ELSEVIER

Carbohydrate Polymers 43 (2000) 81–87

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

# Studies on α-amylase degradation of retrograded starch gels from waxy maize and high-amylopectin potato

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

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

Accepted 13 October 1999

**Abstract**

Gelatinized starch gels of waxy maize and high-amylopectin potato were subjected to different time–temperature conditions, aiming at producing extensive amounts of retrograded amylopectin. The purpose was to investigate the impact of amylopectin retrogradation on the resistant starch formation and on the rate of starch digestion with porcine pancreatic α-amylase. Dried non-cycled gelatinized starch gels were used for comparison. Although differential scanning calorimetry measurements indicated higher amounts of retrograded material in the dried temperature-cycled gels no enzyme resistant starch was detected in any of these samples. However, all cycled starch gels were less-readily degraded by the enzyme than the non-cycled gels. The chain length distribution in the α-amylolysates was studied by high performance anion exchange chromatography after debranching with isoamylase. The main products of hydrolysis were low molecular weight carbohydrates with a degree of polymerization of 1–9 and various branched dextrins. The chain distribution was uniform in the hydrolysates at the different stages of hydrolysis and independent of storage conditions, indicating that the mode of enzyme action remained unaffected by retrogradation. The waxy maize and high-amylopectin potato starches responded similarly to temperature cycling. It was concluded that temperature cycling resulted in a slower hydrolysis of the amylopectin, a phenomena that could be exploited when developing starchy foods with improved nutritional characteristics. 

*Keywords*: α-amylase; Degradation; Amylopectin; Retrogradation

## 1. Introduction

Gelatinized starch gels are thermodynamically unstable structures, and on cooling, reassociation of the starch molecules may occur. A collective term describing these changes is retrogradation. In starch-rich products such as bread, amylose retrogradation is known to be a rapid process taking only a few hours. Amylopectin retrogradation, on the other hand, which is known to be involved in bread staling, continues over a period of several days or weeks (Colonna, Leloup & Buleon, 1992). From a technological point of view, retrogradation is mainly considered as a problem, causing deterioration of product quality in e.g. the baking industry. However, from a nutritional point of view, controlling the retrogradation phenomena would open new possibilities to optimize the nutritional properties of starchy foods.

The nutritional properties of starch are very much related to availability for digestion and absorption in the gastrointestinal tract. Consequently, a reduced rate of digestion will lower the post-prandial blood glucose and insulin responses after a meal. Accumulating data suggest that foods that produce low glycemic responses, often measured as glycemic indices (GI), have metabolic advantages (Brand Miller, 1994). Recent recommendations by the FAO/WHO expert group therefore advocate an increased intake of low GI foods for people in general (FAO/WHO, 1998). A major drawback in this respect is the shortage of low-GI starchy foods, which makes it relevant to identify methods to reduce the enzymatic availability of starch in current staple foods.

Another nutritionally interesting feature of low GI foods is that they frequently contain a starch fraction that resists amylases altogether. Such starch, commonly referred to as resistant starch (RS), share properties in common with dietary fiber in that it provides the colonic flora with substrate (Annison & Topping, 1994; Muir, Young & O'Dea, 1993). Considerable work has been done regarding the formation and nutritional effects of retrograded amylose in particular (Björck & Siljeström, 1992; Liljeberg, Åkerberg & Björck, 1996). Starches containing this type of RS also appear to

* Corresponding author.

0144-8617/00/$ - see front matter 
PII: S0144-8617(99)00205-2

release the bulk of starch more slowly upon amylolysis in vitro (Åkerberg, Liljeberg, Granfeldt, Drews & Björck, 1998), and to lower the glycemic response in healthy humans (Åkerberg et al., 1998; Brand Miller, Pang & Bramall, 1992; Goddard, Young & Marcus, 1984; Juliano & Goddard, 1986). Less is known about the nutritional features of amylopectin retrogradation. Retrogradation of amylopectin appears to render the starch less available for α-amylolysis. Studies by Cui and Oates (1997) and Eerlingen, Jacobs and Delcour (1994) have thus indicated a lowered rate of in vitro hydrolysis, suggesting that retrogradation of amylopectin could lower the glycemic response. In contrast, retrogradation of amylopectin such in staling of bread under prolonged storage did not influence the rate of in vitro starch hydrolysis (Siljeström, Björck, Eliasson & Nyman, 1988). Little direct information is available concerning whether or not retrograded amylopectin contributes to RS.

