dry matter), relative distribution of amylopectin unit-chains (%) and weight-average degree of polymerization (\overline{DP}_w) of debranched amylopectins

	[Amylopectin content]	[Chain length distribution]						\overline{DP}_w
		F1	F2	F3	F4	[F1 + F2]	[F3 + F4]	
Wheat	71.6	1.3	18.8	34.1	45.7	20.1	79.8	25.7
Rye	71.4	1.7	18.7	34.6	45.0	20.4	79.6	26.2
Barley								
Normal-amylose	70.7	1.2	19.2	34.8	44.8	20.4	79.6	25.8
Waxy	92.9	1.9	19.4	34.6	44.1	21.3	78.7	26.7
High-amylose	61.0	0.6	15.5	36.7	47.3	16.1	84.0	24.0
Waxy maize	98.4	2.1	21.6	35.6	40.8	23.7	76.4	28.0
Potato								
Normal-amylose (Desiree)	77.0	6.1	28.1	30.6	35.3	34.2	65.9	33.9
Normal-amylose (Prevalent)	79.6	4.4	25.6	32.7	37.4	30.0	70.1	31.4
High-amylopectin	99.0	6.5	28.1	33.1	32.4	34.6	65.5	34.8
Pea	66.3	1.6	20.7	33.9	43.8	22.3	77.7	26.6

polymodal amylopectin distribution profile having five peaks; A, B1, B2, B3 and B4 and suggested that these populations may be involved in the formation of up to four different clusters. In the present study, fraction F1 approximately corresponds to peaks B3 and B4, F2 to B2, F3 to B1 and F4 to A. However, since this type of analysis is based on decomposition of the amylopectin molecule it is hard to reconstruct the actual structure. Even though the distribution profiles were more or less identical for several of the starches studied, the appearance of the intact amylopectin molecules may still differ.

Starch gelatinization

The gelatinization of the various starches was studied by DSC at a water content of approximately 50%. Due to the limited water content, the typical broadening on the high-temperature side of the gelatinization endotherm was evident for all the samples studied (Eliasson, 1980) (Fig. 3). However, the position and appearance of this broadening varied and, consequently, affected the temperature range of gelatinization. The separation between this high-temperature shoulder and the first part of the endotherm was most apparent for the pea starch, whereas for the three potato starches, the separation was poor.

The gelatinization enthalpy (ΔH_{gel}) is a measure of the overall crystallinity of the amylopectin, i.e. the quality and quantity of starch crystals (Tester and Morrison, 1990b). Pure crystalline A-spherulites exhibit a melting enthalpy of approximately 35 J g^{-1} , which is very similar to the melting enthalpy for pure crystalline B-spherulites (Whittam et al., 1990). For native starches, the molecular order in form of double helices is significantly higher than the crystalline order (Gidley, 1985). In cereal starches and tapioca, but not in potato starch, both levels of structure, i.e. double-helical and crystalline, are disrupted concurrently during gelatinization, and it has been suggested that ΔH_{gel} primarily reflects the loss of double-helical order (Cooke and Gidley, 1992). The amount of double-helical order in the native starches should be strongly correlated to the amylopectin content,

and the crystallinity in the granules increases with the amylopectin content (Gernat et al., 1993). Therefore, all ΔH_{gel} values in this study were calculated on an amylopectin basis. These values of ΔH_{gel} were between 15.6 and 23.9 J g^{-1} amylopectin (Table 4). The highest values were obtained for potato cultivars Prevalent and Desiree, the lowest for the cereals, while pea and high-amylopectin potato showed intermediate values. Although the enthalpy values for pure A- and B-spherulites are nearly the same, it seemed that, even when calculated on an amylopectin basis, the ΔH_{gel} values for the cereal starches were considerably lower than those for the potato and pea starches.

The minor differences between wheat and potato starches in the data of Cooke and Gidley (1992), representing double-helical order, appear to be insufficient to explain the large differences between the ΔH_{gel} values found in the present study.

During the gelatinization, part of the free lipids present in the cereal starches will probably form a helical inclusion complex with the amylose molecules. This complex formation is an exothermic process and will result in a decrease in the observed endothermic gelatinization enthalpy (Eliasson, 1986). This may be one reason why the ΔH_{gel} values for the pea and potato starches (which do not contain any native lipids) are higher than those for the lipid-containing cereal starches. However, it does not explain why the ΔH_{gel} values were higher for wheat than for normal-amylose barley, despite the similar contents of LAM and FAM in these two cereals.

In starch, a biopolymer of partially crystalline nature, the melting of the crystallites cannot start before the glass transition of the glassy regions is exceeded. The second-order glass to rubber transition is accompanied by an incremental change in heat capacity (C_p), preceding the gelatinization endotherm during a d.s.c. scan. For rice starch, Biliaderis et al. (1986) estimated C_p for this glass transition to be $0.11 \pm 0.01 \text{ J/gK}$. During the estimation of the ΔH_{gel} values for the 10 starches in the present study, no consideration has been taken to any possible glass transition related to the melting endotherms, i.e. ΔH_{gel} was calculated

