Available online at www.sciencedirect.com
SCIENCE DIRECT
Carbohydrate Polymers 65 (2006) 306–313
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Resistant starch formation in temperature treated potato starches varying in amylose/amylopectin ratio

A. Margareta Leeman [a], Malin E. Karlsson [b,*], Ann-Charlotte Eliasson [b], Inger M.E. Björck [a]

[a] Department of Applied Nutrition and Food Chemistry, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden
[b] Department of Food Technology, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden

Received 2 May 2005; received in revised form 12 January 2006; accepted 13 January 2006
Available online 10 March 2006

Abstract

Two genetically modified potato starches derived from the same mother line (64%, 1% and 23% amylose, respectively) were used to study the bioavailability after various heat treatments. The conditions for the treatments were of minor importance for resistant starch (RS) formation and hydrolysis results, as compared to the proportion of amylose. A high amylose content gave lower hydrolysis index (HI) and higher amounts of RS than starches with less amylose. Retrograded amylopectin contributed to a decreased HI, although only the high amylose line showed sufficient reduction in predicted glycaemic indices (GI). The line with high amylose content contained 25–30% RS vs. in the range of 0–5% for the other starches. Results could neither be explained by the presence of intact granules, nor by the content of retrograded amylose. Therefore, a synergistic effect between the starch components was suggested to affect the RS and starch hydrolysis.

Keywords: Potato; Starch; Amylose; Amylopectin; Resistant starch; Hydrolysis

1. Introduction

Until the early eighties starch was believed to be completely digested and absorbed in the human small intestine. However, today it is known that the bioavailability of starch may vary and that some starch may enter the colon. This resistant starch (RS) is defined as "the sum of starch and the products of starch degradation not absorbed in the small intestine of healthy individuals" (Asp, 1992). RS has been divided into three main fractions: (1) physically inaccessible starch, (2) resistant B-type granules and (3) retrograded starch (Englyst & Kingman, 1990). Since RS is not absorbed in the small intestine, it provides the colonic micro-flora with a fermentable carbohydrate substrate, promoting colonic production of short-chain fatty acids (SCFA). It has been suggested that RS promotes a higher proportion of butyric acid than other indigestible carbohydrates (Scheppach, Fabian, Sachs, & Kasper, 1988). Butyrate constitute a major energy substrate for the colonocytes and is associated with benefits in relation to colonic health (Brouns, Kettlitz, & Arrigoni, 2002; Hallert et al., 2003).

The amount of RS is increased in retrograded starchy foods, e.g., boiled potatoes after storage in a refrigerator (Åkerberg, Liljeberg, Granfeldt, Drews, & Björck, 1998b; Englyst & Cummings, 1987; Leeman, Bårström, & Björck, 2005; Tschäppät, 2000). Increased RS contents and/or decreased enzyme susceptibility of starches may be attained by time/temperature treatments, which has been shown previously for starches of different botanical origin and/or with varying amylose/amylopectin ratios (Berry, 1986; Eerlingen, Jacobs, & Delcour, 1994; Fredriksson et al., 2000; Silverio, Fredriksson, Andersson, Eliasson, & Åman, 2000). Melting of retrograded amylopectin and amylose after time/temperature treatments, as well as of RS has

* Corresponding author. Tel: +46 46 222 83 11; fax: +46 46 222 95 17.
E-mail address: Malin.Karlsson@livsteki.lth.se (M.E. Karlsson).

0144-8617/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2006.01.019

been studied by use of differential scanning calorimetry (DSC) and/or X-ray diffraction techniques (Eerlingen, Crombez, & Delcour, 1993; Gidley, 1989; Gidley et al., 1995; Sievert, Czuchajowska, & Pomeranz, 1991; Sievert & Pomeranz, 1990; Sievert & Würsch, 1993; Silverio et al., 2000). Amylose retrogradation is considered to be a rapid process completed within 48 h, whereas amylopectin retrogradation may continue for weeks (Miles, Morris, Orford, & Ring, 1985). Retrograded amylopectin melts in the approximate temperature range 40–70 °C, whereas retrograded amylose melts in a higher temperature range, or from 120 to 170 °C (Sievert & Pomeranz, 1990). Retrograded amylose and amylopectin, and also native potato starch, is known to give a B-crystalline pattern as studied by X-ray diffraction.