The degree or extent of amylopectin retrogradation is greatly affected by the botanical source. The high average chain length distribution of potato amylopectin seems to favor retrogradation compared to the shorter unit-chains in cereal amylopectins (Fredriksson, Silverio, Andersson, Eliasson & Åman, 1998; Kalichevsky, Orford & Ring, 1990; Orford, Ring, Carroll, Miles & Morris, 1987; Ring et al., 1987; Silverio, Fredriksson, Andersson, Eliasson & Åman, 1999). In the retrogradation process of amylopectin, the length of the outer chains, or branches, seem to be of importance (Würsch & Gumy, 1994).

The starches chosen for this study were previously described with respect to chemical characteristics, gelatinization and retrogradation properties (Fredriksson et al., 1998) as well as the relationship between temperature cycling and amylopectin recrystallization (Silverio et al., 1999). The purpose of this study was to investigate the impact of amylopectin retrogradation on the RS formation and rate of in vitro starch digestion. Gelatinized starches with different unit-chain length distributions, i.e. waxy maize and high-amylopectin potato, were thus subjected to different time–temperature conditions (cycling) to promote retrogradation of amylopectin. The presence of retrograded amylopectin was checked using differential scanning calorimetry (DSC), and the chain length distribution of the starch hydrolysis products determined by use of high performance anion exchange chromatography (HPAEC).

## 2. Materials and methods

### 2.1. Sample preparation

For sample preparation, containers of stainless steel (inner diameter 50 mm, height 25 mm; outer diameter 60 mm, height 31 mm), tightly sealed with a rubber gasket, were used. Waxy maize and high-amylopectin potato starch, obtained from Lyckeby Stärkelsen (Kristianstad, Sweden), were used throughout all experiments. Starch (20 g) was mixed with water in the open container at a starch water ratio of 45:55 for waxy maize and 40:60 for high-amylopectin potato. The intended starch/water ratio was 1:1, but at the water content and temperature used, full gelatinization was not obtained and the level of water had to be increased. In order to obtain an even water distribution, all samples were pre-swelled for 15 min at 60°C with moderate stirring. To fully gelatinize the starch, the sample containers were then hermetically sealed and placed in a boiling water bath for 30 min. After cooling to room temperature, the samples were either immediately cut into pieces ($\sim 5 \times 10 \times 10$ mm$^3$) and left to air dry at ambient temperature, which took about 16 h, or stored for different intervals of time and temperature conditions. Gels of both starches were either stored for 24 h at 6°C followed by 24 h at 30°C, 24 h at 6°C and 24 h at 30°C (6/30/6/30) or for 24 h at 6°C followed by 24 h at 40°C, 24 h at 6°C and 24 h at 40°C (6/40/6/40). These were the same conditions as used in a previous study (Silverio et al., 1999) aiming to promote extensive retrogradation in a short period of time. The low temperature (6°C) was chosen to favor nucleation and the higher temperature (30 and 40°C) to promote propagation. After this temperature cycling, the samples were cut into pieces and dried as the non-cycled starch gels. The dried samples were then ground in a cyclotec mill (Tecator, Höganäs, Sweden) to pass a 1 mm sieve.

### 2.2. General analyses

The dry matter content was determined following drying in an oven at 105°C for 16 h. Resistant starch was analyzed essentially according to Åkerberg et al. (1998), with the exception of the incubation which was performed at 37°C instead of at 40°C in order to avoid melting of the retrograded starch. Moreover, the chewing procedure used in the initial incubation step for analysis of intact foods was omitted. Instead, the procedure described for powdery materials, which involves stimulated saliva sampling through chewing of glass beads was followed. The dried and milled starch gels were used for the DSC measurements. A DSC 6200 from Seiko Instruments Inc. was used, and the sample preparation and analyses were carried out as described by Fredriksson et al. (1998).