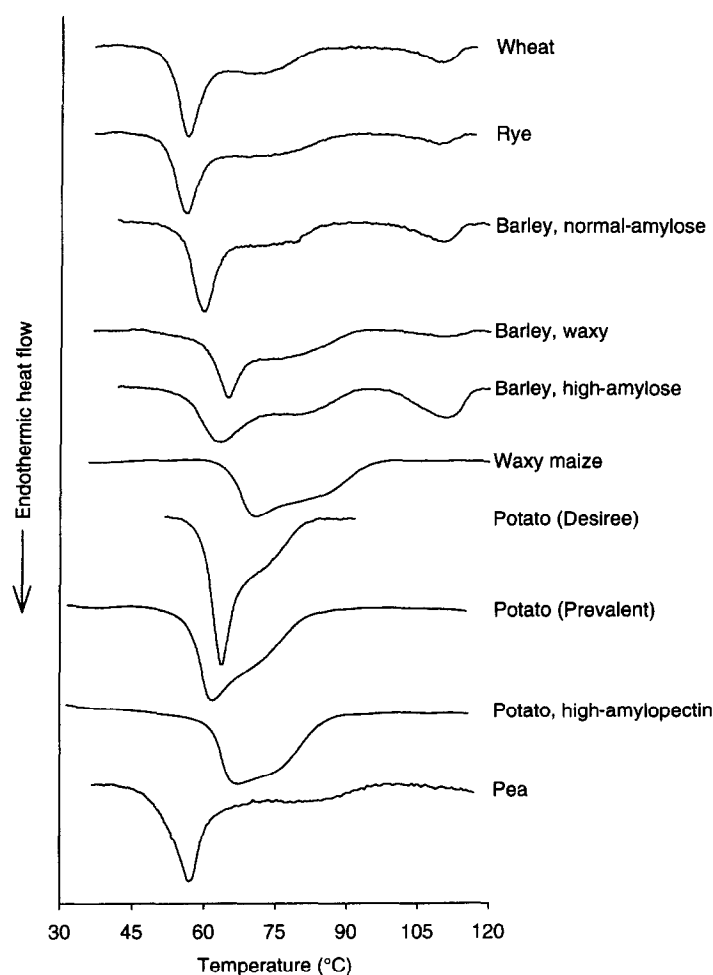


Fig. 3. DSC gelatinization endotherms of the 10 starches studied at a starch:water ratio of approximately 1:1

by estimation of the area under the endotherm with a fitted straight base line.

The gelatinization always takes place over a temperature interval (ΔT_{gel}), which in excess water may be 1–2°C for a single granule, and for the whole population of granules the interval may be >10°C (Eliasson and Gudmundsson, 1996). At intermediate or high water content, the onset (T_o) and peak minimum temperatures (T_m) are independent of the

Table 4. Enthalpy of gelatinization (ΔH_{gel}) and retrogradation related enthalpies of the starch samples after storage for 2 (ΔH_2) and 4 days (ΔH_4) at 6°C (J g^{-1} amylopectin)

	ΔH_{gel}	ΔH_2	ΔH_4
Wheat	17.5	8.1	9.8
Rye	15.8	7.3	8.7
Barley			
Normal-amylose	15.6	10.3	10.6
Waxy	15.7	10.6	11.1
High-amylose	16.1	12.5	13.1
Waxy maize	17.6	12.3	13.0
Potato			
Normal-amylose (Desiree)	22.1	13.3	12.9
Normal-amylose (Prevalent)	23.9	13.6	14.4
High-amylopectin	19.7	13.6	13.5
Pea	20.8	12.5	12.5

water content, whereas the offset temperature (T_f) is much influenced by the water available during gelatinization (Eliasson, 1980). In the present investigation, the highest $T_{o,\text{gel}}$ values, 59.6–64.4°C, were obtained for the high-amylopectin varieties of both A- and B-type starch (Fig. 4). This was consistent with the hypothesis that crystallinity increases with amylopectin content (Gernat et al., 1993). Starch from potato Desiree also showed a relatively high $T_{o,\text{gel}}$ value. Starches from potato Prevalent, normal-amylose barley and high-amylose barley exhibited an intermediate $T_{o,\text{gel}}$, while the lowest $T_{o,\text{gel}}$ was recorded for the pea, rye, and wheat starches. Shi and Seib (1992) investigated starches of the A-type from four waxy cereals, viz. maize, barley and two different rice samples, and found that the gelatinization temperatures (onset, peak and offset) in 75 wt.% water increased in the same order as their X-ray crystallinity. In their study, $T_{o,\text{gel}}$ for the maize and barley was 67.0 and 58.0°C, respectively, which roughly seems to be in line with the results in the present study. They concluded that the molecular structure of the microcrystalline region is the same in the four granular starches, and that the melting temperature of the crystallites in these native waxy starches is controlled indirectly by the surrounding amorphous regions. By contrast, an investigation of maize