Both retrograded amylose and retrograded amylopectin could be expected to have an impact on different nutritional characteristics, though the nutritional consequences of promoting amylose retrogradation have been more extensively studied. Increased RS content and decreased glycaemic response (Åkerberg, Liljeberg, & Björck, 1998a; Hoebler, Karinthi, Chiron, Champ, & Barry, 1999) have been reported in starchy foods with an increased amylose content. Less is known about the nutritional effects of retrograded amylopectin. However, a decreased rate of α-amylase degradation and an increased RS content was found by Eerlingen et al. (1994) in gelatinised temperature treated waxy maize starch. In retrograded waxy maize and high amylopectin potato starches, a decreased rate of amylase digestion was found in vitro following incubation with pancreatic α-amylase, despite low yields of RS (Fredriksson et al., 2000). Although not a prerequisite, food factors which reduce glycaemic indices (GI) also lead to formation of RS (Björck, Liljeberg, & Östman, 2000; Granfeldt, Drews, & Björck, 1995). However, the reduced available starch content, i.e., the reduced available carbohydrate load *per se* does not explain the reduced GI (Björck et al., 2000; Jenkins et al., 1987; Wolever, 1990). Low GI foods are increasingly being referred to as beneficial adjunct to their potential in reducing risk factors for metabolic disease (Frost et al., 1999; Järvi et al., 1999), and the disease risk for the insulin resistance syndrome (McKeown et al., 2004). Based on above, it is therefore of interest from a nutritional perspective to further elucidate mechanisms by which the rate of starch digestion may be reduced, hence promoting a lower GI, and in addition elucidate mechanisms which promote increased yields of RS.

The aim of the present study was to evaluate potential differences in nutritional characteristics of retrograded starch containing mainly amylose vs amylopectin; and to evaluate the relation between the rate of starch digestion and the RS yield employing in vitro enzymatic models. More specifically the bioavailability of potato starch in one genetically modified high amylose starch and one genetically modified amylopectin starch derived from the same mother line was studied in starch-water model systems following selected time/temperature treatments. The in vitro nutritional characteristics studied included analysis of available starch and RS, determination of hydrolysis index (HI) and prediction of GI from HI values. In addition, crystallinity of the treated starches as measured by DSC and X-ray diffraction was studied.

2. Materials and methods

2.1. Materials

Isolated starch material from two transgenic potato lines and one control potato line (*Solanum tuberosum* cv Prevalent, 23% amylose) cultivated 2002 were supplied by BASF Plant Science Sweden. The transgenic lines were derived by inhibition of granule bound starch synthase (GBSS) (line 527-1, 1% amylose) and of two starch branching enzymes (SBE1 and SBE2) (line 342, 64% amylose). Information regarding amylose/amylopectin ratios determined by size exclusion chromatography was provided by BASF Plant Science Sweden.

2.2. Sample preparation

Samples were pre-swelled at 60 °C and thereafter boiled at 100 °C in a hermetically sealed container as described by Tufvesson, Skrabanja, Björck, Elmståhl, and Eliasson (2001), at a starch:water ratio of 1:3. Autoclaving was omitted, since this is not a common procedure for food preparation. After cooling, the samples were subjected to selected time/temperature treatments. The temperature conditions used were selected to promote the retrogradation of the amylopectin and the amylose fractions, respectively. Consequently, 4 °C/31 °C was used to favour amylopectin retrogradation, whereas 4 °C/100 °C was used to promote the retrogradation of amylose. In addition, a treatment at 4 °C was included. The set time was 24 h at each temperature. Samples were then cut into pieces, air dried at ambient temperature, and ground in a cyclotec mill (Tecator, Höganäs, Sweden) to pass a 1 mm sieve (Fredriksson et al., 2000; Tufvesson et al., 2001).

2.3. Starch content

Total starch content was analysed in the starches according to Björck and Siljeström (1992). Starch analyses were performed in duplicate.

2.4. Resistant starch and available starch content

Resistant starch (RS) analysis was performed on the time/temperature treated starches. The RS analysis was performed according to Åkerberg et al. (1998b) where chewing is a pre-step before incubation with pepsin, pancreatin and amyloglucosidase. However, the incubation was performed at 37 °C instead of 40 °C (Fredriksson et al., 2000). To avoid exposure of the test subjects to

the genetically modified starches, the chewing step was omitted and instead glass beads were chewed to stimulate saliva production. The method allows parallel determination of RS and available starch by utilising a physiological approach mimicking the conditions in the gastrointestinal tract. The sum of available starch and RS should equal total starch content, however, deviations may occur due to test persons involved or to pre-preparation of samples, e.g., milling. Nevertheless, the method is reproducible and the same test persons are used for all samples.

2.5. In vitro rate of starch hydrolysis

The method for determination of rate of in vitro starch hydrolysis (Granfeldt, Björck, Drews, & Tovar, 1992) was followed with some modifications. Also for this analysis the chewing step was omitted and glass beads were used to stimulate saliva production. Further, withdrawn dialysate from the hydrolysis performed at 37 °C, was analysed according to Holm, Björck, Drews, and Asp (1986) including incubation with thermostable α-amylase at 100 °C (Termamyl, Novozymes, Bagsvaerd, Denmark) and amyloglucosidase at 60 °C (Roche diagnostics GmbH, Germany). As a measure of the rate of starch hydrolysis, the released glucose was assayed. White wheat bread was used as reference material for the calculation of a HI which is defined as the area under the hydrolysis curve for a test product, expressed as the percentage of the corresponding area for white bread. GI values were predicted from the hydrolysis graphs using the equation $GI = 6.272 + 0.912 \times HI$ (Leeman et al., 2005).

