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Some physical and nutritional characteristics of genetically modified potatoes varying in amylose/amylopectin ratios

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Abstract

Transgenically modified potatoes with a large spread in amylose/amylopectin ratios were analysed both as tubers and in the form of isolated starch. Different microscopic techniques were used to study starch granules and tuber tissue. Starch gelatinisation properties and recrystallisation of amylopectin and amylose were studied by differential scanning calorimetry. Starch bioavailability and resistant starch (RS) were evaluated using enzymatic in vitro procedures. Glycaemic indices (GI) were predicted from low molecular weight carbohydrates (LMWC) contents and the in vitro hydrolysis rate of the starch moiety. For many of the examined parameters, differences of varying magnitude were found between the potato lines, especially for high amylose lines. High amylose starch granules had irregular shapes and showed only a limited swelling. Moreover, contents of RS and recrystallised amylose were elevated. GI's for the starch moiety were reduced, though elevated contents of LMWC caused a high over-all predicted GI.

Keywords: *Solanum tuberosum*; Potato; Starch; Amylose; Amylopectin; Resistant starch; Microscopy

1. Introduction

Starches with different amylose/amylopectin ratios are of interest since the two components behave very differently both from a technological (Jobling, 2004; Kalichevsky, Orford, & Ring, 1990) and nutritional (Åkerberg, Liljeberg, & Björck, 1998a; Amelsvoort & Weststrate, 1992; Granfeldt, Drews, & Björck, 1995) perspective. In wild type potatoes, there are no major differences in amylose/amylopectin ratios. Normally, potato starch contains 20–27% amylose (w/w) of total starch depending on variety and method of determination (Fredriksson, Silverio, Andersson, Eliasson, & Åman, 1998; Swinkels, 1985). To obtain potatoes with very high amylose or amylopectin contents, genetic modification is required. Granule bound starch synthase (GBSS), is responsible for the synthesis of amylose. Suppression of GBSS results in starch with very high amylopectin contents (Blennow et al., 2003; Fulton et al., 2002; Jobling, 2004; Visser, Suurs, Bruinenberg, Bleeker, & Jacobsen, 1997a). On the other hand, attaining potato starch high in amylose (more than 60%) require simultaneous inhibition of two starch branching enzymes (SBE1 and SBE2) (Schwall et al., 2000).

The amylose/amylopectin ratios affect starch gelatinisation as well as recrystallisation properties. A shift of gelatinisation to higher temperatures has been reported both for high amylose maize starch (Shi, Capitani, Trzasko, & Jeffcoat, 1998) and for amylopectin potato starch (Fredriksson et al., 1998; McPherson & Jane, 1999; Svegmark et al., 2002; Visser, Suurs, Steeneken, & Jacobsen, 1997b). The recrystallisation of amylose and amylopectin differs considerably. Recrystallisation of amylose is normally completed within 48 h, while recrystallisation of amylopectin may continue for weeks (Miles, Morris,

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Orford, & Ring, 1985). The melting onset temperature of recrystallised amylopectin is around 40 °C, whereas recrystallised amylose melts above 120 °C (Sievert & Pomeranz, 1990). Differential scanning calorimetry (DSC) can be used to study starch gelatinisation as well as recrystallisation of both the amylopectin and amylose fractions of starch.

