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Carbohydrate Polymers 42 (2000) 175–184

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

The effect of temperature cycling on the amylopectin retrogradation of starches with different amylopectin unit-chain length distribution

J. Silverio[a], H. Fredriksson[b], R. Andersson[b], A.-C. Eliasson[b,*], P. Åman[b]

[a]*Department of Food Technology, Lund University, P.O. Box 124, S-221 00 Lund, Sweden*
[b]*Department of Food Science, Swedish University of Agricultural Sciences, P.O. Box 7051, S-750 07 Uppsala, Sweden*

Received 6 March 1998; received in revised form 5 July 1999; accepted 6 August 1999

Abstract

The amylopectin retrogradation of six cereal starches (wheat, rye, barley (normal-amylose, high-amylose and waxy), and waxy maize), three potato starches (two normal-amylose, and a high-amylopectin potato), and a single pea starch was studied by differential scanning calorimetry (DSC). The recrystallization of amylopectin was measured after 2 and 4 days of storage. In order to affect the crystallinity of the amylopectin, samples were exposed to different temperature cycles during the storage period, favouring the nucleation or the propagation of the crystallites. The temperature cycles during the first 2 days were: 24 h at 6°C (facilitating nucleation), followed by 24 h at either 30 or 40°C (promoting propagation). For the 4 days storage test the temperature cycles were repeated. Moreover, the amylopectin unit-chain length distribution of the starches was determined by high-performance anion exchange chromatography (HPAEC) after debranching of isolated amylopectins.

The recrystallization of amylopectin was greatly affected when gelatinized starch was treated with different time–temperature cycles. The melting enthalpy of recrystallized amylopectin (ΔH) decreased in most cases. The onset temperature of melting of recrystallized amylopectin (T_o) was controlled by the propagation temperature (T_p), and increasing the latter resulted in an increase in T_o. As a result of the increase in T_o, the melting range of the recrystallized amylopectin (ΔT_r) decreased. A development of crystallites that melted at higher temperatures, noticed as an increase in the offset temperature (T_f) of melting, was observed for the cereal starches after all temperature treatments. The possibility of predicting how a specific T_p would affect and increase the T_o of recrystallized amylopectin when gelatinized starch was treated with the two day cycles was demonstrated.

The DSC and HPAEC results showed that amylopectin unit-chains with DP 6 and the population of chains with DP 18–19 were positively correlated to ΔH, whereas the correlation to the population of chains of DP 8–11 was negative. Indications of a negative correlation between ΔH and chains of DP 22–34 as well as a positive correlation to chains with DP $>$ 40 were also found. The changes found in T_f correlated to the same distinct amylopectin populations as the previous ones for the relation to ΔH. However, the signs of the correlation coefficients were changed.

Keywords: Temperature cycling; Amylopectin retrogradation; Unit-chain length distribution

1. Introduction

The semicrystalline or partly crystalline nature of starch has now been long recognised (Katz, 1934; Slade & Levine, 1988; Zobel, Young & Rocca, 1988). Starch granule crystallinity is attributed to amylopectin, and it is rather poor, resulting in diffuse X-ray diffraction patterns (Zobel et al., 1988). The crystallinity of native starch can be improved, through certain combinations of time, temperature and moisture, known in the starch literature as annealing or heat–moisture treatments (Cameron & Donald, 1992). The crystallization of gelatinized starch molecules, i.e. retrogradation, has received considerable attention because of its relation to bread staling. In this respect amylopectin is of special interest, since the crystallization of amylose has already received much attention from nutritionists due to its relation to resistant starch (Berry, 1986; Eerlingen, Crombez & Delcour, 1993; Sievert & Pomeranz, 1989; Siljeström & Asp, 1985).

Starch retrogradation is a non-equilibrium thermoreversible recrystallization process which is governed by a consecutive three step mechanism of nucleation, propagation and maturation (Slade & Levine, 1987). Because nucleation and propagation is a liquid state event which requires orientational mobility of the polymer chains in the amylopectin molecule, the crystallization process can only occur in the temperature range between the glass transition temperature and the melting temperature, e.g. in the range of approximately

* Corresponding author.

0144-8617/00/$ - see front matter
PII: S0144-8617(99)00140-X

Table 1
The different temperature cycles used during the two and four days of retrogradation

Temperature cycle (°C)	Storage temperature day 1 (°C)	Storage temperature day 2 (°C)	Storage temperature day 3 (°C)	Storage temperature day 4 (°C)
6/6	6	6	–	–
6/30	6	30	–	–
6/306/30	6	30	6	30
6/40	6	40	–	–
6/40/6/40	6	40	6	40

−5.0 and 60°C for a starch gel containing 50% water. The glass transition temperature for fully gelatinized and hydrated wheat starch (55% water) has been given as −5°C (Levine & Slade, 1990), and the onset of melting of recrystallized wheat starch at a water content of ≈50%, and stored at room temperature is below 50°C (Zeleznak & Hoseney, 1987). The kinetics of starch retrogradation exhibit a strong temperature dependence because the nucleation rate increases exponentially with decreasing temperature down to the glass transition temperature, while the propagation rate increases exponentially with increasing temperature up to the melting temperature. In white bread crumb the greatest extent of staling was attained by nucleation at 0°C followed by propagation at 40°C, and the extent of nucleation and overall crystallization in wheat starch–water mixtures (1:1) increased with increasing time of nucleation (Slade & Levine, 1987). Heating to temperatures within or above the gelatinization temperature range has been shown to influence the extent of the subsequent retrogradation (Fisher & Thompson, 1997). Although annealing treatments have been used to change the crystallinity of native starch, such treatments have not been used for retrograded starch, although the possibility has been pointed out (Slade & Levine, 1987).