2.6. Differential scanning calorimetry (DSC)

The dried and milled starch powders were weighed into coated aluminum pans (TA Instruments, New Castle, USA) with doubly distilled water to a starch:water ratio of 1:3. The samples were allowed to equilibrate for at least 1 h before DSC analysis. Analyses were performed as described previously (Karlsson & Eliasson, 2003) over the temperature range of 6–195 °C. Transition enthalpy (ΔH expressed as Joules per gram dry matter), onset (T_o, °C) and conclusion temperature (T_c, °C) were determined. All results are the means of at least three measurements.

2.7. X-ray diffraction

X-ray diffraction patterns were studied using a Ni-filtered Philips X-ray generator type 2223/20 (Philips, Eindhoven, the Netherlands) giving a $Cu_{K\alpha}$ radiation with a wavelength of 1.542 Å at 40 kV and 20 mA. The measurements were performed at 22 °C and the exposure time was 6 h for native starches and 16–24 h for time/temperature treated starches. As calibrating reference tristearin ($d = 0.46$ nm) was used.

2.8. Light microscopy

Dried and milled sample powders (“4/31 °C” and “4/100 °C”) were suspended in water, stained with a diluted Lugols solution (I_2/KI solution; 1:2 w/w), and immediately viewed under an Olympus BX-50 microscope (Olympus, Japan).

2.9. Statistical analysis

Statistical calculations were performed with MINITAB Statistical Software (release 13 for Windows, Minitab Inc, State College, PA, USA) and STATGRAPHICS Plus Version 5.0 (Manugistics Inc., Rockville MD, USA). Significances were evaluated with the general linear model (ANOVA). For analyses including chewing test persons, ANOVA was followed by Tukey's multiple comparison test and for DSC results means were compared by least significant difference (LSD). P values <0.05 were considered significant.

3. Results

3.1. RS content

Total starch contents as well as contents of available starch and RS are provided in Table 1. The contents of available starch were significantly lower, and RS contents significantly higher for the high amylose line (342) compared with the mother line Prevalent and the amylopectin

Table 1
Total starch (Total), available starch (Available), and resistant starch (RS) contents in boiled and time/temperature treated potato starches varying in amylose/amylopectin ratio

Sample	Temperature								
	4 °C			4/31 °C			4/100 °C		
	Total (%)	Available (%)	RS (%)	Total (%)	Available (%)	RS (%)	Total (%)	Available (%)	RS (%)
342	92.7	77.6 ± 0.6^{bx}	26.0 ± 0.6^{ay}	90.0	70.2 ± 0.4^{cz}	28.1 ± 0.3^{ax}	92.4	75.7 ± 0.4^{by}	29.0 ± 0.2^{ax}
527-1	97.0	108.4 ± 3.2^{ax}	0.1 ± 0.0^{cy}	95.7	96.2 ± 1.6^{bx}	0.3 ± 0.0^{cx}	99.8	93.5 ± 6.7^{ax}	0.1 ± 0.0^{cy}
Prevalent	96.7	106.1 ± 0.4^{ax}	5.3 ± 0.2^{bx}	96.0	107.8 ± 0.3^{ax}	3.8 ± 0.2^{by}	96.3	99.8 ± 4.2^{ax}	5.2 ± 0.1^{bx}

Total starch values on dry matter (DM) basis, $n = 2$. Values on available starch and RS are means ± SEM (starch basis) of six replicates. Values within a column (a–c) or row (x–z, available and RS compared separately) not containing the same letter are significantly different ($P < 0.05$).

Table 2
Degree of hydrolysis, in vitro hydrolysis index (HI) and predicted GI's obtained from HI in boiled and time/temperature treated potato starches varying in amylose/amylopectin ratio

Sample	Temperature								
	4 °C			4/31 °C			4/100 °C		
	% hydrolysed	HI	Predicted GI	% hydrolysed	HI	Predicted GI	% hydrolysed	HI	Predicted GI
342	42 ± 1	68 ± 3^{bx}	68	43 ± 1	69 ± 1^{bx}	69	39 ± 1	65 ± 2^{bx}	66
527-1	59 ± 1	94 ± 3^{ay}	92	55 ± 1	88 ± 3^{az}	87	62 ± 1	103 ± 2^{ax}	100
Prevalent	57 ± 1	95 ± 4^{ax}	93	55 ± 1	87 ± 3^{ay}	86	61 ± 1	100 ± 4^{ax}	97

Values are means ± SEM of six replicates. White wheat bread as reference, HI = 100. HI values within a column (a–b) or row (x–z) not containing the same letter are significantly different ($P < 0.05$).

line (527-1) at all temperature treatments used. Further, the treatment "4/31 °C", resulted in significantly lower available starch content in line 527-1 compared with Prevalent. Also, RS content was lower in 527-1 compared with Prevalent, irrespectively of temperature treatment.