The recrystallisation process may affect the bioavailability of starch in the gastro-intestinal tract, and recrystallised starch constitute a starch fraction which may be delivered to the large bowel, so-called resistant starch (RS). Other established forms of RS are physically inaccessible starch and crystalline starch granules of the B-type. Recrystallised amylose is considered to be an important source of RS in processed foods, whereas the contribution of RS in the form of recrystallised amylopectin is less well known. From a nutritional point of view, RS share properties in common with other indigestible carbohydrates, e.g., dietary fibre. Recrystallisation may also affect the readiness by which the available starch fraction is digested and absorbed; thus, influencing post-prandial glycaemia and hormonal responses (Åkerberg et al., 1998a). Consequently, in vitro measurements of starch hydrolysis rate and calculation of hydrolysis index (HI) may be used to predict a glycaemic index (GI) of starch and starchy foods (Granfeldt, Björck, Drews, & Tovar, 1992). A number of studies indicate that an increase in amylose content is accompanied with lowered blood glucose and insulin responses (Amelsvoort & Weststrate, 1992; Behall & Hallfrisch, 2002; Goddard, Young, & Marcus, 1984; Granfeldt et al., 1995). Such foods, i.e., foods with a low GI, are considered to produce metabolic advantages (Björck, Liljeberg, & Östman, 2000; Järvi et al., 1999; Salmeron et al., 1997a; Salmeron et al., 1997b). Potato products are, with few exceptions, known to produce high GI's. Therefore, the identification of a potato variety which maintains a GI in the lower range upon cooking would be desirable, and the potential of potatoes with a large spread in amylose/amylopectin ratio is interesting.

Most studies regarding the gelatinisation and recrystallisation phenomena have been performed on isolated starches, and it could be argued that the physical behaviour of starch in situ might be different and influenced by the potato tissue. Moreover, starch and other components are unevenly distributed in the tuber (Fedec, Ooraikul, & Hadziyev, 1977; Karlsson & Eliasson, 2003; Reeve, Hautala, & Weaver, 1969). The outer parts, cortex, has the highest dry matter content, whereas the pith tissue in the centre of the tuber has high water content. The distribution within the tissue may affect experiments based on tuber samples and is therefore of importance to study. Light microscopy (LM) is suitable for the study of isolated starches, whereas for tissue samples extensive pretreatments are needed. Pretreatments as fixation, embedding and cutting may introduce artifacts. Cryo-fixation is an alternative fixation method that is widely used for scanning electron microscopy (SEM). SEM has been used to study both starch and other cell components in the potato (Martens & Thybo, 2000; McComber, Horner, Chamberlin, & Cox, 1994; van Marle, Clerkx, & Boekstein, 1992). One advantageous microscopy method for tissue samples is confocal laser scanning microscopy (CLSM) (Alvarez, Saunders, & Vincent, 2000; Dürrenberger, Handschin, Conde-Petit, & Escher, 2001; Ferrando & Spiess, 2000). CLSM provides the opportunity to study the sample in situ. Artifacts from fixation, embedding and cutting are avoided due to the possibility to use thick samples that can be sectioned optically.

In this study, potatoes with large variation in amylose/amylopectin ratios were used. The distribution and morphology of starch within the tuber as well as isolated starch was studied using CLSM, SEM or LM. Starch gelatinisation and recrystallisation properties were examined by DSC, both in starch–water mixtures and in situ. The possible influence of amylose content on starch bioavailability and RS formation was evaluated using enzymatic in vitro procedures. A GI was predicted from the in vitro hydrolysis rate and the content of low molecular weight carbohydrates, which was substantial in the high amylose lines.

2. Materials and methods

2.1. Materials

Three mother control potato lines (*Solanum tuberosum* cv Dinamo (Din), Prevalent (Pre) and Producent (Pro)) and five transgenic potato lines derived from these (Table 1) were included in the study. Tubers and isolated starch materials were supplied by BASF Plant Science Sweden. The transgenic lines were derived by inhibition of GBSS (line 527-1), SBE2 (line 2017), or SBE1 and SBE2 (lines 342, 418 and 715). Tubers were planted in May and harvested in late September 2002. Analyses were performed 17–24 weeks after harvest; the storage temperature was 6 °C. For some analyses, tubers grown under similar conditions and harvested in 2003 were used. These tubers were stored at 6 °C and analysed 15–17 weeks after harvest. Information regarding amylose/amylopectin ratios

Table 1
Amylose content (g/100 g), mean starch granule diameter (μm) and dry matter contents (DM, g/100 g) for five genetically modified potato lines and the three mother lines

Sample	Amylose content	Granule size	DM 2002	DM 2003	Mother line
715	78	27.1	11.0 ± 0.2	12.1 ± 1.8	Din
342	64	31.7	17.3 ± 0.8	na	Pre
418	66	31.8	23.4 ± 0.9	21.9 ± 3.5	Pro
2017	22	46.0	28.7 ± 1.4	27.6 ± 2.1	Din
527-1	1	48.1	25.7 ± 0.5	24.4 ± 2.1	Pre
Din	22	55.7	24.3 ± 4.7	27.6 ± 1.0	
Pre	23	47.6	24.9 ± 2.2	27.8 ± 1.1	
Pro	23	46.0	27.8 ± 0.7	29.1 ± 2.1	

na, not analysed.

determined by size exclusion chromatography after enzymolysis was provided by BASF Plant Science Sweden.