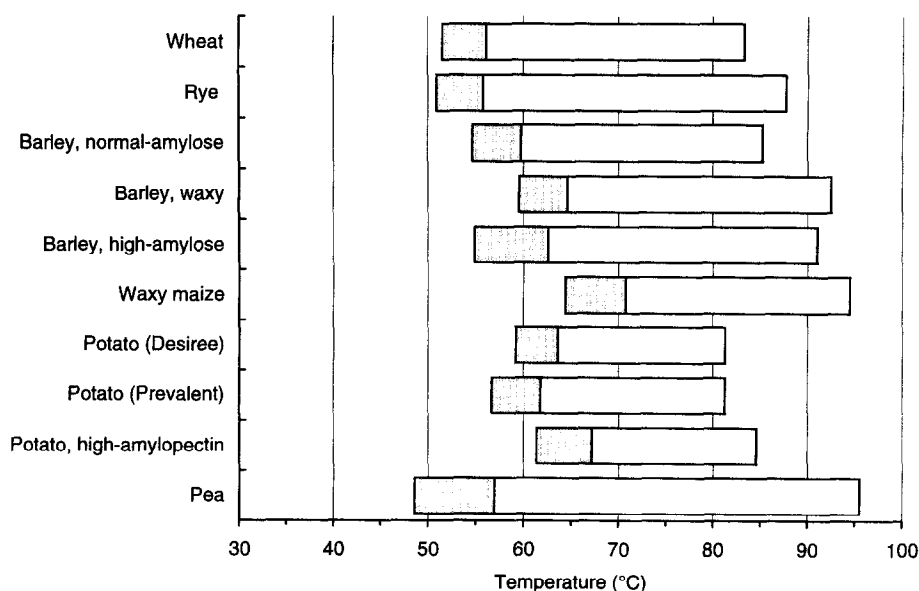


Fig. 4. Bars indicating the temperature range of gelatinization (T_g to T_m) of starch samples, border verifies the position of T_m

starches with varying amylose content (0–66.1%) indicated that gelatinization temperatures increased with increasing amounts of amylose (Knutson, 1990). A study of 11 different waxy rice starches with varying gelatinization properties showed that starches with low gelatinization temperatures had more amorphous and less crystalline material compared to starches with high gelatinization temperatures (Tester and Morrison, 1990b). Their explanation to this phenomenon was that crystallite perfection could be the principal mechanism controlling the gelatinization temperatures. Other factors, such as the amount of damaged starch, isolation techniques (Morrison, 1995) and variation in climatic conditions during growth (Shi et al., 1994; Tester et al., 1991), also influence these temperatures.

The pea starch exhibited the largest ΔT_{gel} (46.9°C) and the three potato starches the smallest (22.1–24.6°C). No correlations were found between the granule size distribution and d.s.c. parameters, such as $T_{o,gel}$, $T_{m,gel}$, ΔT_{gel} and ΔH_{gel} .

Starch retrogradation

The features of the retrogradation endotherms were very similar after 2 and 4 days of storage at 6°C, except that the areas of the DSC peaks were somewhat smaller after the shorter storage time. Hence, only the endotherms after 4 days of storage are shown (Fig. 5). The endotherms for starches from wheat, rye, normal-amylose barley and high-amylose barley were bell-shaped, and that for waxy barley starch was slightly bimodal. The endotherms for the three potato starches were all bimodal as well as those for the waxy maize and pea starches. The mono- and bimodal features of the retrogradation endotherms can be related to differences in the quality of the recrystallized amylopectin crystals, a factor which may depend on the botanical origin

of the starch. The bimodal shape of the aforementioned retrogradation endotherms could also be an evidence of an amylopectin reorganization that takes place during the d.s.c. scan. Because of increased molecular mobility, there will be a chance for chain rearrangements in the amylopectin crystallites just above the onset temperature (Biliaderis et al., 1986). The structure of the recrystallized amylopectin will approach a new equilibrium, and a fraction of the crystallites will melt at a slightly higher temperature.

The melting enthalpy (ΔH_2 and ΔH_4) of recrystallized amylopectin was calculated from the area of the retrogradation-related endotherm and is expressed on an amylopectin basis (Table 4). After storage for 2 days at 6°C the starches of rye and wheat exhibited the lowest ΔH_2 values (7.3 and 8.1 J g⁻¹ amylopectin, respectively). The highest ΔH_2 values were obtained for the three potato starches (13.3–13.6 J g⁻¹ amylopectin) followed by the pea and high-amylose barley starches. These results confirmed earlier findings that amylopectins from cereals retrograde to a lesser extent than pea (Kalicevsky et al., 1990) and potato amylopectins (Kalicevsky et al., 1990; Silverio et al., 1996). This has been attributed to the shorter average chain lengths in the cereal amylopectins (Orford et al., 1987; Kalicevsky et al., 1990). In contrast to their results, the ΔH_2 and ΔH_4 values obtained for the pea starch were lower than those for the three potato starches.

After 4 days of storage at 6°C, a similar ranking of the different samples as that obtained after storage for 2 days was observed (Table 4) but it seems that the rate of retrogradation was slower for the wheat and the rye starches compared to the others. The varying retrogradation enthalpies, determined in the present study, reflected the large differences between the overall quality and quantity of the recrystallized amylopectin in starches of different botanical origin. Wide-angle X-ray diffraction studies (WAXS) of