When comparing different temperature treatments for each sample, line 342 contained a significantly lower amount of available starch at "4/31 °C" compared with "4 °C" and "4/100 °C", and a lower amount of RS at "4 °C" compared with "4/31 °C" and "4/100 °C", respectively. For line 527-1 no differences were seen in the available starch content, but RS was increased at "4/31 °C" compared with "4 °C" and "4/100 °C". Neither did any differences occur in the case of Prevalent in the available starch content, though the amount of RS was significantly lower at "4/31 °C" compared with the other treatments.

3.2. In vitro rate of starch hydrolysis

Degree of hydrolysis and HI's are given in Table 2. The high amylose line displayed a lower degree of hydrolysis than the other lines, irrespectively of temperature treatment. When comparing the different temperature treatments for each line, the temperature "4/31 °C" resulted in significantly lower HI values compared with the other temperature combinations for lines 527-1 and Prevalent. Further, for line 527-1, the HI at "4 °C" was significantly lower compared with "4/100 °C".

3.3. DSC measurements

Variable amounts of retrograded amylopectin were found in all samples (Table 3). Most retrograded amylopectin was found in line 527-1, although there were no differences in amounts if comparing treatments "4 °C "with "4/31 °C" either for line 527-1 or Prevalent. Line 342 contained small amounts of retrograded amylopectin regardless of preparation conditions. The lowest onset temperature for melting of retrograded amylopectin was 40.1 ± 3.0 °C, giving that the selected temperature for promoting retrogradation of amylopectin, 31 °C, did not cause any melting. Retrograded amylopectin in line 342 melted at higher temperatures (T_o 57.9–63.8 °C) compared with line 527-1 and Prevalent (T_o 40.1–45.9 °C).

No significant differences in the amount of retrograded amylose were found, either between starches or time/temperature treatments (Table 3). The values were in the range of 0.7–2.0 J/g dry matter, also for the amylopectin line 527-1. Melting occurred within the temperature range 109.7–148.3 °C, where T_o were slightly lower for line 342 compared with line 527-1 and Prevalent. An isolated RS fraction derived from the RS analysis was analysed for comparison and gave onset and conclusion temperatures of 116.0 and 140.2 °C, respectively.

3.4. X-ray diffraction patterns of time/temperature treated starches

Untreated samples of all starches were analysed and gave a clear crystalline B-pattern. Of "4/31 °C" samples, line 527-1 was clearly crystalline, whereas Prevalent gave a somewhat weaker crystalline pattern and line 342 only showed a weak tendency of crystallinity. Samples "4/100 °C" gave unambiguous but weak crystalline patterns. Samples treated at only 4 °C were not analysed.

3.5. Light microscopy

In line 342 intact starch granules were clearly seen, though more granules were affected after the treatment "4/100 °C" (Fig. 1). Discrete starch granules were detected also in Prevalent samples, although in a very small amount. No intact granules were found in samples from line 527-1. Granule remnants were seen in all samples except 527-1 after treatment "4/100 °C".

4. Discussion

The proportion of amylose was the main factor influencing HI and RS in temperature treated starches. As expected, the line with high amylose content contained appreciable amounts of RS, around 25–30% (starch basis) vs. in the range of 0–5% (starch basis) in the case of the mother line and the amylopectin line. Further, the HI's and thereby the predicted GI values were significantly lower for the high amylose line compared with the lines with lower amylose contents. However, only the digestion rate profile of the high amylose line showed

Table 3
Onset (T_o, °C) and conclusion (T_c, °C) temperatures and transition enthalpy (ΔH) for melting of retrograded amylopectin and amylose, respectively, in boiled and time-temperature treated potato starches varying in amylose/amylopectin ratio

Sample	Amylopectin								Amylose							
	4 °C			4/31 °C			4/100 °C			4 °C			4/31 °C			4/100 °C		
	T_o	T_c	ΔH	T_o	T_c	ΔH	T_o	T_c	ΔH	T_o	T_c	ΔH	T_o	T_c	ΔH	T_o	T_c	ΔH
342	63.8 ± 5.9	89.8 ± 3.9	1.0 ± 0.9	57.9 ± 5.1	83.4 ± 7.5	1.2 ± 0.8	63.8 ± 6.4	86.8 ± 4.3	1.4 ± 0.8	109.9 ± 8.2	138.3 ± 6.4	1.6 ± 1.1	109.7 ± 5.0	137.7 ± 7.5	1.6 ± 0.6	116.7 ± 2.7	143.3 ± 1.6	2.0 ± 1.0
527-1	40.4 ± 4.3	71.9 ± 1.7	9.0 ± 1.0	41.8 ± 4.9	74.6 ± 1.2	8.9 ± 0.8	44.7 ± 1.7	69.8 ± 5.3	3.3 ± 1.2	122.6 ± 11.7	144.2 ± 7.1	1.1 ± 0.9	126.8 ± 6.6	148.3 ± 2.5	0.7 ± 0.1	118.0 ± 11.8	138.7 ± 13.9	1.4 ± 1.0
Prevalent	43.0 ± 1.3	70.9 ± 1.2	5.4 ± 0.8	40.1 ± 3.0	74.6 ± 1.0	6.2 ± 1.3	45.9 ± 1.4	73.4 ± 3.3	2.6 ± 0.5	121.4 ± 13.7	142.0 ± 10.9	1.2 ± 0.9	125.9 ± 6.1	147.0 ± 2.0	1.4 ± 1.2	119.3 ± 12.8	143.7 ± 8.3	1.2 ± 0.5