2.2. Boiling of tubers

Potato tubers were boiled unpeeled in approximately 1 l of water. The ready to eat consistency was checked mechanically with a metal stick (diameter 1 mm) and time and core temperatures were registered. Depending on potato line, the final core temperature differed between 85 and 96 °C, and the cooking time ranged from 20 to 30 min. After boiling, the tubers were placed in plastic bags and cooled in tap water for 10 min.

2.3. Dry matter content

Slices were cut from boiled and peeled potatoes and pressed through a commercial ricer (diameter 3 mm). The pressed tuber substance was dried over night at 105 °C. Reported values (Table 1) are averages based on at least three measurements.

2.4. Granule size distribution

The size distribution and area-weighted average diameter of granules was determined by using a Coulter LS 130 (Beckman Coulter, High Wycombe, UK) with refractive index according to Bromley and Hopkinson (2002).

2.5. Microscopy

2.5.1. Confocal laser scanning microscopy (CLSM)

Slices of 1 mm height with the sides 3×3 mm were cut from pith, cortex, and stem end parenchyma tissues of raw potato. From boiled potatoes cortex tissue was used. Slices were stained with Acridine Orange, 0.01 g/100 ml 0.1 M phosphate buffer (pH 7). A confocal laser scanning microscope (Multiprobe 2001, Molecular Dynamics, Inc., USA), equipped with an Ar/Kr laser and an inverted Nikon Diaphot TMD microscope was used. Samples were excited by an Argon laser beam at 488 nm and emitted light was selected by filters detecting starch and cell walls at 530–565 nm, and proteins above 570 nm. Digitalised images were processed and analysed with ImageSpace software (Molecular Dynamics, Inc., USA) on a Silicon Graphics 310GTX Power Series workstation.

2.5.2. Light microscopy (LM)

Starch grains were suspended in water and viewed with polarised light or stained with a diluted Lugols solution (I_2/KI solution; 1:2 w/w). A Linkam THMS 600 heating stage, controlled with Linkam LNP and TMS 93 systems (Tadworth, UK) was used to heat starch in excess water from 25 to 100 °C with a rate of 5 °C min^{-1}. Micrographs were captured at selected temperatures. Observations were made using an Olympus BX-50 microscope (Olympus, Japan).

2.5.3. Scanning electron microscopy (SEM)

From cortex tissue of raw and boiled tubers, cylinders with a diameter of 3.5 mm were punched and cut to approximately 3 mm thick slices. The samples were frozen in liquid nitrogen and freeze-dried over night. Samples were stored in a desiccator before analysis. Both broken and cut samples were mounted onto aluminium specimen stubs with conductive carbon cement, coated with Au/Pd in a Polaron SC7640 sputter coater (Polaron Range, East Grinstead, UK) and examined in a Jeol 5600LV scanning electron microscope (JEOL, Tokyo, Japan) at 5 kV.

2.6. Differential scanning calorimetry (DSC)

Sample preparation and DSC analyses were performed as described previously (Karlsson & Eliasson, 2003). From fresh potatoes samples were taken from cortex tissue. Starch was analysed with double-distilled water in a 1:3 ratio. The scans were performed over the temperature range of 6–105 °C for gelatinisation and 6–195 °C for recrystallisation, each with a scanning rate of 10 °C min^{-1}. Samples for recrystallisation experiments were gelatinised and then stored at 6 °C for up to 7 days before DSC analysis. Transition enthalpy (ΔH expressed as J per g), onset temperature (T_o), peak temperature (T_p) and conclusion temperature (T_c) were determined. The specific dry matter content for each sample, determined by puncturing the pans and drying them in an oven at 105 °C for 16 h after the DSC scan, was used to calculate ΔH. All results are the means of at least three measurements from different tubers for potato tissue, or three measurements for isolated starch.