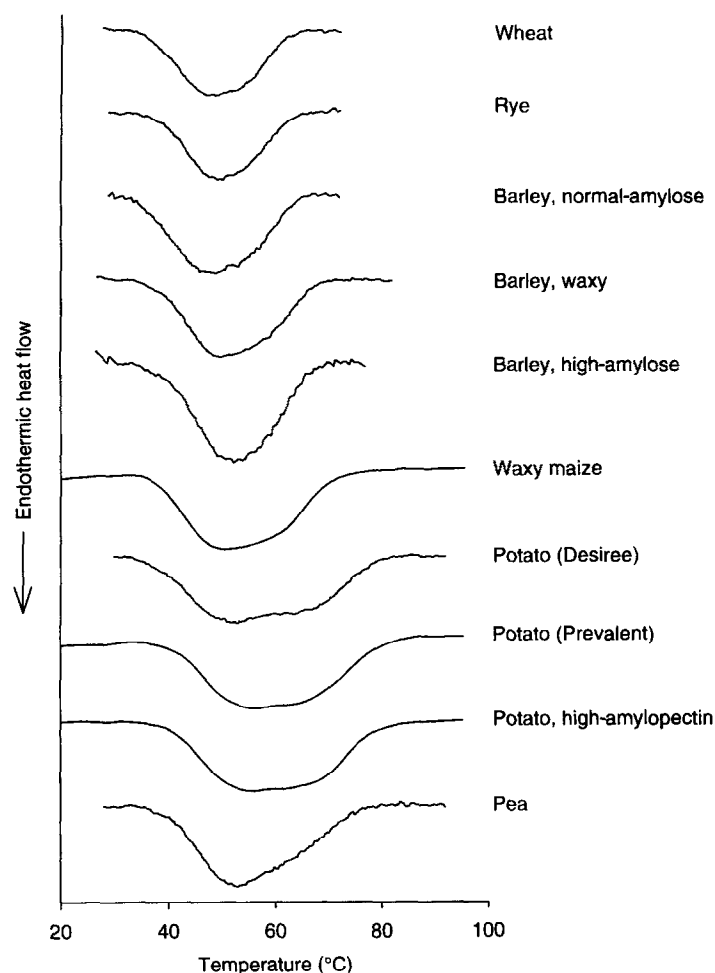


Fig. 5. DSC endotherms of recrystallized amylopectin from starch samples, after gelatinization at a starch:water ratio of approximately 1:1 and storage for 4 days at 6°C

cooked starch showed a slow development of the B form of crystallinity (Katz, 1934), which is independent of the polymorphic form the native starch had prior to gelatinization. Although retrograded amylopectin from different sources evidently shows the same B-polymorphic crystal pattern, other factors, such as structural differences in the amylopectin molecule, could cause the variations found in the rate and extent of amylopectin recrystallization. It is clear that high ΔH_{gel} values resulted in high ΔH_2 and ΔH_4 values, as obtained for the potato starches, while low ΔH_{gel} values gave low ΔH_2 and ΔH_4 values, as obtained for starches from cereals, such as rye and wheat.

It could be expected that the three barley starches should retrograde to the same extent, especially since the enthalpy of retrogradation was expressed on an amylopectin basis. The present investigation showed that the normal-amylose and the waxy barley starches indeed retrograded to the same level, but that the high-amylose barley starch retrograded to a higher extent. This observation may increase the interest for high-amylose barley from a nutritional point of view. Retrograded waxy maize starch has been shown to be more resistant to α -amylase than freshly gelatinized starch (Eerlingen et al., 1994), and may partly escape digestion

and absorption in the human small intestine. In a retrogradation study of gels from nongranular mixtures of amylose and amylopectin in different ratios, synergistic interactions were seen between amylopectin and amylose at high amylose contents, i.e. unexpectedly high melting enthalpies were obtained for gels with very high amylose content (75–90%) (Gudmundsson and Eliasson, 1990). The possibility of cocrystallization of the two polymers has been proposed in relation to retrogradation, when amylose is found in high amounts (Russell, 1987).

Crystallization, the rate-limiting step in retrogradation, is a nucleation-controlled growth process, and the advantage of low-temperature storage is that the nucleation rate increases exponentially with decreasing temperature down to the glass transition. On the other hand, crystal growth is favoured at a temperature just below T_m , and the rate of propagation also increases exponentially with increasing temperature up to T_m (Slade and Levine, 1987). The melting temperature range (ΔT_2 and ΔT_4) gives an indication of the quality and heterogeneity of the recrystallized amylopectin. Thus, a wide melting range might imply a large amount of crystals of varying stability, whereas a narrow range could suggest crystals of a more homogeneous quality and similar

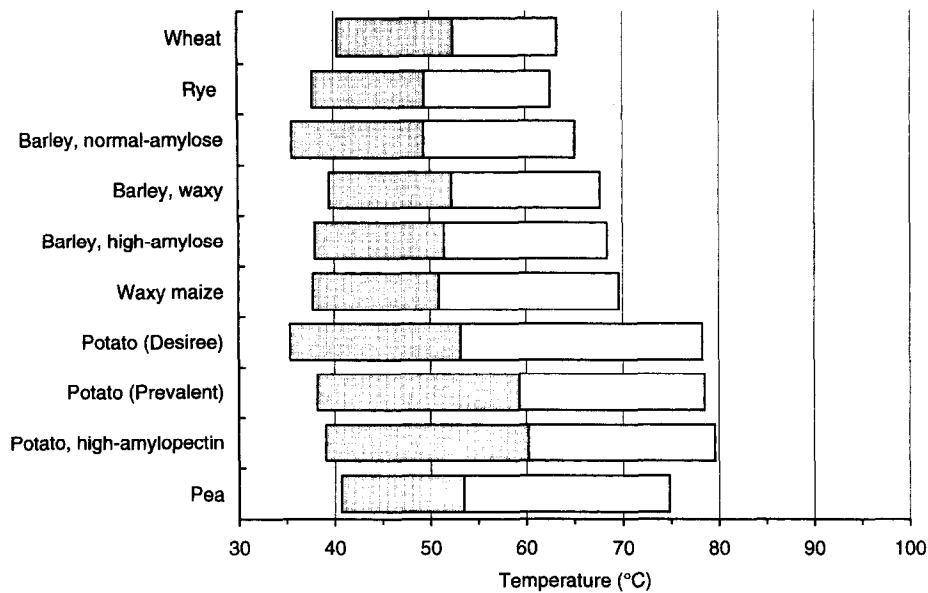


Fig. 6. Bars indicating the melting temperature ranges ($T_{o,2}$ to $T_{f,2}$) of recrystallized amylopectin after 2 days of storage at 6°C, border verifies the position of $T_{m,2}$.