The degree or extent of retrogradation also depends on the botanical source (Kalichevsky, Orford & Ring, 1990; Orford, Ring, Carroll, Miles & Morris, 1987; Silverio, Svensson, Eliasson & Olofsson, 1996). In general, cereal amylopectin retrograde to a lesser extent than pea and potato amylopectin, which has been attributed to the shorter average chain lengths in the cereal amylopectin (Fredriksson, Silverio, Andersson, Eliasson & Åman, 1998; Kalichevsky et al., 1990; Orford et al., 1987). A method for analysis of the amylopectin unit chain length distribution that provides individual peaks for linear (1→4)-α-D-glucans with a degree of polymerization (DP) between 6 and 60 is high-performance anion exchange chromatography (HPAEC) with a pulsed amperometric detector (PAD). Such systems have been used to detect differences in amylopectins with different retrogradation behaviour (cf. Shi & Seib, 1992; Shi & Seib, 1995; Shi, Seib & Bernardin, 1994; Suzuki, Kaneyama, Shibanuma, Takeda, Abe & Hizukuri, 1992; Ward, Hoseney & Seib, 1994), and was used in the present study.

Physico-chemical properties of starch from wheat, rye, barley (waxy, high-amylose, normal-amylose), waxy maize, pea and potato (normal-amylose, high-amylopectin) have been previously reported (Fredriksson et al., 1998). In the present study the possibility of increasing amylopectin recrystallization was investigated for these starches. Temperature cyclings were done in order to obtain growth of the crystalline regions, perfection of crystallites and/or possibly a change to a more stable crystal structure (Wunderlich, 1976). The amylopectin crystallization was correlated to the amylopectin unit chain lengths.

2. Materials and methods

2.1. Starches

Ten starches from different botanical sources were studied: six cereal starches (wheat, rye, normal-amylose barley, waxy barley, high-amylose barley and waxy maize), three potato starches (normal-amylose from cultivar Prevalent and Desiree and high-amylopectin potato, PAP) and a single pea starch. The material and the isolation procedure of amylopectin was previously described by Fredriksson et al. (1998).

2.2. DSC measurements

The phase transition of the starch due to the melting of recrystallized amylopectin was investigated with a Perkin–Elmer DSC 2c (starch from wheat, rye, normal-amylose barley, waxy barley, high-amylose barley, potato Desiree and pea) and a DSC 6200 from Seiko Instruments Inc. (starch from waxy maize, high-amylopectin potato and potato Prevalent). The performances of the calorimeters were checked with reference substances to ensure comparable results. Sample preparations were essentially performed as described by Fredriksson et al. (1998). The water content was approximately 50% (w/w) for all samples studied, and 5–10 mg of starch was analysed during each DSC scan. The sample pans were heated in an oven for 15 min at 105°C and then stored for two or four days. During the storage the samples were exposed to different temperature conditions according to the scheme in Table 1. The storage time at each temperature was one day (24 h). The effect of these temperature cyclings was compared to the

results after two days of storage at 6°C (reference treatment 6/6°C) (Fredriksson et al., 1998). All samples were analysed at 17–127°C, with a heating rate of 10°C/min using an empty aluminium pan as a reference.

The DSC results given are the average of at least two measurements. The enthalpies and melting temperatures are all within at least 7 and 2% of the given values, respectively. The DSC-endotherm related to starch retrogradation was evaluated by determining the onset temperature (T_o), the peak temperature (T_m), and the offset temperature (T_f) of melting of recrystallized amylopectin, the interval of melting of recrystallized amylopectin ($\Delta T_r = T_f - T_o$) and the enthalpy of melting of recrystallized amylopectin (ΔH). The maximum storage temperature in each cycle, i.e. 30 or 40°C, was referred to as the propagation temperature (T_p). When the influence of T_p on the retrogradation of amylopectin was studied T_{m1} was used to denote the onset temperature of melting of crystallites in the untreated reference sample (i.e. T_{m1} was taken as T_o for the 6/6°C treatment when the 6/30°C treatment was analysed).

2.3. Characterization of amylopectin

Isolated amylopectin samples were debranched with isoamylase, and analysed using HPAEC (Fredriksson, Andersson, Koch & Åman, 1997). The detector response of the PAD is not quantitative with respect to carbohydrate content. Therefore the individual peaks of (1→4)-α-D-glucans in the amylopectin chromatograms were corrected for by their relative molar PAD response (Koch, Andersson & Åman, 1998). The amounts of chains between DP 6–56 in each chromatogram were normalised to an equal sum.

2.4. Statistical analysis

Analysis of linear regression was performed by using Microsoft Excel 7.0. Correlation coefficients (r) were calculated using Matlab (The Mathworks, Inc., Natick, MA, USA).

3. Results

3.1. Starch retrogradation

3.1.1. DSC-thermograms

The DSC-thermograms of the various gelatinized starch–water mixtures, after storage for two and four days, exhibited the expected retrogradation endotherm in the temperature range between 30–90°C caused by the melting of recrystallized amylopectin (Fig. 1). The two- and four-step temperature cycling of the gelatinized starches thus resulted in bell shaped retrogradation endotherms. This was also the case for the rye, wheat, normal- and high-amylose barley starches stored for two or four days at 6°C, whereas the endotherms of the potato, pea, waxy maize and waxy barley starches were bimodal (Fredriksson et al., 1998). The thermograms of rye starch after treatments 6/30/6/30°C and 6/40/6/40°C are compared with the thermogram obtained after four days of storage at 6°C in Fig. 1a. The corresponding thermograms of wheat and barley were very similar. The thermograms of potato starch Desiree were typical for all three potato starches (Fig. 1b). Thermograms of the pea starch are shown in Fig. 1c.