2.7. Starch content

Potentially available starch was determined in boiled and peeled tubers and gelatinised isolated starches (5% concentration) according to Holm, Björck, Drews, and Asp (1986). This method utilises thermo stable α-amylases which may degrade certain types of RS. Total starch content was analysed in isolated starches and in tubers harvested in 2003 according to Björck and Siljeström (1992) and this analytical procedure includes a solubilisation of the retrograded starch with KOH. Starch analyses were performed on three to six tubers from each line and year. Calculations were based on the specific dry matter content for each sample.

2.8. Low molecular weight carbohydrate content

Glucose, fructose and sucrose contents were determined in the boiled and peeled tubers with an enzymatic kit (Diffchamb AB, Gothenburg, Sweden). Analyses were performed on one to two tubers from each potato line and year. Calculations were based on the specific dry matter content for each sample.

2.9. Resistant starch (RS) content

RS analysis was performed on the isolated starches. Prior to analysis, the starch was suspended in millipore water (concentration 5%) and heated in a water bath at 60 °C for 15 min. The samples were stirred every third minute. Thereafter, the samples were placed in a boiling water bath for 15 min, with continued stirring every third minute. Samples were boiled since the aim was not to fully melt amylose but to analyse RS contents from a practical cooking perspective. The RS analysis started 5–10 min after the heat treatment and was performed according to Åkerberg, Liljeberg, Granfeldt, Drews, and Björck (1998b) with the exception that incubation was performed at 37 °C instead of 40 °C (Fredriksson et al., 2000). The method allows parallel determination of RS and available starch by utilising a physiological approach mimicking the conditions in the gastrointestinal tract. To avoid exposure of the test subjects to the genetically modified potatoes, the chewing step was omitted and instead glass beads were chewed to stimulate saliva production, whereas potato slices were pressed through a commercial ricer. Calculations were based on the specific dry matter content for each sample.

2.10. In vitro rate of starch hydrolysis

Potatoes were boiled as described above and peeled. Samples were taken as previously described (Leeman, Bårström, & Björck, 2005) with the modification that slices were pressed through a commercial ricer (diameter 3 mm). Determination of HI in tubers harvested 2003 was performed on tubers that were boiled and frozen prior to freeze-drying. Freeze-dried samples were gently grinded in a mortar and stored in a desiccator before analysis.

Prior to evaluation of isolated starches, the starch was suspended in phosphate buffer (concentration 5%) and heated in a water bath at 60 °C for 15 min. The samples were stirred every third minute. Thereafter, the samples were placed in a boiling water bath for 15 min, with continued stirring every third minute. The analysis started 5–10 min after the heat treatment.

The rate of in vitro starch hydrolysis was analysed according to Granfeldt et al. (1992). However, the chewing step was omitted and instead potato slices were pressed through a commercial ricer and glass beads were used to stimulate saliva production. The reducing capacity of low molecular weight carbohydrates was withdrawn, by using a similar procedure as for determination of HI, though omitting the amylolytic enzymes. The HI values presented are thus the HI values obtained in the in vitro system less the HI values obtained when amylolytic enzymes were omitted. 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 for the starch moiety (GI_{HI}) were predicted from the hydrolysis graphs using the equation $GI = 6.272 + 0.912 \times HI$ (Leeman et al., 2005). To predict a GI, taking into account also the presence of carbohydrates other than starch (GI_c), literature data for GI of pure fructose, glucose and sucrose, 27, 138 and 92, respectively (Foster-Powell & Miller, 1995), and GI_{HI} for the starch moiety, were used.