stability. The widest melting ranges (40.3–43.3°C) were obtained for the three potato starches (Figs. 6 and 7), indicating that a heterogeneous mixture of amylopectin crystals of differing stability was formed during ageing of these starches. Interestingly, the potato starches also exhibited the highest gelatinization and retrogradation enthalpies. By contrast, the potato starches showed the narrowest melting range (ΔT_{gel}) during gelatinization. During biosynthesis, the potato starch granules may therefore create more homogeneous crystal regions than the other starches studied. Fairly large melting ranges (ΔT_2 and ΔT_4) were also observed for the pea and waxy maize starches. For the pea starch, a wide melting range (ΔT_{gel}) was also observed

during gelatinization. For the remaining starches, ΔT_2 was 22.8–30.4°C and ΔT_4 25.7–29.9°C.

The onset temperature, $\Delta T_{o,2}$ ranged from 35.4 to 40.7°C and $\Delta T_{o,4}$ from 35.1 to 39.6°C. The temperatures $\Delta T_{m,2}$ and $\Delta T_{m,4}$ at peak minimum were 49.4–60.3 and 47.8–60.1°C, respectively. The variation within the $T_{o,2}$ and $T_{o,4}$ values (ca. 5°C) for the various retrograded starches was fairly small compared to the differences among the $T_{o,gel}$ values (ca. 16°C) for the native starches. This is probably due to the mechanism by which the storage temperature controls the melting temperature of the least stable recrystallized amylopectin crystal. At a particular storage temperature, only a certain amount of crystals of similar stability is formed. The

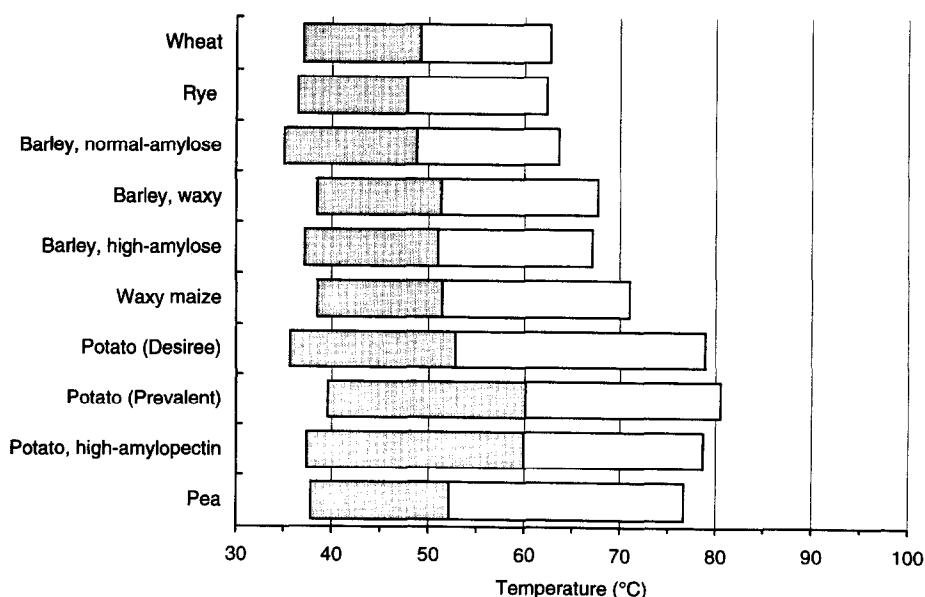


Fig. 7. Bars indicating the melting temperature ranges ($T_{o,4}$ to $T_{f,4}$) of recrystallized amylopectin after 4 days of storage at 6°C, border verifies the position of $T_{m,4}$.

$\Delta T_{m,4}$ were all lower than ΔT_{gel} for each of the 10 starches studied and varied between 1.7°C for potato Prevalent and 19.4°C for the waxy maize. This is all in accordance with the theory of predicting the behaviour of recrystallization and melting of fully water-plasticized starch gels (Slade and Levine, 1987).

Amylose–lipid complex

A transition endotherm due to the melting of the amylose–lipid complex was observed for the wheat, rye and three barley starches (Table 2). The highest transition enthalpy (ΔH_{cx}) was obtained for the high-amylose barley starch, while starches from the waxy barley and rye showed the lowest enthalpies. The melting endotherms of these cereal starches were related to the LAM content. It has been suggested that the retarding mechanism of lipids on starch retrogradation is connected to their ability of forming an inclusion complex with the amylose fraction (Krog and Nybo-Jensen, 1970). The striking results obtained in the present investigation, regarding the amylose–lipid complex transitions, were that the rye starch, showing the lowest ability to retrograde, had the lowest ΔH_{cx} value, while the high-amylose barley starch, having the highest LAM content, exhibited a high retrogradation capacity. Despite the traces of LAM detected in the starches from the waxy maize and the high-amylopectin potato, no melting endotherm was detected for these starches. This was also the case for the pea starch and the two remaining potato starches. The traces of LAM possibly present in these starches may partly explain their high retrogradation capability.