### 2.3. Rate of amylolysis

The rate of hydrolysis was evaluated according to Granfeldt, Björck, Drews and Tovar (1992) with some modifications. The chewing procedure was omitted and the incubation with pancreatic α-amylase was performed unrestrictedly. For the enzymatic hydrolysis, 500 mg (DM) of the ground starch gels were mixed in a screw capped bottle with 50 ml 0.022 M K–Na–phosphate buffer (pH 6.9) containing sodium chloride (0.4 M) and tempered at 37°C for 10 min under constant magnetic stirring (300

rpm, 25 mm magnet) before a 0.2 ml sample was taken (0 min incubation). Porcine pancreatic α-amylase (PPA) was immediately added (EC 3.2.1; A 6255, Sigma Chemical Co., St Louis, MO; 110 U) and samples (0.2 ml) taken after 4, 8, 12, 16, 24 and 40 min of incubation. The samples were rapidly transferred to tubes containing 0.8 ml of the K–Na–phosphate buffer and 1 ml 3,5-dinitrosalicylic acid reagent. The tubes were immediately boiled for 10 min, and 13 ml distilled water was added before measuring the reducing power spectrophotometrically at 530 nm using maltose as reference. The extent of hydrolysis was calculated as the proportion of starch degraded to maltose (per cent maltose equivalents) at the different incubation times.

### *2.4. Determination of chain length distribution of starch hydrolysates*

The samples were incubated with PPA, as described above. However, a 5 ml sample was taken at each time point (0, 4, 8, 12, 16 and 40 min). The reaction was stopped by boiling (5 min), followed by freeze-drying of the sample. A 5.5 mg sample was then dissolved in 0.025 ml 1 M sodium acetate buffer (pH 3.8) and 0.375 ml water by boiling for 30 min. Subsequently, after cooling to room temperature, the sample was debranched with 1 μl isoamylase (EC 3.2.1.68; from *Pseudomonas amyloderamosa*; Hyashibara, Biochemical Labs, Inc., Okayama, Japan) at 40°C overnight. After isoamylase hydrolysis, a number of samples were also treated with pullulanase (EC 3.2.1.41; from *Klebsiella pneumoniae*; Hyashibara, Biochemical Labs, Inc., Okayama, Japan), in order to remove any remaining branched residues. For an aliquot of isoamylolysate, pH was adjusted to 5.5 using 1 M sodium acetate and the sample was then further diluted with water (ca 3 mg/ml) followed by incubation with 5 μl pullulanase at room temperature overnight. The debranching enzymes were inactivated by boiling for 15 min prior to injection. The HPAEC conditions were as previously described by Fredriksson, Andersson, Koch and Åman (1997), except for a slightly modified elution gradient. The eluents A and B were, respectively, 150 mM sodium hydroxide and 150 mM sodium hydroxide containing 500 mM sodium acetate. The gradient program was as follows: 70% of eluent A at 0 min, 55% at 5 min, 33% at 55 min, 10% at 80 min, 70% at 81 min and thereafter isocratic for 15 min. The detector response of the pulsed amperometric detector (PAD) is not quantitative with respect to carbohydrate content. Therefore, each individual α-glucan peak between degree of polymerization (DP) 6 and 65 was corrected for by its relative molar PAD response (Koch, Andersson & Åman, 1998). For material with DP $< 6$, standards of glucose, maltose, maltotriose, maltotetraose (Sigma Chemical Co, St Louis, MO) and maltopentaose (Boehringer Mannheim, Mannheim, Germany) were used for calibration. In cases where a second peak arose next to a linear α-glucan peak, the same response factors were used for both peaks.

## 3. Results and discussion

### *3.1. Sample preparation and characterization of gels*

Gelatinized starch gels were subjected to different temperature treatments aiming to produce extensive amounts of retrograded amylopectin. After drying and milling of the gels DSC would have been a useful tool to determine the extent of crystallinity of the samples. However, to obtain reproducible results, the ground samples would need to remain at room temperature for a prolonged time to become fully hydrated. During this period of time substantial changes of the samples, such as increased retrogradation, may have occurred. The measurements were therefore made shortly after adding the water, resulting in a rather poor reproducibility but probably more true values, and therefore the results could only be used as a guideline as to whether retrogradation had occurred in the samples or not. Heating of freshly gelatinized starch does not produce any thermal transition, indicating that no retrogradation has occurred at this stage (Colonna et al., 1992; Cui & Oates, 1997; Eerlingen et al., 1994); this was also more or less the case in the present study, since only traces of retrogradation were observed in the non-cycled samples. The temperature-cycled starches, on the contrary, had retrograded to a greater extent. The retrogradation enthalpies, furthermore, indicated a lower degree of retrogradation in the samples propagated at 40°C as opposed to 30°C, which is in agreement with previous findings for temperature-cycled starches (Silverio et al., 1999).