The thermograms of waxy barley, high-amylose barley and waxy maize were very similar to the ones of the other cereal starches after treatment with the temperature cycles. The features of the endotherms after treatments 6/30°C and 6/40°C were comparable to the endotherms after the 6/30/6/30°C and 6/40/6/40°C treatments, respectively.

3.1.2. Effects on melting temperature

The different time–temperature cyclings caused a general increase in the onset temperature of melting of the recrystallized amylopectin (T_o) compared with the values obtained after two days of storage at 6°C (Table 2). After treatment 6/30°C and 6/40°C the increase was highest for the potato Desirée (13.6 and 24.0°C, respectively) and normal-amylose barley (13.2 and 22.4°C, respectively), and lowest for pea (7.8 and 17.3°C, respectively) and wheat (8.0 and 17.5°C, respectively). These starches also had the lowest and highest T_o temperatures, respectively, after two days of storage at 6°C. A second cycle (i.e. the total treatments 6/30/6/30°C and 6/40/6/40°C) resulted in, at the most, an increase of 1.6°C in T_o for high-amylopectin potato after treatment 6/40/6/40°C. The cycled starches thus showed more similar T_o temperatures, compared with two days storage at 6°C.

Due to the strong positive correlation between T_o and T_m (Fredriksson et al., 1997), the peak temperature of the retrogradation endotherm was affected in a similar way as T_o, and an increase was obtained after the temperature cycling (Fig. 1). The potato starches showed the highest T_m, the cereal starches the lowest and the pea starch had an intermediate T_m-value, regardless of the treatment.

The offset temperature of the retrogradation endotherm (T_f) concurs with the temperature where the most stable amylopectin crystallites formed during the temperature cycles melt. T_f for the cereal starches were lowest (62.5–70.7°C), highest for the potato starches (78.2–79.3°C), and intermediate for the pea starch (74.8°C) after the 6/6°C treatment (Table 2).

Small changes in T_f were generally observed for the three potato and the pea starches when treated with the four different temperature cycles (Table 2). An increase in T_f was, on the contrary, observed for wheat, rye and normal-amylose barley after all temperature treatments (Table 2), with the largest increase after treatment 6/40/6/40°C (5.5–7.1°C). For waxy barley, high-amylose barley and waxy maize a smaller increase in T_f (2.4–3.9°C) was found after treatment 6/40/6/40°C.

A distinct reduction in the melting range of the recrystallized amylopectin (ΔT_r) was thus noticed during temperature cycling compared to the non-cycled samples (Table 2 and

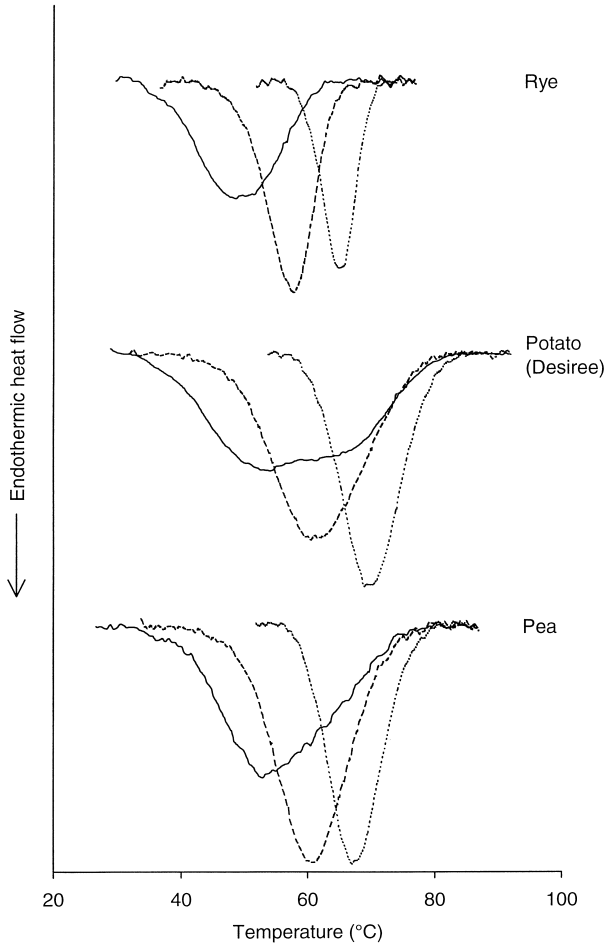

Fig. 1. The retorgradatin related endotherms after treatments 6/6°C (—), 6/30/6/30°C (– – –) and 6/40/6/40°C (…) of rye starch, potato starch (Desiree) and pea starch.

Fig. 1). The melting range for the starches after two days at 6°C was broad; the cereal starches were in the range 22.9–32.9°C, ΔT_r of the pea starch was 34.2°C, and the potato starches exhibited a melting range of 40.2–43.0°C. After treatment 6/30/6/30°C ΔT_r was in the range 15.3–20.0°C for the cereal starches, 26.4–28.4°C for the potato starches, and 23.2°C for the pea starch. After treatment 6/40/6/40°C the corresponding range was, for cereal starches 10.8–14.1°C, for potato starches 18.8–19.1°C and for pea starch 17.3°C. The longer treatment (e.g. 6/30/6/30°C compared with 6/30°C) did not result in a narrower ΔT_r, but the increase in temperature (from 6 to 30°C, and from 30 to 40°C) did.