The peak minimum temperature (T_{cx}) for the melting transition ranged from 108.0 to 112.3°C.

Principal component analysis—overview of the material

PCA was used to visualise the variation in amylose and amylopectin characteristics as well as in gelatinization and retrogradation properties (Table 5). With this statistical method, a large number of variables are reduced to a few orthogonal variables called principal components (PC), which describe the greatest covariance in the data analysed. The first and the second PC, describing 56 and 18% of the variance, respectively, provided an overview of the starch samples. The three potato starches had high positive scores in PC1, whereas the cereal starches, except that from the waxy maize, had negative scores (Fig. 8a). The starches from rye, wheat, normal-amylose barley and high-amylose barley were found close to PC1, indicating little influence from PC2. The three high-amylopectin starches, had negative scores in PC2. The pea starch had a high positive score in PC2. The starches from pea, waxy barley and waxy maize were located close to zero in PC1.

The loading plot of the two first PC described 74% of the variance in the chemical and thermo-analytical variables (Fig. 8b). The variance explained by these two PC was

Table 5. The different variables examined with PCA

Variable	Description
FAM	Lipid-free amylose
LAM	Lipid-complexed amylose
AmI	Amylose content, iodine staining
AmG	Amylose content, GPC
ΔH_{gel}	Gelatinization enthalpy
$T_{o,gel}$	Onset temperature of gelatinization
$T_{m,gel}$	Peak min. temperature of gelatinization
$T_{f,gel}$	Offset temperature of gelatinization
ΔT_{gel}	Temperature interval of gelatinization
ΔH_2	Retrogradation enthalpy, 2 days at 6°C
$T_{o,2}$	Onset temperature of retrogradation endotherm, 2 days at 6°C
$T_{m,2}$	Peak min. temperature of retrogradation endotherm, 2 days at 6°C
$T_{f,2}$	Offset temperature of retrogradation endotherm, 2 days at 6°C
ΔT_2	Temperature interval of retrogradation endotherm, 2 days at 6°C
ΔH_4	Retrogradation enthalpy, 4 days at 6°C
$T_{o,4}$	Onset temperature of retrogradation endotherm, 4 days at 6°C
$T_{m,4}$	Peak min. temperature of retrogradation endotherm, 4 days at 6°C
$T_{f,4}$	Offset temperature of retrogradation endotherm, 4 days at 6°C
ΔT_4	Temperature interval of retrogradation endotherm, 4 days at 6°C
F1	Relative amylopectin unit-chain length distribution, fraction with longest chains
F2	Relative amylopectin unit-chain length distribution
F3	Relative amylopectin unit-chain length distribution
F4	Relative amylopectin unit-chain length distribution, fraction with shortest chains
DP _w	Average DP after debranching of the amylopectin, calculated on weight basis

above 60% for all variables, except for T_{gel} , $T_{f,gel}$, $T_{o,2}$ and $T_{o,4}$, indicating no correlation between the latter four variables and any other studied variable. Variables found close to each other in pairs or groups indicate a positive correlation. The group of variables describing \overline{DP}_w , the size of the two amylopectin fractions consisting of long unit-chains (F1 and F2), ΔH_2 , ΔH_4 , ΔT_2 , ΔT_4 , $T_{m,2}$, $T_{m,4}$, $T_{f,2}$ and $T_{f,4}$, as well as ΔH_{gel} , confirmed previous findings that a high \overline{DP}_w favours retrogradation (Orford et al., 1987; Kalichevsky et al., 1990). This relationship is illustrated by the correlation between the sum of the amylopectin fractions F1 and F2 and ΔH_4 in Fig. 9a. The loading plot also showed that \overline{DP}_w correlated more weakly to ΔH_{gel} than to ΔH_2 and ΔH_4 , even if the trend was the same. In addition, $T_{o,gel}$ and $T_{m,gel}$ were negatively correlated to the amylose content since these variables (AmG, AmI and FAM), were found on the opposite side of a diagonal (intersecting origo) in the plot. Fig. 9b shows the negative correlation between AmG and $T_{o,gel}$. Variables found in orthogonal directions, as indicated by the arrows in Fig. 8b, varied independently of each other. Thus the amylose content and the gelatinization temperatures ($T_{o,gel}$, $T_{m,gel}$) varied independently from variables describing the amylopectin unit-chain distribution, the LAM content and several of the gelatinization and retrogradation variables (Fig. 8b).