2.11. 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 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 and discussion

3.1. Starch granule distribution and morphology

Starch granules in the high amylose lines, 715, 342 and 418, were very small compared to granules in potato lines with high or normal amylopectin contents (Table 1). These results were in line with previously reported values (Hofvander, Andersson, Larsson, & Larsson, 2004). The starch granules were smaller than in the mother control line also in line 2017. Dry matter contents were generally lower for the high amylose lines compared with the control lines.

The distribution of starch within the tubers differed between the potato lines. In general, most starch is located in cortex tissue while pith tissue contains minor amounts of starch (Karlsson & Eliasson, 2003). This was true for most of the potato lines studied; however, line 418 had comparatively high starch contents in all parts of the tuber (Fig. 1). Line 715 had the lowest starch content, and as a consequence also the cortex tissue contained only small amounts of starch.

The starch in high amylose lines had a shape resembling that of normal starch granules; however, the surface was irregular and many of the granules possessed asymmetrical fissures (Figs. 1–3). This has also been seen in other transformed potato starches with high amylose contents (Blennow et al., 2003; Edwards et al., 1999; Schwall et al., 2000). The granules also demonstrated a tendency to form clusters (Bustos et al., 2004; Edwards et al., 1999) (Figs. 2 and 3). Starch granules from line 715 had a rather rough surface (Fig. 2). The granules were generally not birefringent as viewed under polarised light. During heating to 100 °C the granules remained essentially intact. Lines 342 and 418, containing somewhat higher ratios of amylopectin, both displayed more normal granule shapes compared to line 715. In addition, more starch granules were birefringent, and some granules showed normal gelatinisation

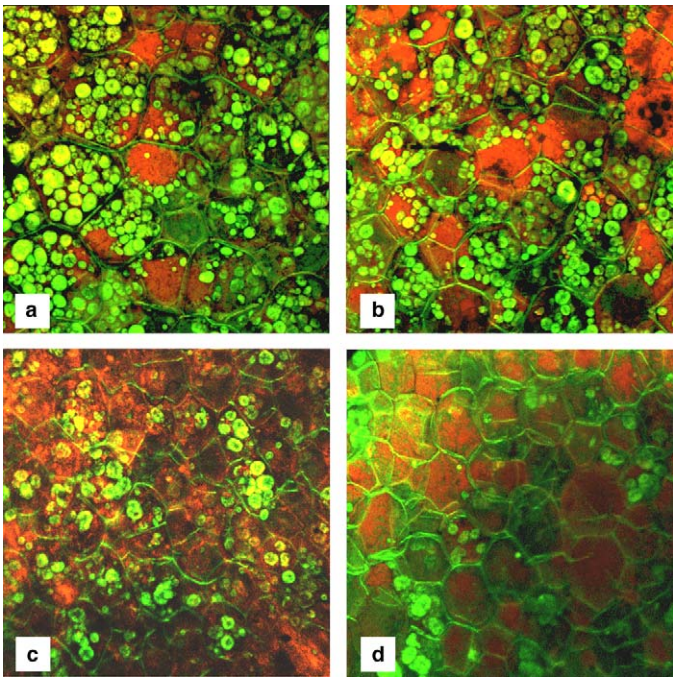

Fig. 1. CLSM image of potato tissue: (a) line 418 cortex, (b) line 418 pith, (c) line 715 cortex, (d) line 715 pith.

behaviour. The incomplete swelling corresponded to the findings of Ormerod, Ralfs, Jobling, and Gidley (2002), reporting that potato starch of 68% amylose content was still crystalline at 70 °C whilst gelatinised at 100 °C as indicated by crossed polars. Even at 100 °C this starch showed only limited swelling. Granules from line 2017 behaved in a similar way as the mother line, though the granules were smaller. With iodine staining, granules from the amylopectin line 527-1 stained red with a blue hilum core, suggesting that amylose was restricted to the core. This seems to be characteristic for GBSS antisense lines (Blennow et al., 2003; Fulton et al., 2002; Kuipers, Jacobsen, & Visser, 1994; Visser et al., 1997a), and indicates that a complete inhibition of GBSS could not be achieved (Kuipers et al., 1994). During heating line 527-1 displayed a normal starch behaviour.