3.1.3. The effects on ΔH

The retrogradation enthalpy is often expressed on starch basis, however, as the information obtained during the DSC scans in the range 30–90°C only reflected the melting of recrystallized amylopectin, and the different temperature cyclings were intended to affect this starch component, so it was appropriate to express the transition enthalpy on amylopectin basis. These calculations were based on the amylopectin contents previously reported from gel permeation chromatography by Fredriksson et al. (1998). The retrogradation enthalpies for the starches after the two and four step temperature cyclings are shown in Table 3. All cereal starches, except for the waxy maize starch, but especially the wheat and rye starch, exhibited low retrogradation enthalpies compared to the pea and potato starches. Regardless of treatment the cereal starches were ranked in the same order of increasing ΔH: rye < wheat < normal-amylose barley < waxy barley < high-amylose barley. The highest retrogradation enthalpies were obtained for the potato starches, and relatively high enthalpies were also found for the pea starch. The order in ΔH-values for the groups of starches with similar X-ray diffraction pattern (i.e. A—pattern low, B—pattern high, and C—pattern intermediate ΔH-values) was thus the same (except the waxy maize whose level of retrogradation was more similar to the pea starch), independently of the treatment. The level of retrogradation was, on the contrary, related to the treatment.

A second cycle (i.e. treatments 6/30/6/30 or 6/40/6/40°C) increased ΔH, whereas the increase in propagation temperature (from 30 or 40°C) resulted in a decrease (Table 3). An overall increase in ΔH compared to the treatment at 6°C was not obtained for the cycled samples except in a few cases. For the wheat, rye, waxy maize, high-amylopectin potato and pea starches a small increase in retrogradation enthalpy was observed after the 6/30/6/30 treatment compared to the 6/6°C treatment. All other treatments resulted in a decrease in ΔH compared to two days at 6°C.

3.2. Amylopectin unit chain length distribution

The chain length distribution of the amylopectins was revealed by HPAEC-PAD after the debranching of isolated amylopectins using isoamylase (Fig. 2). In the low molecular material, the cereal starches had the smallest proportions of chains with DP 6, and the same observation was made for chains with DP 8 in the pea and potato amylopectins. All potato amylopectins had a minimum at DP 8, however, within this group the distribution pattern differed slightly, and the amylopectin from potato Desiree had somewhat smaller proportions of chains with DP 6 than the high-amylopectin potato and potato Prevalent. The cereal amylopectins of wheat, rye and barley showed very similar distribution patterns with peaks at DP 12 and 15, a peak or shoulder at DP 19–20 and a larger proportion of material around DP 45. The distribution profiles of the waxy maize and pea amylopectin both had the highest peak at DP 16 and a second peak around DP 45 that was less distinct. The two potato amylopectins of Desiree and Prevalent had rather similar profiles with a peak at DP 14, a shoulder at DP 19 and a second peak around DP 48–49. The high-amylopectin potato, on the other hand, had the highest peak at DP 16, no shoulder at DP 19 and a peak around DP 48.

Table 2
The onset (T_o) and final melting (T_f) temperature of recrystallized amylopectin after treatment with different time–temperature cycles

Starch	Time–temperature treatment ($T_o - T_f$ (°C))				
	6/6°C	6/30°C	6/30/6/30°C	6/40°C	6/40/6/40°C
Wheat	40.4–63.3	48.5–64.7	49.3–65.4	58.0–68.5	58.2–69.0
Rye	37.8–62.5	48.3–63.5	49.2–64.5	57.8–68.1	58.8–69.6
Barley	35.6–65.1	48.8–65.8	49.0–65.6	58.0–69.0	59.0–70.6
High-amylose barley	37.6–68.6	48.7–67.0	49.7–67.6	58.2–70.4	59.5–71.9
Waxy barley	40.0–67.7	48.8–67.1	49.7–67.1	58.4–70.4	59.4–71.6
Waxy maize	37.8–70.7	48.9–69.1	49.8–69.8	57.6–71.9	59.0–73.1
Potato(Desiree)	35.2–78.2	48.8–78.3	49.7–78.1	59.2–78.3	60.7–79.8
Potato(Prevalent)	38.2–78.9	50.3–78.0	51.8–78.2	59.4–78.9	60.7–79.5
High-amylopectin potato	39.1–79.3	49.9–77.9	51.3–78.0	58.8–78.9	60.4–79.4
Pea	40.6–74.8	48.6–72.8	49.7–72.9	58.1–75.5	59.0–76.3

3.3. Correlation between amylopectin composition and retrogradation

In the present study the relationships between amylopectin composition and retrogradation for the cycled and uncycled starches were evaluated. The correlation coefficients between the relative amount of individual amylopectin unit-chains (DP 6–56) and ΔH after the temperature treatments were calculated (Fig. 3). The correlations observed were very similar for all treatments. In this material, the proportions of chains with DP 6 and the population of chains with DP 18–19 were positively correlated to ΔH, whereas the correlation to the population of chains of DP 8–11 was negative. The calculations also indicated a negative correlation between ΔH and chains of DP 22–34 as well as a positive correlation to chains with DP $>$ 40.