When comparing the score and loading plots (Fig. 8a,b),

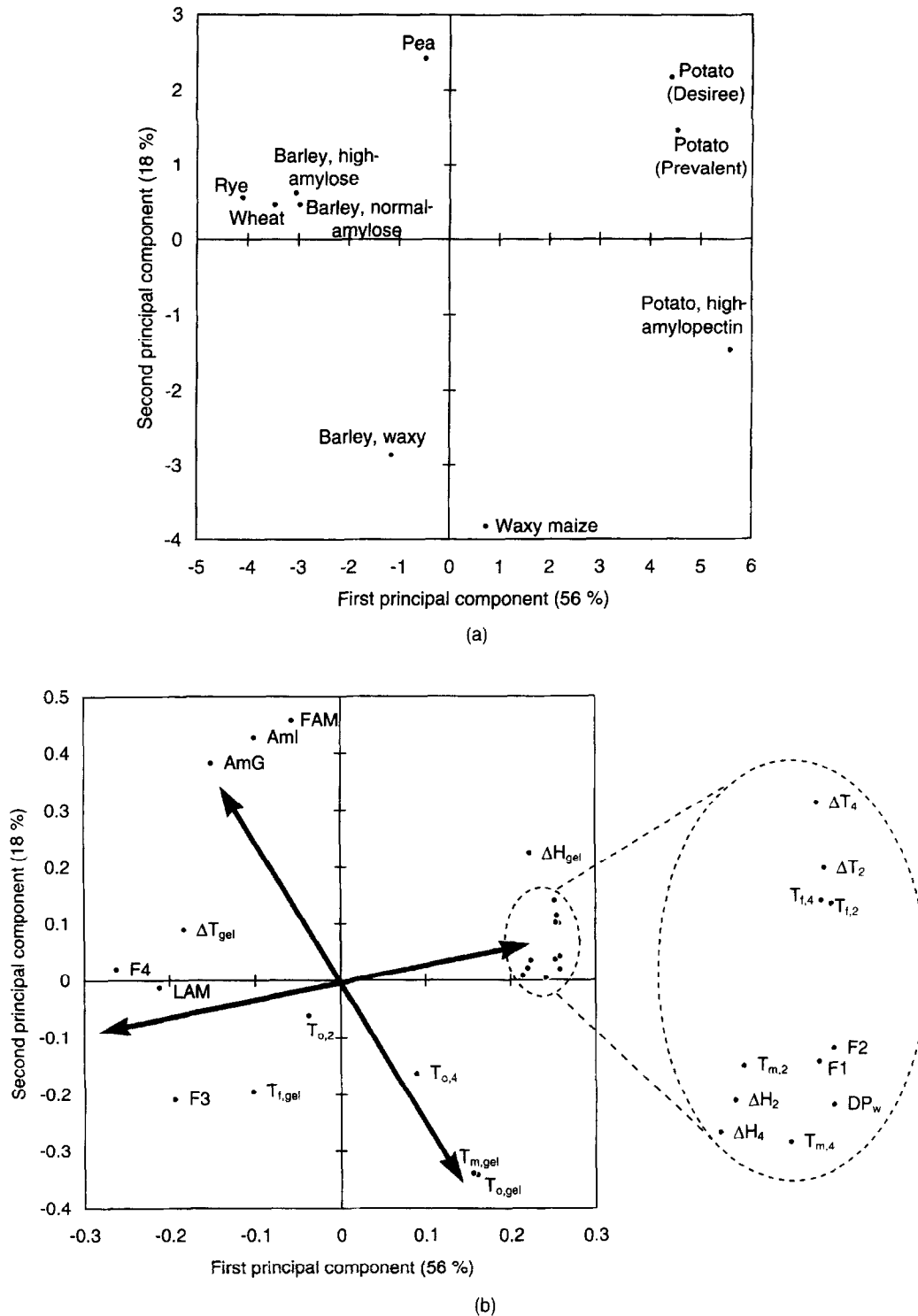


Fig. 8. (a) Score plot and (b) loading plot of the principal components 1 and 2, describing the variation in chemical and thermal properties of the 10 starch samples. Arrows indicate main trends in correlations between the different measured variables in the studied material

the three potato starches showed high \overline{DP}_w values, large proportions of fractions F1 and F2, as well as high values of ΔH_2 , ΔH_4 , ΔT_2 , ΔT_4 , $T_{m,2}$, $T_{m,4}$, $T_{f,2}$ and $T_{f,4}$ and ΔH_{gel} . On the other hand, the cereal starches with a normal or high amylose content, had high negative scores in PC1 because of large fractions of F3 and F4, the presence of LAM and low values of those thermo-analytical variables that were

high for the potato starches. The negative scores in PC2 for the waxy maize and waxy barley starches mainly corresponded to the absence of amylose and the high gelatinization temperatures. The pea and the waxy maize starches had scores close to zero in PC1, since the chain distribution of their amylopectin unit-chains was in between those of the potato and the other starches studied.