In high amylose lines, the cell structure collapsed during cooking (Fig. 3). The cell wall seemed to shrink around the cell content but not to break. The collapsed cell structure caused the cells to separate. In line 2017, line 527-1 and in the mother lines, the swollen starch granules filled the cells causing a rounder shape. These cells did not show any severe cell separation.

3.2. Starch and low molecular weight carbohydrate contents

Large differences were found in sugar contents between the different lines (Table 2). Tubers from high amylose lines, especially line 715 contained large amounts of glucose, fructose and sucrose. High amylose content in potatoes seemed to be accompanied by higher sugar contents though Hofvander et al. (2004) only found an increase in glucose and fructose, and not in sucrose content.

In general, high amylose lines contained lower amounts of potentially available starch, both when analysed in tubers and in isolated starch (Table 3). Also, there was a tendency to lower starch contents in tubers from 2002 harvest compared with 2003 harvest, which may partly be explained by the higher sugar content in the 2002 harvest (Table 2).

3.3. Starch gelatinisation and recrystallisation

The gelatinisation properties of the different potato lines are given in Table 4 and Fig. 4. The high amylose line 715 gave a hardly detectable gelatinisation peak during the DSC-scan. The gelatinisation transition enthalpy (ΔH_{gel}, J/g dry matter) for lines 342 and 418 was significantly lower than for other samples, both for starch in situ and for isolated starch, and the transition temperatures were higher. Similar results have been reported for high amylose maize starches (Matveev et al., 2001; Shi et al., 1998). Line 2017 did not differ significantly from the mother line (Din). Starch isolated from the amylopectin line 527-1 gave a substantially higher ΔH_{gel} and transition temperatures than the mother line (Pre). This observation was in line with previous findings (Fredriksson et al., 1998; McPherson & Jane, 1999; Svegmark et al., 2002; Visser et al., 1997b). However, for starch in situ, ΔH_{gel} was lower for line 527-1 than for Pre, indicating an influence from other tuber constituents. As reported for other potato varieties (Karlsson & Eliasson, 2003), ΔH_{gel} was lower and T_o higher for starch in situ compared to isolated starches.

Recrystallisation of amylopectin increased during storage, both in situ and in isolated starches (Fig. 5). When starches with normal or high amylopectin contents were compared, no significant differences were found in ΔH as calculated by the amylopectin content (ΔH_{AP}, J/g amylopectin). In high amylose starches melting peaks of recrystallised amylopectin were less distinct. ΔH_{AP} for line 715 was lower than for the mother line. Lines 342 and 418, having comparable amylopectin contents, showed very different recrystallisation properties. Line 418 had the highest ΔH_{AP} of all starches whereas line 342 had the lowest. This indicates large differences in the amylopectin structure between these starches. Amylopectin recrystallisation of starch in situ gave results comparable to isolated starch for Pre, Pro and 527-1, whereas lines 2017 and Din gave slightly lower ΔH values, and for high amylose lines no recrystallised amylopectin was detected in situ (not shown).

Since the recrystallisation of amylose is rather fast, (Miles et al., 1985; Silverio, Svensson, Eliasson, & Olofsson, 1996), and no differences in amount of amylose recrystallised during 1, 3 or 7 days were detected, the reported values are averages from all samples measured (Table 5). Most recrystallised amylose was found in starch from line 715, furthermore, line 418 tended to give more recrystallised amylose than line 312. A peak in the temperature