sufficient reduction in predicted GI from a metabolic point of view.

It should be noted that degree of hydrolysis was determined on a total starch basis, thus including also the RS fraction. In selected time/temperature treated starches degree of hydrolysis was further determined on basis of potentially available starch as analysed according to Holm et al. (1986). Degree of hydrolysis and HI's were higher (ca. 13–28%) for the high amylose line based on potentially available starch compared with total starch as basis, whereas the difference was negligible for the other starches. Recently, it was recommended that for the purpose of GI determination, the carbohydrate load should be based on available carbohydrates (Brouns et al., 2005).

The conditions for the time/temperature treatments of potato starches were of minor importance for RS formation and hydrolysis results. In the present study, a significantly decreased degree of hydrolysis was found at "4/31 °C" compared with the "4 °C" and "4/100 °C" treatments in the case of the Prevalent and amylopectin starch. An accompanying small, but significant increase in RS content was seen in the amylopectin line at "4/31 °C". This was, however, not the case for Prevalent, were the RS content was significantly lower at "4/31 °C" compared with the other temperature treatments.

Retrograded amylopectin was found in all samples when studied by DSC. Treatments "4 °C" and "4/31 °C" did not result in any significant differences in amylopectin retrogradation. The reason for this could be either that the temperature 31 °C was not close enough to the melting temperature, or that the treatment time was too short to give detectable discrepancies. The amount of retrograded amylopectin found in "4/100 °C" samples indicates that some amylopectin retrogrades during the drying step within the preparation procedure. Differences in melting of retrograded amylopectin in line 342 compared with line 527-1 and Prevalent may be due to the low amylopectin content. As indicated by the high T_o, the genetic alteration of line 342 may also have caused structural changes of the amylopectin.

The absence of differences in amounts of retrograded amylose as judged from DSC was unexpected. Line 342 was expected to produce the highest amounts of retrograded amylose, due to the high amylose content. However, only a weak tendency in this direction was found. The temperature treatments, e.g., boiling at 100 °C may have been too weak to cause considerable differences, alternatively, repeated treatments may be needed. Previously, retrograded amylose and RS fractions have been reported to give transition enthalpy values of 2–40 J/g (Eberstein, Höpcke, Koniecny-Janda, & Stute, 1980; Shamai, Bianco-Peled, & Shimoni, 2003; Sievert & Pomeranz, 1989). The conclusion temperatures 138–148 °C were low compared with previously reported results (Sievert & Pomeranz, 1989; Sievert & Würsch, 1993). The melting transition in the region for retrograded amylose found in samples from line 527-1, containing almost exclusively amylopectin, indicated that outer chains of