None of the high-amylopectin starches contained RS, whether temperature-cycled or not, despite differences in the rate of amylolysis (see below). Resistant starch was, in this study, determined according to Åkerberg et al. (1998), i.e. with an analytical assay aimed at mimicking physiological conditions. As a reference, approximately 3% of total starch was RS in the case of non-cycled as well as cycled potato starch with a normal amylose content (cv. Desirée). The origin of this RS was probably retrograded amylose. In contrast, Eerlingen et al. (1994), defining the RS as the fraction of starch not digested to glucose after incubation with pancreatin and amyloglucosidase for 2 h at 34°C, found extremely high levels of RS between 5 and 42% in waxy maize starch stored at different time and temperature conditions. This suggests that the recovery of retrograded amylopectin as RS is dependent not only on storage conditions, but also on the in vitro method used; the true in vivo digestibility of retrograded amylopectin remains to be established. It should be noted, however, that storage of boiled potatoes in a refrigerator increased RS recovery in excreta, from ileostomy subjects, from 3 to 12% of ingested starch. Reheating of the cooled potato product lowered the RS recovery to 7% indicating that retrograded amylopectin possibly contributed with indigestible carbohydrates in boiled potatoes submitted to storage at refrigerator temperature (Englyst & Cummings, 1987).

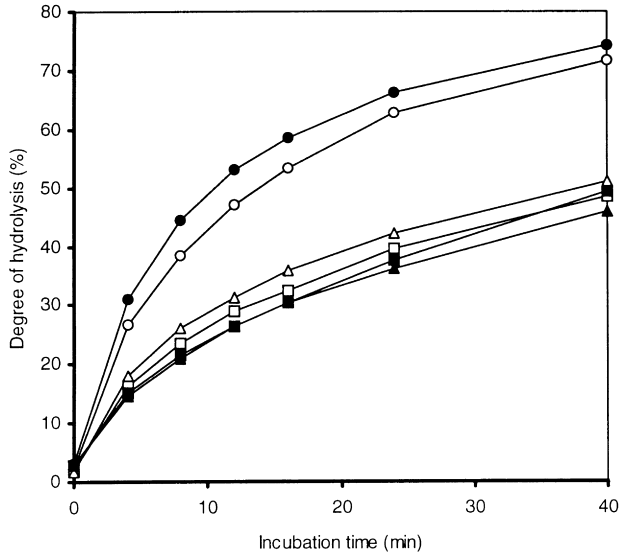


Fig. 1. Hydrolysis of starch samples after different incubation times with porcine pancreatic α-amylase: (●) non-cycled waxy maize; (○) non-cycled high-amylopectin potato; (■) temperature-cycled waxy maize 6/30/6/30; (▲) temperature-cycled waxy maize 6/40/6/40; (□) temperature-cycled high-amylopectin potato 6/30/6/30; and (△) temperature-cycled high-amylopectin potato 6/40/6/40. The extent of hydrolysis was calculated as the proportion of starch degraded to maltose (maltose equivalents) at the different incubation times.

### 3.2. Rate of enzymatic hydrolysis with PPA

The temperature-cycled starch gels were less rapidly hydrolyzed by PPA than the non-cycled gels (Fig. 1). The non-cycled high-amylopectin potato starch gel was hydrolyzed at a slightly slower rate than the waxy maize starch, and the extent of hydrolysis reached 50% after about 14 min for the potato starch versus 11 min for the waxy maize starch. After 40 min of hydrolysis, around 70–75% of the non-cycled gels was hydrolyzed, i.e. the maximal plateau of approximately 80% α-amylolysis was almost reached (Holm, Lundquist, Björck, Eliasson & Asp, 1988). Only minor endothermic transitions were observed in the DSC measurements in the case of these samples. The extent of hydrolysis of the temperature-cycled starch gels was rather similar and independent of propagation temperature and/or starch source, even if the waxy maize starch seemed somewhat less available to hydrolysis than the high-amylopectin potato starch. After hydrolysis for 40 min, the degree of hydrolysis was significantly lower than for the non-cycled gels and in the same range for all cycled gels, i.e. around 45–50%. The lowered enzyme susceptibility is in accordance with a more extensive retrogradation, as judged from the DSC measurements. From these results it can be concluded that amylopectin retrogradation significantly decreased the rate of hydrolysis by PPA. The results of this study seem generally to be in line with those obtained by Eerlingen et al. (1994). However, the experimental conditions were not the same as in the present study, for instance, in addition to hydrolysis with porcine α-amylases, their study also included an incubation step with amyloglucosidase. Moreover, in the study by Eerlingen et al. (1994), the incubation temperature was only 34°C, which is below the physiological temperature, and this may have contributed to the comparatively lower enzyme availability of the cycled amylopectin gels studied.