In order to further evaluate the relation between amylopectin composition and retrogradation behaviour, the correlation coefficients between unit-chains and changes in T_f (i.e. ΔT_f) after cycling were calculated in the same manner as for ΔH (Fig. 4). ΔT_f was taken as the difference between T_f of the 6/30°C, 6/40°C, 6/30/6/30°C, 6/40/6/40°C treatments, respectively, and T_f of the 6/6°C treatment (e.g. $T_{f(6/40/6/40)} - T_{f(6/6)}$). The amylopectin unit-chains correlating to the changes in T_f were found in the same populations as those obtained in the previous calculations of the relation to ΔH. However, the signs of the correlation coefficients were changed. The strongest correlation found was positive and included the population of chains with DP 8–10. The negative correlation for chains with DP around 18–19 was weaker. The correlations between ΔT_f and chains with DP 22–37, and DP $>$ 40 increased with propagation temperature, although they were less significant, especially for the temperature cycles with the lower propagation temperature. The time of storage seemed to have less effect on the correlations between ΔT_f and DP.

4. Discussion

When the temperature effects on starch retrogradation are discussed it is important to keep in mind that all starches were kept at the same propagation temperature (T_p), i.e. the temperature difference between T_p and the onset of melting (T_{m1}) of the least stable crystallites formed during retrogradation differ between the starches, as the starches differ in their T_o values. It has been shown that the temperature difference between annealing temperature and onset temperature of starch gelatinization is important for annealing effects on native starches (Larsson & Eliasson, 1991).

Table 3
The retrogradation enthalpies obtained after treating starch with different time–temperature cycles

Starch	Time–temperature treatment (ΔH (J/g AP))				
	6.6°C	6/30°C	6/30/6/30°C	6/40°C	6/40/6/40°C
Wheat	8.1	7.7	9.4	4.5	5.9
Rye	7.3	6.4	8.0	4.3	5.0
Barley	10.3	8.2	9.5	5.2	6.8
High-amylose barley	12.5	11.1	11.8	8.2	9.7
Waxy barley	10.6	10.0	10.4	7.3	8.2
Waxy maize	12.3	12.2	13.2	10.0	10.8
Potato (Desiree)	13.3	11.7	13.1	9.9	10.6
Potato (Prevalent)	13.6	13.2	13.6	11.6	12.6
High-amylopectin potato	13.6	13.5	13.9	11.7	12.3
Pea	12.5	11.8	13.6	8.9	9.7

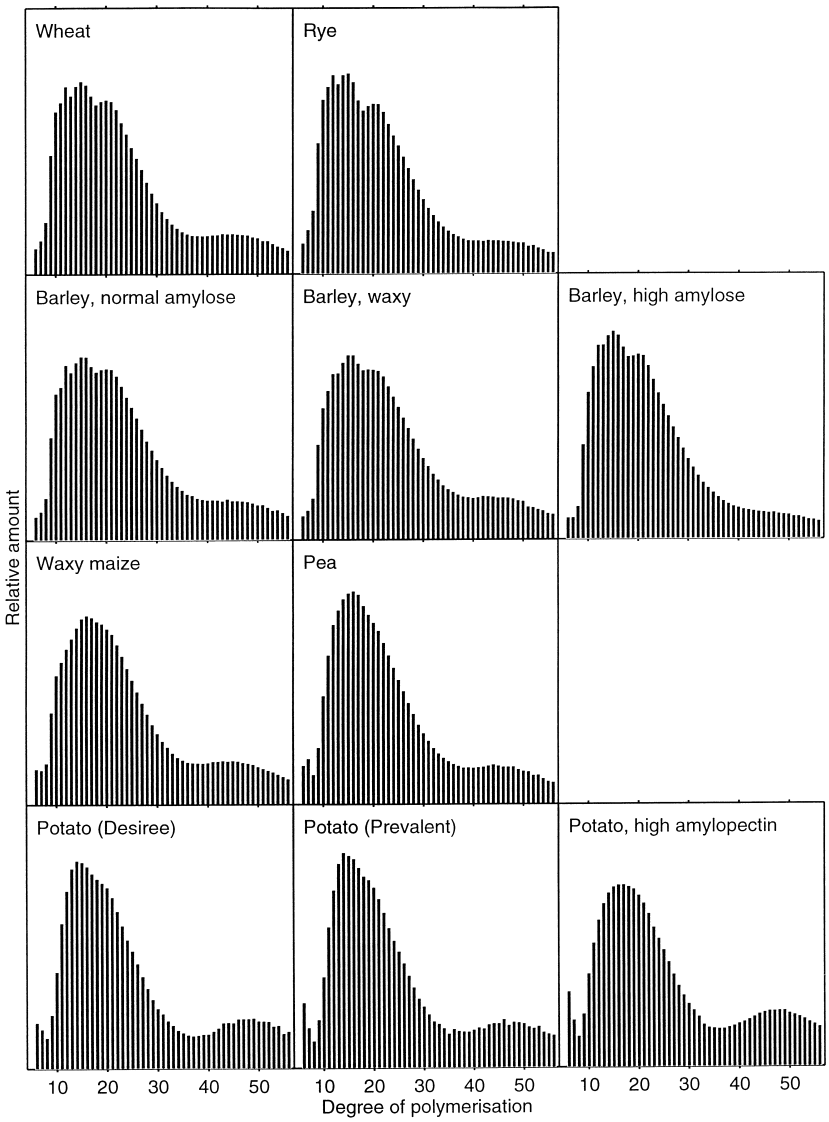

Fig. 2. Amylopectin unit-chain length distribution of the ten starches by high-performance anion-exchange chromatography.