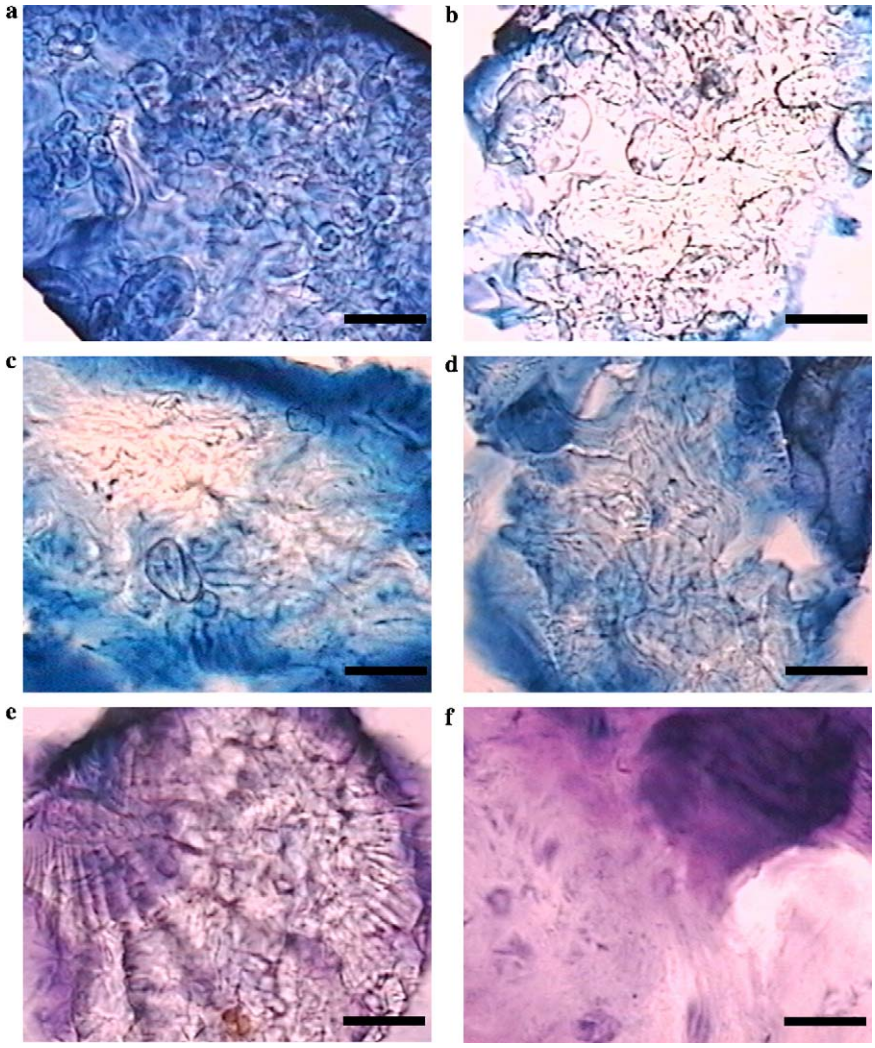

Fig. 1. Micrographs of boiled and time/temperature treated potato starches varying in amylose/amylopectin ratio. (a) 342 "4/31 °C", (b) 342 "4/100 °C", (c) Prevalent "4/31 °C", (d) Prevalent "4/100 °C", (e) 527-1 "4/31 °C", (f) 527-1 "4/100 °C". Scale bar is 50 μm.

amylopectin may act similarly to amylose. This was supported by the results obtained with the mother line Prevalent.

All samples were crystalline according to X-ray diffraction patterns, although DSC results could not satisfactory explain whether the crystallinity originated from amylose or amylopectin. Microscopy pictures clearly showed that some samples contained intact starch granules. Consequently, some of the resistant starch determined, at least in line 342, was most likely RS type 2.

One interesting question is whether the retrograded amylopectin was recovered by the in vitro RS method used in this study. Most studies have shown that RS type 3, retrograded starch, present in common heat treated starchy foods most frequently consists of retrograded amylose. The contribution from retrograded amylopectin has been shown (Cui & Oates, 1997; Eerlingen et al., 1994), though it seems as if retrograded amylopectin is less resistant to digestive enzymes than retrograded amylose. In contrast, Rabe and Sievert (1992) concluded that retrograded amylopectin is not involved in RS formation. In the present study, the RS contribution from amylopectin starch was negligible, although a decreased degree of hydrolysis was found after the temperature treatment "4/31 °C". These findings were in line with previous work using similar in vitro analysis methods (Fredriksson et al., 2000). A possible explanation may be that the in vitro incubation step at 37 °C, chosen to mimic physiologic conditions (Åkerberg et al., 1998b), melts some of the retrograded amylopectin, although this effect is likely to be negligible (Table 3). More importantly, the incubation conditions are critical for the extent of hydrolysis in the case of retrograded starches (Cui & Oates, 1997; Eerlingen et al., 1994). Consequently, when incubation time exceeds 2 h, RS levels were considerably diminished, suggesting that enzyme resistance is not an absolute feature.

Increased RS contents have previously been found in boiled potato tubers following cold storage over night (Leeman et al., 2005; Tschäppät, 2000). However, HI was significantly increased after reheating to 70 °C for 24 h (Leeman et al., 2005). After reheating to 100 °C of cool

stored cooked potatoes or retrograded amylopectin, RS contents decreased (Englyst & Cummings, 1987; Tschäppät, 2000), suggesting that retrograded amylopectin at least partly is responsible for incomplete digestion of starch in cool stored potatoes. Further, Cui and Oates (1997) discuss the potential decrease in amylase availability emanating from the combined influence of the development of the amylose gel network and increased molecular order due to amylopectin retrogradation, indicative of amylase barriers emanating from interactions between amylose and amylopectin.

In the present study, it was clearly shown that retrograded amylopectin contributed to a decreased HI, whereas no influence could be observed on RS contents. The absence of retrograded amylopectin appearing as RS might be due to the incubation method used. However, this method was chosen to mimic physiological conditions and has been suggested as the preferred method after comparing three different methods for RS determination (Li, Blackwell, Behall, & Liljeberg Elmståhl, 2001). The content of amylose was found to be the main factor influencing RS contents, although neither the presence of intact granules, nor the content of retrograded amylose could completely explain the results. Therefore, a synergistic effect between the starch components could be suggested to affect both the RS and starch hydrolysis.