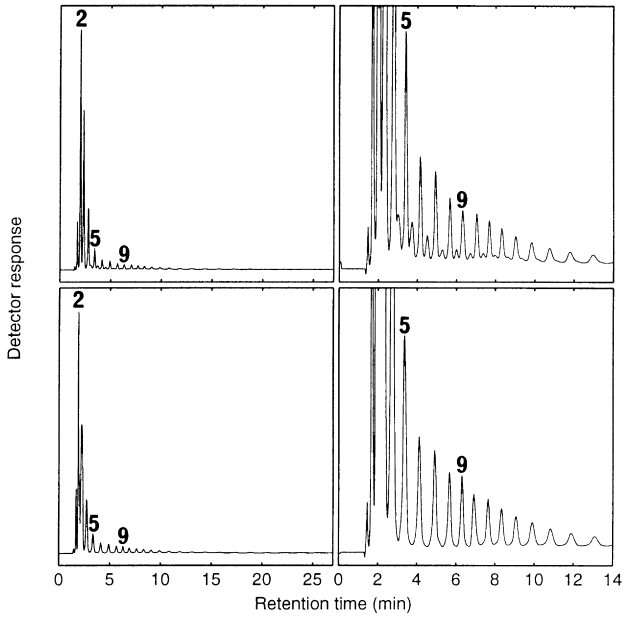


Fig. 2. HPAEC chromatograms of non-cycled waxy maize starch, treated with porcine pancreatic α-amylase for 40 min followed by debranching with isoamylase, (upper) before and (lower) after debranching with pullulanase. Enlargement of the chromatograms (right). Certain chain lengths are indicated in the figures.

### 3.3. Determination of chain length distribution after enzymatic hydrolysis

The small intestine is the major site of starch breakdown. In this in vitro study, PPA was chosen to mimic the rate of starch digestion in the human intestine. PPA hydrolyzes α-1,4-glucosidic linkages of starch and the action of the enzyme is endowise (Sakano, 1988). The chain length distribution in the hydrolysate was studied by HPAEC after hydrolysis with PPA for different periods of time, followed by debranching with isoamylase (Fig. 2).

The main products of hydrolysis were maltose, maltotriose and smaller amounts of oligosaccharides with a DP of 4–9, as well as minute amounts of glucose (Fig. 3). In addition, PPA is known to produce various branched dextrins (Sakano, 1988). The chromatography provided information of linear chains within the DP range of 1–65. However, between some of these peaks adjacent peaks were detected with somewhat different retention times (Fig. 2). No adjacent peaks were found in the non-hydrolyzed samples after debranching with isoamylase, showing that these peaks were formed only in the PPA treatment. PPA may reduce the length of some side chains to maltosyl groups which makes debranching with isoamylase ineffective,

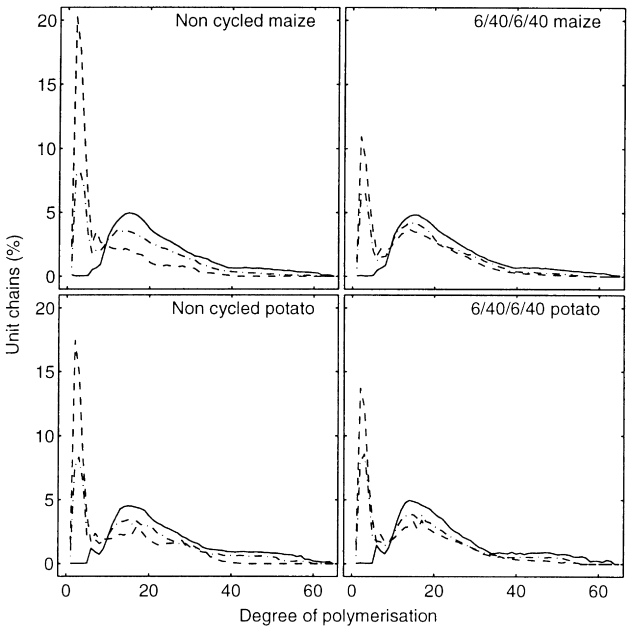


Fig. 3. Representative chain length distributions of both non-cycled (left) and temperature-cycled 6/40/6/40 (right) waxy maize starch (upper) and high-amylopectin potato starch (lower). Prior to analysis, the samples were treated with porcine pancreatic α-amylase for 0 (—), 8 (– · – · –) and 40 min (– – –) followed by debranching with isoamylase. The amount of chains with DP 1–65 was normalized to an equal sum in each chromatogram.

leaving branched dextrins with short side chains in the sample. Therefore, a second debranching with pullulanase was introduced to some of the samples, because pullulanase readily releases maltosyl side chains (Manners, 1997). Debranching with pullulanase removed the adjacent peaks confirming that the α-amylolysate did contain some branched material after the treatment with isoamylase.