The increase in T_o (ΔT_o) obtained in the present study was calculated as a function of $T_{m1} - T_p$ for the treatments 6/30 and 6/40°C, and is plotted in Fig. 5. The T_o values for all the samples treated for two days at 6°C were taken as T_{m1} for treatments. Linear regression analysis resulted in the equation

$$\Delta T_o = 18.5 - 0.955(T_{m1} - T_p) \qquad (R^2 = 0.985) \qquad (1)$$

This equation shows that with a propagation temperature coinciding with T_{m1} the T_o can be increased with only 18.5°C. It is interesting to note that all starches at both propagation temperatures follow the Eq. (1). For a larger increase in T_o the propagation temperature thus has to be above T_{m1}. This was in fact the situation in several of the treatments here, especially for $T_p = 40$°C.

If gelatinized starch was kept at a temperature 18.5°C below the onset of melting of retrogradation neither the melting temperature nor ΔH would be affected. On the contrary, if the gelatinized starch was kept at a temperature corresponding to the melting temperature an increase in the subsequent melting temperature of 18.5°C would be expected. These results were independent of the type of starch investigated. To increase the quality of crystalline amylopectin, which could possibly contribute to "resistant starch", gelatinized starch should be kept at the onset temperature of melting of amylopectin.

The calculations above were repeated for the treatments 6/30/6/30 and 6/40/6/40°C (with $T_{m1} = T_o$ after 6/6°C), and resulted in the following equation

$$\Delta T_o = 19.6 - 0.964(T_{m1} - T_p) \qquad (R^2 = 0.973) \qquad (2)$$

It could be debated, regarding the calculations of Eq. (2), which values are valid to be assigned to T_{m1}, since the T_o after 6/6°C does not correspond to T_{m1} after the first cycle in

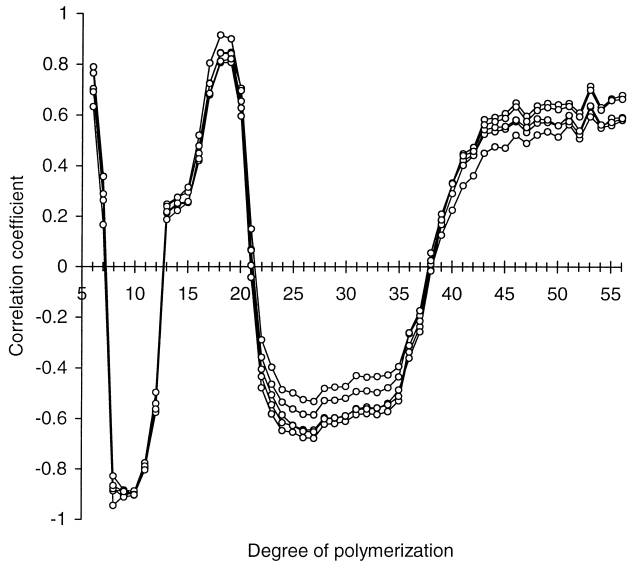

Fig. 3. The correlation coefficients between the ΔH-values after treatment 6/6, 6/30, 6/40, 6/30/6/30, and 6/40/6/40°C and the amount of amylopectin unit-chains with DP6–56.

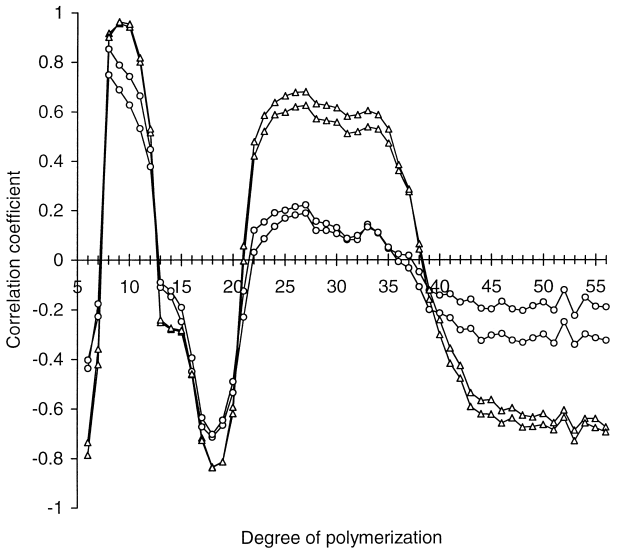

Fig. 4. The correlation coefficients between the changes in T_f, taken as the difference between T_f for treatments 6/6 and 6/30, 6/40, 6/30/6/30, or 6/40/6/40°C, respectively, and the amount of amylopectin unit-chains with DP 6–56. $T_p = 30$°C (○), $T_p = 40$°C (△).

the treatment, as T_o has evidently increased after treatment with the first cycle (i.e. after the treatments 6/30 and 6/40°C). The increase in T_o after further treatment with the second cycles (i.e. after treatments 6/30/6/30 and 6/40/6/40°C) was only 0.2–1.6°C, and applying Eq. (1) with these two ΔT_o-values indicated that the temperature difference $T_{m1} - T_p$ was in the range 17.7–19.2°C. An alternative could be using the T_o-values after the 6/30 and 6/40°C treatments as T_{m1}. The temperature range $T_{m1} - T_p$ is then equivalent to 17.6–20.3°C, which reasonably corresponds with the temperature range obtained from the previous calculations using Eq. (1). If ΔT_o is calculated as the difference between T_o after treatments for two days and T_o after treatment for four days (i.e. $\Delta T_o = T_{o(6/30/6/30)} - T_{o(6/30)}$ and $\Delta T_o = T_{o(6/40/6/40)} - T_{o(6/40)}$), and if the same assumption is made as for the calculations of Eq. (2) that $T_{m1} - T_p = T_{c(6/6)} - T_p$, the correlation between ΔT and $T_{m1} - T_p$ was very poor ($\Delta T_o = 1.091 - 0.016(T_{m1} - T_p)$, $r = 0.228$). It thus seems that the second cycle with an additional storage period the third day at 6°C did not result in new nucleation seeds.