Acknowledgements

Financial support was obtained from VINNOVA, Orkla Foods AB and Plant Science Sweden AB.

References

Åkerberg, A., Liljeberg, H., & Björck, I. (1998a). Effects of amylose/amylopectin ratio and baking conditions on resistant starch formation and glycaemic indices. *Journal of Cereal Science, 28*(1), 71–80.

Åkerberg, A. K., Liljeberg, H. G., Granfeldt, Y. E., Drews, A. W., & Björck, I. M. (1998b). 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*(3), 651–660.

Asp, N. G. (1992). Resistant starch – Proceedings from the 2nd Plenary Meeting of Euresta – European Flair Concerted Action 11 on Physiological Implications of the Consumption of Resistant Starch in Man. *European Journal of Clinical Nutrition, 46*, S1.

Berry, C. S. (1986). Resistant starch: formation and measurement of starch that survives exhaustive digestion with amylolytic enzymes during the determination of dietary fibre. *Journal of Cereal Science, 4*, 301–314.

Björck, I., Liljeberg, H., & Östman, E (2000). Low glycaemic-index foods. *British Journal of Nutrition, 83*, 149–155.

Björck, I. M., & Siljeström, M. A. (1992). In-vivo and in-vitro digestibility of starch in autoclaved pea and potato products. *Journal of the Science of Food and Agriculture, 58*, 541–553.

Brouns, F., Björck, I., Frayn, K. N., Gibbs, A. L., Lang, V., Slama, G., et al. (2005). Glycaemic index methodology. *Nutrition Research Reviews, 18*, 145–171.

Brouns, F., Kettlitz, B., & Arrigoni, E. (2002). Resistant starch and "the butyrate revolution". *Trends in Food Science and Technology, 13*, 251–261.

Cui, R., & Oates, C. G. (1997). The effect of retrogradation on enzyme susceptibility of sago starch. *Carbohydrate Polymers, 32*(1), 65–72.

Eberstein, K., Höpcke, R., Koniecny-Janda, G., & Stute, R. (1980). DSC investigations of starches 1. Feasibility of thermoanalytical methods to characterize starches. *Starch/Stärke, 32*(12), 397–404.

Eerlingen, R. C., Crombez, M., & Delcour, J. A. (1993). Enzyme-resistant starch. I. Quantitative and qualitative influence of incubation time and temperature of autoclaved starch on resistant starch formation. *Cereal Chemistry, 70*(3), 339–344.

Eerlingen, R. C., Jacobs, H., & Delcour, J. A. (1994). Enzyme-resistant starch 5. Effect of retrogradation of waxy maize starch on enzyme susceptibility. *Cereal Chemistry, 71*(4), 351–355.

Englyst, H. N., & Cummings, J. H. (1987). Digestion of polysaccharides of potato in the small intestine of man. *American Journal of Clinical Nutrition, 45*(2), 423–431.

Englyst, H. N., & Kingman, S. M. (1990). Dietary fiber and resistant starch. In D. Kritchevsky, C. Bonfield, & J. W. Anderson (Eds.), *Dietary fiber – Chemistry, physiology, and health effects* (pp. 49–65). New York: Plenum Press.

Fredriksson, H., Björck, I., Andersson, R., Liljeberg, H., Silverio, J., Eliasson, A. C., et al. (2000). Studies on alpha-amylase degradation of retrograded starch gels from waxy maize and high-amylopectin potato. *Carbohydrate Polymers, 43*(1), 81–87.

Frost, G., Leeds, A. A., Dore, C. J., Madeiros, S., Brading, S., & Dornhorst, A. (1999). Glycaemic index as a determinant of serum HDL-cholesterol concentration. *Lancet, 353*(9158), 1045–1048.

Gidley, M. J. (1989). Molecular mechanisms underlying amylose aggregation and gelation. *Macromolecules, 22*, 351–358.

Gidley, M. J., Cooke, D., Darke, A. H., Hoffmann, R. A., Russell, A. L., & Greenwell, P. (1995). Molecular order and structure in enzyme-resistant retrograded starch. *Carbohydrate Polymers, 28*, 23–31.

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*(9), 649–660.

Granfeldt, Y., Drews, A., & Björck, I. (1995). Arepas made from high amylose corn flour produce favorably low glucose and insulin responses in healthy humans. *Journal of Nutrition, 125*(3), 459–465.

Hallert, C., Björck, I., Nyman, M., Pousette, A., Granno, C., & Svensson, H. (2003). Increasing fecal butyrate in ulcerative colitis patients by diet: Controlled pilot study. *Inflammatory Bowel Diseases, 9*(2), 116–121.