The content of low molecular weight carbohydrates with DP 1–9 increased during the PPA treatment (Fig. 3). In general, the production of low molecular weight carbohydrates was somewhat higher for the non-cycled waxy maize starch compared to the high-amylopectin potato starch, a result in line with the hydrolysis curves (Fig. 1). Since the debranching was carried out on the complete hydrolysate, the chain length distribution, as analyzed by HPAEC, did not reveal which of the chains that were released by α-amylase treatment or during debranching. However, the degree of degradation could be expressed as the molar percent of glucose residues present in unit-chains with DP $< 6$, since the content of those chains was close to zero in the native amylopectin. There were linear relationships between the degree of degradation and the proportion of unit-chains with DP 10–11, 12–21, 22–37 and 38–65 (Figs. 4 and 5). These DP intervals were chosen because of their different correlation to retrogradation according to Silverio et al. (1999). The longest chains appeared to be degraded more rapidly than the shorter chains, as indicated by the different slopes. These differences may be due to

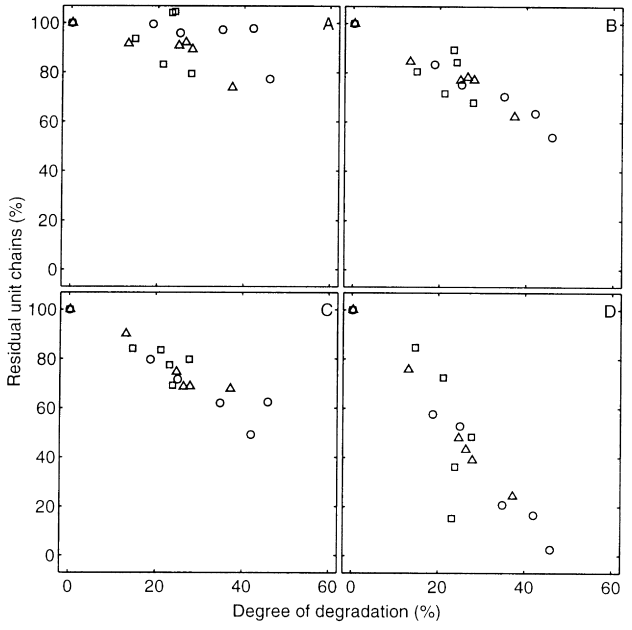


Fig. 4. Relationship between degree of degradation (sum of glucose, maltose and oligomers with DP $< 6$ as percent of total amount of glucose residues recovered) and residual unit-chains of: (A) DP 10–11; (B) 12–21; (C) 22–37; and (D) 38–65 expressed as percent of the amount before α-amylase treatment, in non-cycled (○), temperature-cycled 6/30/6/30 (□) and temperature-cycled 6/40/6/40 (△) high-amylopectin potato starch.

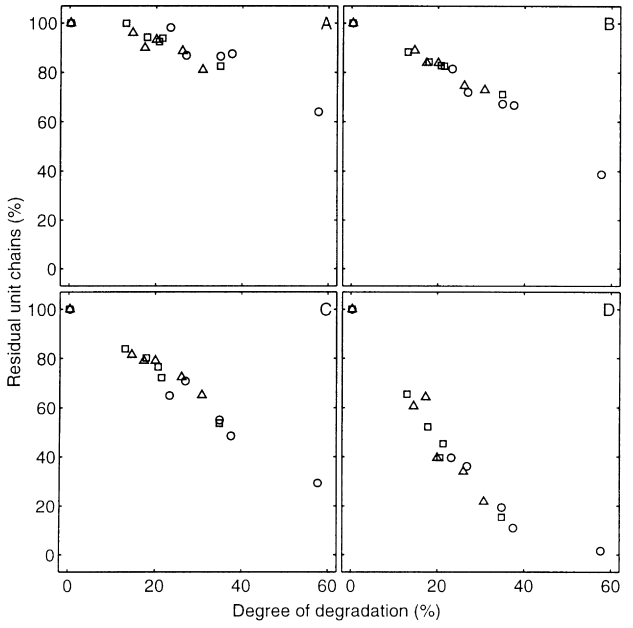


Fig. 5. Relationship between degree of degradation (sum of glucose, maltose and oligomers with DP $< 6$ as percent of total amount of glucose residues recovered) and residual unit-chains of: (A) DP 10–11; (B) 12–21; (C) 22–37; and (D) 38–65 expressed as percent of the amount before amylase treatment, in non-cycled (○), temperature-cycled 6/30/6/30 (□) and temperature-cycled 6/40/6/40 (△) waxy maize starch.