The onset temperature of the retrogradation endotherm appeared to become more uniform when the starches were treated with the different temperature cycles during storage (Table 2). This was an indication that, even though the overall crystallinity was reduced in most cases during the temperature cycling, the remaining amylopectin crystallites were of a better quality, and that the storage temperature during the propagation step (i.e. the second and fourth day) influenced the melting temperature of the least stable amylopectin crystallites. Also the increase in T_f for the cereal starches suggested that during the temperature cycling a more stable kind of amylopectin crystallite was formed. This could possibly be regarded as an annealing effect, where the growth of more stable crystallites can occur at the expense of the less stable ones. A decrease in ΔT_r was observed and can be interpreted as a shift from a heterogeneous set of amylopectin crystals with varying stability to a more homogenous set with similar stability.

The amylopectin unit-chain length distribution was studied by HPAEC, after debranching of the isolated amylopectins. All cereal amylopectins, except for that of waxy maize, had similar distribution profiles. The amylopectin from waxy maize, on the contrary, had a profile rather similar to that of the pea. Within the potato group, the main differences observed were the small proportions of chains with DP 6 in potato Desiree and, in the high-amylopectin potato, the lack of a shoulder around DP 19. Compared to previously reported results of this material from high-performance size-exclusion chromatography (Fredriksson et al., 1998) the main differences were the more detailed data obtained in the low molecular weight material area and the lack of information of chains with a DP above 56. For amylopectin chains with DP < 56, HPAEC turned out to be a useful tool for identification of certain amylopectin unit-chains or populations of importance for retrogradation. However, as this type of analysis is based on decomposing the amylopectin molecule structural information such as branch point positions are lost.

It is well documented that the amylopectin unit-chain length distribution of a starch affects its retrogradation behaviour (cf. Fredriksson et al., 1998; Kalichevsky et al., 1990; Lu, Chen & Lii, 1997; Shi & Seib, 1992; Shi et al., 1994; Suzuki et al., 1992; Ward et al., 1994). In the present study the influence of the composition of amylopectin on the retrogradation behaviour was evaluated (Figs. 3 and 4). The chain length distribution patterns for chains between

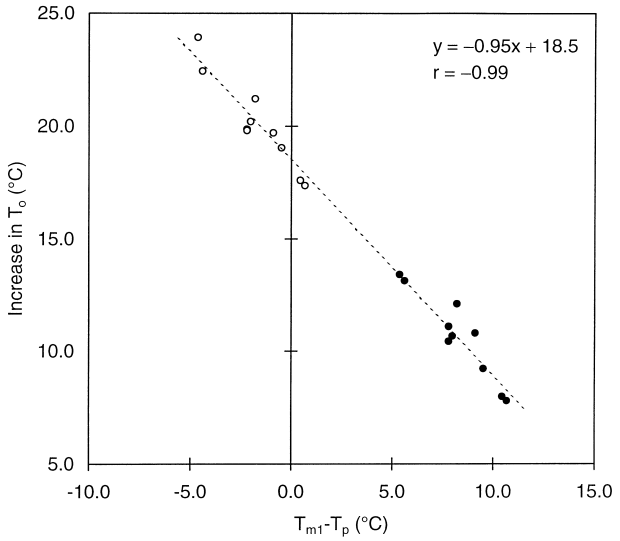

Fig. 5. The increase in T_o after treating starch with cycles 6/30°C (●) and 6/40°C (○) as a function of the difference between the onset onset temperature of melting after the 6/6°C treatment ($T_{m1} = T_{o(6/6)}$) and the propagation temperature (T_p).

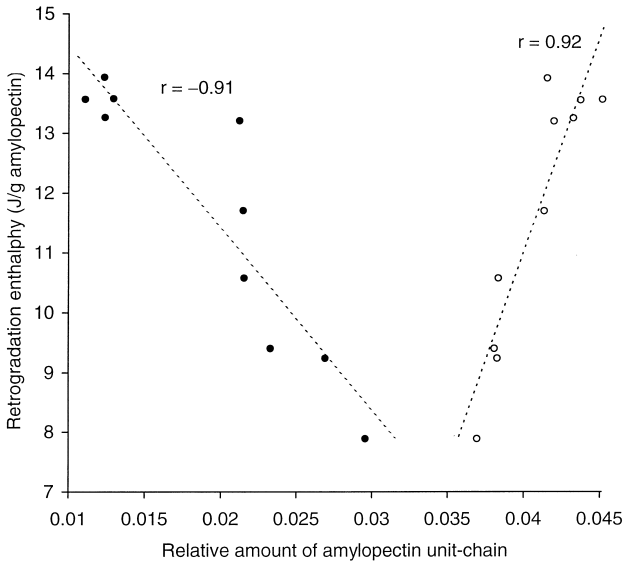

Fig. 6. The ΔH after treatment 6/30/6/30°C as a function of the relative amount of amylopectin unit-chains with DP 9 (●) and DP 18 (○), which showed the best correlations as indicated in Fig. 3.

DP 6 and 9 seem to be characteristic for a species (Hanashiro, Abe & Hizukuri, 1996; Koizumi & Fukuda, 1991) and, in general, the cereal amylopectins had the smallest proportions of chains with DP 6, the pea intermediate and the potato the largest. This was the same relative ranking as that obtained for the average chain length (DPw) reported by Fredriksson et al. (1998) which altogether probably had a stronger impact on the retrogradation than the presence of the short amylopectin chains.