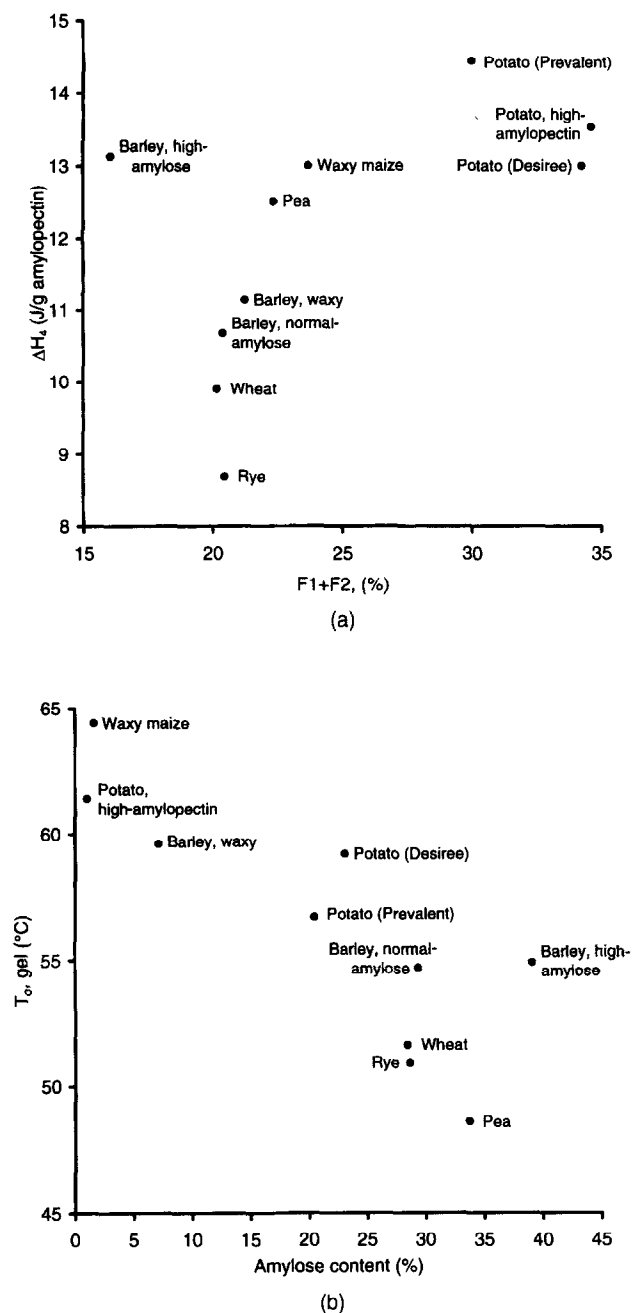


Fig. 9. (a) Retrogradation enthalpies (ΔH_4), after 4 days of storage at 6°C, as a function of the sum of amylopectin fractions F1 and F2. (b) Onset temperature of gelatinization (T_o) as a function of the amylose content (AmG)

Some of the gelatinization and retrogradation variables describe similar features but were found apart in the loading plot. The melting temperatures of native and retrograded starches both measure the quality and heterogeneity of the amylopectin crystals. For the retrograded starch, $T_{f,2}$ and $T_{f,4}$ were positively correlated to ΔT_2 and ΔT_4 , respectively, since $T_{o,2}$ and $T_{o,4}$ were at a similar level in all samples. A different pattern was found for native starch, where both $T_{o,gel}$ and $T_{f,gel}$ varied among the various starches, and their correlation to ΔT_{gel} was poor. Furthermore, the retrogradation enthalpies (ΔH_2 and ΔH_4) were positively

correlated to the melting intervals (ΔT_2 and ΔT_4), which was not the case during gelatinization. The main reason for these differences is probably, as already discussed in the starch retrogradation section, that the crystallization processes of biosynthesis and retrogradation are influenced by different factors. During retrogradation, the storage temperature was constant, whereas the biosynthetic pathway is far more complex and influenced by several other factors.

Although the results of the chemical analyses revealed a close resemblance in the amylopectin chain length distribution and the amylose content, the cereal starches (wheat, rye and barley) with a normal amylose content showed some individual variations, both in gelatinization and retrogradation properties. The differences in ΔH_{gel} were similar to previously published results (Kalichevsky et al., 1990; Radosta et al., 1991). One explanation of the variations in ΔH_{gel} could be the differences in LAM content, if the formation of the amylose-lipid complex causes a reduction in the endothermic heat flow (Eliasson, 1986). However, in the present study the lowest ΔH_{gel} value was obtained for the rye starch, although the LAM content of this starch was lower than that of the wheat and barley starches. The high-amylose barley had notably high retrogradation enthalpies, despite the presence of only a small proportion of long amylopectin unit-chains. With no anomalous starch material found, one possible explanation could be synergistic interactions between amylopectin and amylose at high amylose contents (Russell, 1987). Another reason could be the large proportion of the amylopectin fraction F4 (DP < 17.8). A contradictory observation was the high LAM content, which also ought to retard retrogradation. Apart from the differences in gelatinization temperatures, the high-amylopectin starches of barley and potato seemed to have thermal properties similar to those of their normal-amylose counterparts. Similar results have been reported previously for barley, maize (Morrison, 1995) and wheat (Yasui et al., 1996). In products and processes where the amylose may have detrimental effects, the high-amylopectin starches should be favoured.

CONCLUSION

The 10 starches studied provided valuable information about various starch properties and characteristics. Using PCA, it was possible to identify two main trends (indicated by arrows in Fig. 8b) and several correlations as well as differences in both chemical and thermal data. One of the trends observed was the correlation between the amylopectin unit-chain distribution and the thermo-analytical variables describing the retrogradation melting intervals, peak minimum and offset temperatures as well as the gelatinization and retrogradation enthalpies. The other trend was the negative correlation between the amylose content and the gelatinization onset and peak minimum temperatures of the starches. The fact that these two correlations were observed in almost orthogonal directions in the loading

plot indicated that they described two independent features of the starch samples.

The large sample diversity made evaluation rather complicated. In order to further elucidate and understand the links between the chemical and functional properties of starch, more knowledge is needed. The mode and location of branching need to be clarified, as well as the molecular weights of the amylose and amylopectin molecules in order to obtain a more complete understanding of the starch structure. In future investigations, new genotypes of existing starch-rich crops with a wide range of amylose/amylopectin ratios may provide useful materials to study.

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