formation of chains of intermediate length during degradation of the longer chains. It may also indicate a selectivity of the α-amylase, not affected by the retrogradation, since all samples followed the same regression line, regardless of treatment.

Supported by the theory that mainly external amylopectin unit-chains are involved in the retrogradation process (Würsch & Gumy, 1994), this study was performed in order to identify the chains involved in retrogradation thereby being less available to hydrolysis. However, regardless of treatment the degradation of the starch gels seemed uniform since no enrichment of material among chains with DP > 9 was shown. It is therefore suggested that besides crystallinity and molecular order, factors such as particle structure and size were important in limiting the enzyme accessibility of the milled gel-powders after rehydration and this conclusion is in accordance with the suggestions of Colonna et al. (1992) and Eerlingen et al. (1994). The diffusion rate of the enzyme through the ground and rehydrated starch gels probably varied for different gels. By the time 40–50% of the total material had been hydrolyzed by PPA it is suggested that the enzyme had fully diffused through the gels, since none or very small amounts of material with DP > 38 was found in the hydrolysates at this stage (Fig. 3). In the amylopectin cluster model (Hizukuri, 1986) such high molecular weight material would intersect at least three clusters and, therefore, only fragments of amylopectin could be left at this stage of hydrolysis.

## 4. Concluding remarks

The waxy maize and high-amylopectin potato starch gels responded to the different treatments in similar ways, regardless of the differences in chain length of the native starches (Fig. 1). The non-cycled starch gels were more readily degraded than the temperature-cycled gels, although, a uniformity in chain length distribution of the amylolysates (Figs. 3–5) indicated that the enzymatic mode of action was not affected by temperature cycling and retrogradation. Processing, in terms of temperature cycling during storage of starchy foods, may result in a slower hydrolysis of the amylopectin in the small intestine, which could be of nutritional interest even if this fraction analytically did not fulfil the properties of RS. Interesting applications include development of starchy foods with low GI properties. A slowdown in the α-amylolysis of amylopectin may also be used in technical applications such as pharmaceutical products with a slow release of the active compound.

## Acknowledgements

Financial support was received from the Cerealia Foundation R&D, Lyckeby-Stärkelsen, Procordia Food AB, the Swedish Farmer's Foundation for Agricultural Research, the Swedish Council for Forestry and Agricultural Research, Wasabröd AB and the SL Foundation.

## References

Åkerberg, A., Liljeberg, H., Granfeldt, Y., Drews, A., & Björck, I. (1998). An in vitro method based on chewing to predict resistant starch content in foods, allows parallel determination of potentially available starch and dietary fiber. *Journal of Nutrition, 128,* 651–660.

Annison, G., & Topping, D. L. (1994). Nutritional role of resistant starch: chemical structure vs physiological function. *Annual Review of Nutrition, 14,* 297–320.

Björck, I., & Siljeström, M. (1992). In vivo and in vitro digestibility of starch in autoclaved pea- and potato products. *Journal of the Science of Food and Agriculture, 58,* 41–553.

Brand Miller, J. (1994). Importance of glycemic index in diabetes. *American Journal of Clinical Nutrition, 59* (suppl), S747–S752.

Brand Miller, J., Pang, E., & Bramall, L. (1992). Rice: a high or low glycemic index food? *American Journal of Clinical Nutrition, 56,* 1034–1036.

Colonna, P., Leloup, V., & Buleon, A. (1992). Limiting factors of starch hydrolysis. *European Journal of Clinical Nutrition, 46* (suppl 2), S17–S32.

Cui, R., & Oates, G. (1997). The effect of retrogradation on enzyme susceptibility of sago starch. *Carbohydrate Polymers, 32,* 65–72.