From the correlations in Fig. 3 the amylopectin unit-chains could be sorted into distinct groups with either positive or negative correlation to ΔH, and only a few chains were difficult to classify. The presence of these distinct populations indicates that the different starches have a biosynthetical feature in common. The relationships between ΔH after treatment 6/30/6/30°C and the level of amylopectin chains with DP 9 and DP 18 are illustrated in Fig. 6. The cereal amylopectins had the highest proportion of chains with DP 8–11, and, consequently, those chains probably decreased the extent of retrogradation more than the same fraction in the potato and pea amylopectin did. According to Ring et al. (1987), amylopectin chains with less than 15 glucose units do not take part in the recrystallization process which may explain the negative correlation between the level of chains with DP 9 (Fig. 6), or, as shown in Fig. 3, in fact the whole population of chains with DP around 9. These potato and pea starches had the highest levels of amylopectin chains with DP 19 that most likely, in contrast to the cereal starches with lower levels of these chains, contributed to retrogradation to a larger extent. The effects on retrogradation of chains with DP $>$ 20 were less clear even if indications of a negative correlation to chains with DP 22–34 and a positive correlation to chains with DP above 40 were observed. The result differed from those obtained by Lu et al. (1997) and Shi and Seib (1992) indicating a negative correlation between the level of chains with DP 6–9 and the extent of retrogradation. Further, the level of amylopectin chains with DP 14–24 (Shi & Seib, 1992) and, 16–30 in another study (Shi & Seib, 1995), has been shown to increase retrogradation.

The correlation between the changes in T_f and the individual peak-areas of the amylopectin unit-chains was also calculated (Fig. 4). This result gave an indication on how different amylopectin unit chains influenced T_f. The cereal starches that showed the largest increase in T_f had high levels of chains with DP around 8–10 (Fig. 7). Starches with high levels of chains with DP 18, i.e. the three potato and the pea starches, had little influence on the changes in T_f.

5. Summary

The recrystallization of amylopectin was greatly affected when gelatinized starch was treated with different time–temperature cycles, i.e. two or four storage periods of 24 h at alternating temperatures favouring the nucleation or the propagation of crystallites. A smaller amount of recrystallized amylopectin was, in most cases, obtained after treatment with the time–temperature cycles. Although the amount of crystallites decreased the remaining crystallites were of a more homogenous and temperature stable character. The onset temperature of melting of recrystallized amylopectin (T_o) was controlled by the propagation temperature (T_p), and increasing the latter resulted in an increase in T_o. As a result of the increase in T_o, the melting

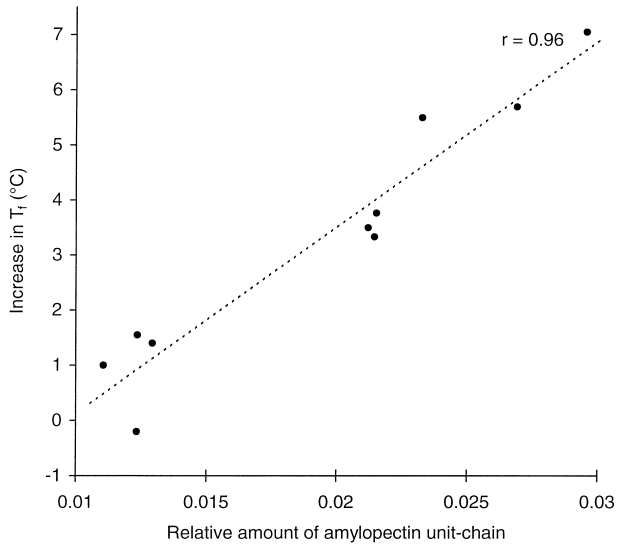

Fig. 7. The difference in T_f after the 6/40/6/40 and 6/6°C treatments as a function of the relative amount of amylopectin unit-chains with DP 9, which showed the best correlation according to Fig. 4.

range of the recrystallized amylopectin decreased when the starch was exposed to the various treatments, meaning that a more homogenous set of crystallites was obtained. The cereal starches showed a development of crystallites that melted at higher temperatures when treated with the temperature cycles. This could possibly be regarded as an annealing effect, where the growth of more stable crystallites could occur at the expense of the less stable ones. It was also possible to predict how a specific T_p would increase the T_o when the starch was treated with the two day cycles, if the T_o of the recrystallized amylopectin after 2 days storage at the nucleation temperature is known.

It was revealed that distinct amylopectin populations with accurately defined chain-lengths correlated to the retrogradation enthalpies of the amylopectin obtained after temperature cycling. The study showed that amylopectin unit-chains with DP 6 and the population of chains with DP 18–19 were positively correlated to ΔH, whereas the correlation to the population of chains of DP 8–11 was negative. Moreover, indications of a negative correlation between ΔH and chains of DP 22–34 as well as a positive correlation to chains with DP $>$ 40 were found. The changes found in T_f correlated to the same distinct amylopectin populations as the previous ones for the relation to ΔH. However, the signs of the correlation coefficients were changed.

Acknowledgements

Financial support was received from the Cerealia Foundation R and D, Lyckeby-Stärkelsen, Procordia Food AB, the Swedish Farmer's Foundation for Agricultural Research (Stiftelsen lantbruksforskning), the Swedish Council for Forestry and Agricultural Research (SJFR), Wasabröd AB and the SL Foundation (Skånska Lantmännen).

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