Hoebler, C., Karinthi, A., Chiron, H., Champ, M., & Barry, J. L. (1999). Bioavailability of starch in bread rich in amylose: Metabolic responses in healthy subjects and starch structure. *European Journal of Clinical Nutrition, 53*(5), 360–366.

Holm, J., Björck, I., Drews, A., & Asp, N.-G. (1986). A rapid method for the analysis of starch. *Starch/Stärke, 38*(7), 224–226.

Järvi, A. E., Karlström, B. E., Granfeldt, Y. E., Björck, I. E., Asp, N. G., & Vessby, B. O. (1999). Improved glycemic control and lipid profile and normalized fibrinolytic activity on a low-glycemic index diet in type 2 diabetic patients. *Diabetes Care, 22*(1), 10–18.

Jenkins, D. J., Jenkins, A. L., Wolever, T. M., Collier, G. R., Rao, A. V., & Thompson, L. U. (1987). Starchy foods and fiber: reduced rate of digestion and improved carbohydrate metabolism. *Scandinavian Journal of Gastroenterology Supplement, 129*, 132–141.

Karlsson, M. E., & Eliasson, A.-C. (2003). Gelatinization and retrogradation of potato (*Solanum tuberosum*) starch in situ as assessed by differential scanning calorimetry (DSC). *Lebensmittel – Wissenschaft und-Technologie/FST, 36*(8), 735–741.

Leeman, A. M., Bårström, L. M., & Björck, I. M. E. (2005). In vitro availability of starch in heat-treated potatoes as related to genotype, weight and storage time. *Journal of the Science of Food and Agriculture, 85*(5), 751–756.

Li, B. W., Blackwell, E. L., Behall, K. M., & Liljeberg Elmståhl, H. G. M. (2001). Resistant starch and total dietary fiber content of oatrim muffins with different levels of amylose, amylopectin, and β-glucan. *Cereal Chemistry, 78*(4), 387–390.

McKeown, N. M., Meigs, J. B., Liu, S., Saltzman, E., Wilson, P. W. F., & Jacques, P. F. (2004). Carbohydrate nutrition, insulin resistance, and the prevalence of the metabolic syndrome in the Framingham Offspring Cohort. *Diabetes Care, 27*(2), 538–546.

Miles, M. J., Morris, V. J., Orford, P. D., & Ring, S. G. (1985). The roles of amylose and amylopectin in the gelation and retrogradation of starch. *Carbohydrate Research, 135*, 271–281.

Rabe, E., & Sievert, D. (1992). Effects of baking, pasta production, and extrusion cooking on formation of resistant starch. *European Journal of Clinical Nutrition, 46*, S105–S107.

Scheppach, W., Fabian, C., Sachs, M., & Kasper, H. (1988). The effect of starch malabsorption on fecal short-chain fatty acid excretion in man. *Scandinavian Journal of Gastroenterology, 23*(6), 755–759.

Shamai, K., Bianco-Peled, H., & Shimoni, E. (2003). Polymorphism of resistant starch type III. *Carbohydrate Polymers, 54*(3), 363–369.

Sievert, D., Czuchajowska, Z., & Pomeranz, Y. (1991). Enzyme-resistant starch. III. X-ray diffraction of autoclaved amylomaize VII and enzyme-resistant starch residues. *Cereal Chemistry, 68*(1), 86–91.

Sievert, D., & Pomeranz, Y. (1989). Enzyme-resistant starch. I. Characterization and evaluation by enzymatic, thermoanalytical, and microscopic methods. *Cereal Chemistry, 66*(4), 342–347.

Sievert, D., & Pomeranz, Y. (1990). Enzyme-resistant starch. II. Differential scanning calorimetry studies on heat-treated starches and enzyme-resistant starch residues. *Cereal Chemistry, 67*(3), 217–221.

Sievert, D., & Würsch, P. (1993). Thermal behaviour of potato amylose and enzyme-resistant starch from maize. *Cereal Chemistry, 70*(3), 333–338.

Silverio, J., Fredriksson, H., Andersson, R., Eliasson, A.-C., & Åman, P. (2000). The effect of temperature cycling on the amylopectin retrogradation of starches with different amylopectin unit-chain length distribution. *Carbohydrate Polymers, 42*, 175–184.

Tschäppät, S. (2000). Influence of processing on in vitro digestibility and fermentability of potatoes. Ph.D. thesis No. 13709, Swiss Federal Institute of Technology, Zurich.

Tufvesson, F., Skrabanja, V., Björck, I., Elmståhl, H. L., & Eliasson, A. C. (2001). Digestibility of starch systems containing amylose–glycerol monopalmitin complexes. *Lebensmittel-Wissenschaft Und-Technologie-Food Science and Technology, 34*(3), 131–139.

Wolever, T. M. (1990). The glycemic index. *World Review of Nutrition and Dietetics, 62*, 120–185.