Eerlingen, R. C., Jacobs, H., & Delcour, J. A. (1994). Enzyme-resistant Starch. V. Effect of retrogradation of waxy maize starch on enzyme susceptibility. *Cereal Chemistry, 71,* 351–355.

Englyst, H. N., & Cummings, J. (1987). Digestion of polysachharides of potato in the small intestine of man. *American Journal of Clinical Nutrition, 45,* 423–431.

FAO/WHO (1998). Carbohydrates in Human Nutrition. FAO–Food and Nutrition Paper, 66, 1–140.

Fredriksson, H., Andersson, R., Koch, K., & Åman, P. (1997). Calibration of a size-exclusion chromatography system by using fractions with defined amylopectin unit-chains. *Journal of Chromatography A., 768,* 325–328.

Fredriksson, H., Silverio, J., Andersson, R., Eliasson, A. -C., & Åman, P. (1998). The influence of amylose and amylopectin characteristics on gelatinization and retrogradation properties of different starches. *Carbohydrate Polymers, 35,* 119–134.

Goddard, M. S., Young, G., & Marcus, R. (1984). The effect of amylose content on insulin and glucose responses to ingested rice. *American Journal of Clinical Nutrition, 39,* 388–392.

Granfeldt, Y., Björck, I., Drews, A., & Tovar, J. (1992). An in vitro procedure based on chewing to predict metabolic response to starch in cereal and legume products. *European Journal of Clinical Nutrition, 46,* 649–660.

Hizukuri, S. (1986). Polymodal distribution of the chain lengths of amylopectins, and its significance. *Carbohydrate Research, 147,* 342–347.

Holm, J., Lundquist, I., Björck, I., Eliasson, A. -C., & Asp, N. -G. (1988). Degree of gelatinisation, digestion rate of starch in vitro, and metabolic response in rats. *American Journal of Clinical Nutrition, 47,* 1010–1016.

Juliano, B. O., & Goddard, M. S. (1986). Cause of varietal differences in insulin and glucose responses to ingested rice. *Qualitas Plantarum. Plant Foods for Human Nutrition, 36,* 835–842.

Kalichevsky, M. T., Orford, P. D., & Ring, S. G. (1990). The retrogradation and gelation of amylopectins from various botanical sources. *Carbohydrate Research, 198,* 49–55.

Koch, K., Andersson, R., & Åman, P. (1998). Quantitative analysis of amylopectin unit-chains by means of high-performance anion-exchange chromatography with pulsed amperometric detection. *Journal of Chromatography A., 800,* 199–206.

Liljeberg, H., Åkerberg, A., & Björck, I. (1996). Resistant starch formation in bread as influenced by choice of ingredients or baking conditions. *Food Chemistry*, *56*, 389–394.

Manners, D. J. (1997). Observations on the specificity and nomenclature of starch debranching enzymes. *Journal of Applied Glycoscience*, *44*, 83–85.

Muir, J. G., Young, G. P., & O'Dea, K. (1993). Resistant starch: the neglected dietary fiber? implication for health. *Dietary Fiber Bibliography Reviews*, *1*, 33–47.

Orford, P. D., Ring, S. G., Carroll, V., Miles, M. J., & Morris, V. J. (1987). The effect of concentration and botanical source on the gelation and retrogradation of starch. *Journal of Science of Food and Agriculture*, *39*, 169–177.

Ring, S. G., Colonna, P., I'Anson, K. J., Kalichvsky, M. T., Miles, M. J., Morris, V. J., & Orford, P. D. (1987). The gelation and crystallisation of amylopectin. *Carbohydrate Research*, *162*, 277–293.

Sakano, Y. (1998). Porcine pancreatic α-amylase. In The Amylase Research Society of Japan (Ed.) *Handbook of amylases and related enzymes: their sources, isolation methods, properties and applications* (pp. 22–6). Tokyo: Pergamon, pp. 22–26.

Siljeström, M., Björck, I., Eliasson, A. -C., & Nyman, M. (1988). Effects on polysaccharides during baking and storage of bread—in vitro and in vivo studies. *Cereal Chemistry*, *65*, 1–12.

Silverio, J., Fredriksson, H., Andersson, R., Eliasson, A.-C., & Åman, P. (1999). The effect of temperature cycling on the amylopectin retrogradation of starches with different amylopectin unit-chain length distribution. Carbohydrate Polymers, accepted for publication.

Würsch, P., & Gumy, D. (1994). Inhibition of amylopectin retrogradation by partial beta-amylolysis. *Carbohydrate Research*, *256*, 129–137.