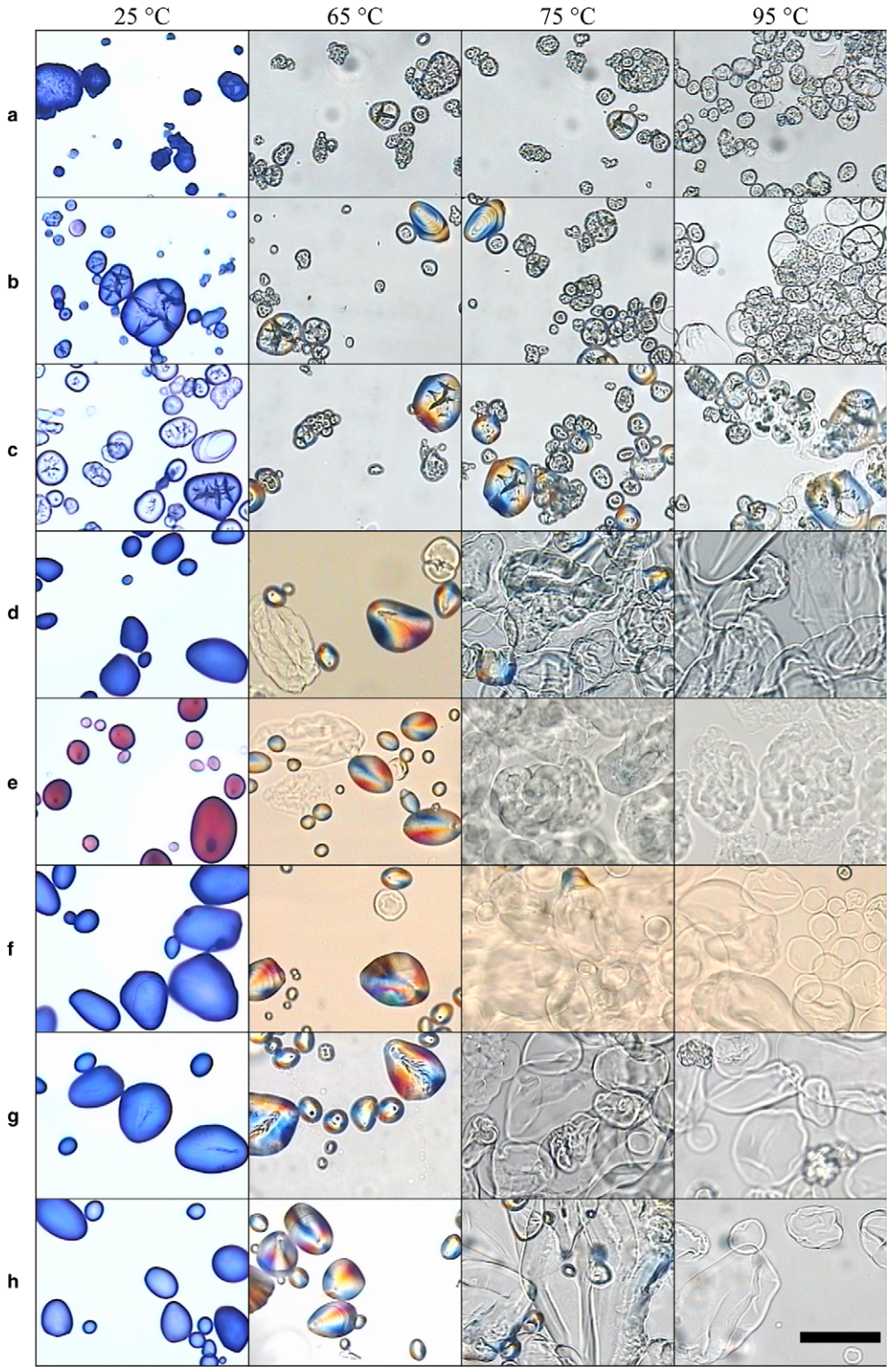

Fig. 2. Micrographs (LM) of isolated starches at different temperatures, (a) 715, (b) 342, (c) 418, (d) 2017, (e) 527-1, (f) Din, (g) Pre, (h) Pro. At 25 °C iodine stained, at 65, 75 and 95 °C viewed under polarised light. Scale bar is 100 μm.

range for melting of recrystallised amylose was detected also for the amylopectin line 527-1 (Fig. 6). A possible explanation for this could be that amylopectin outer chains of this transgenic line may display an amylose-like behaviour. Transition enthalpies for melting of recrystallised amylose (ΔH_{Am}, J/g dry matter) were low compared to previously reported results. Recrystallised amylose and RS fractions have been reported to give transition enthalpy values of 2–40 J/g depending on starch source and previous heat-cooling treatments (Biliaderis & Galloway, 1989;

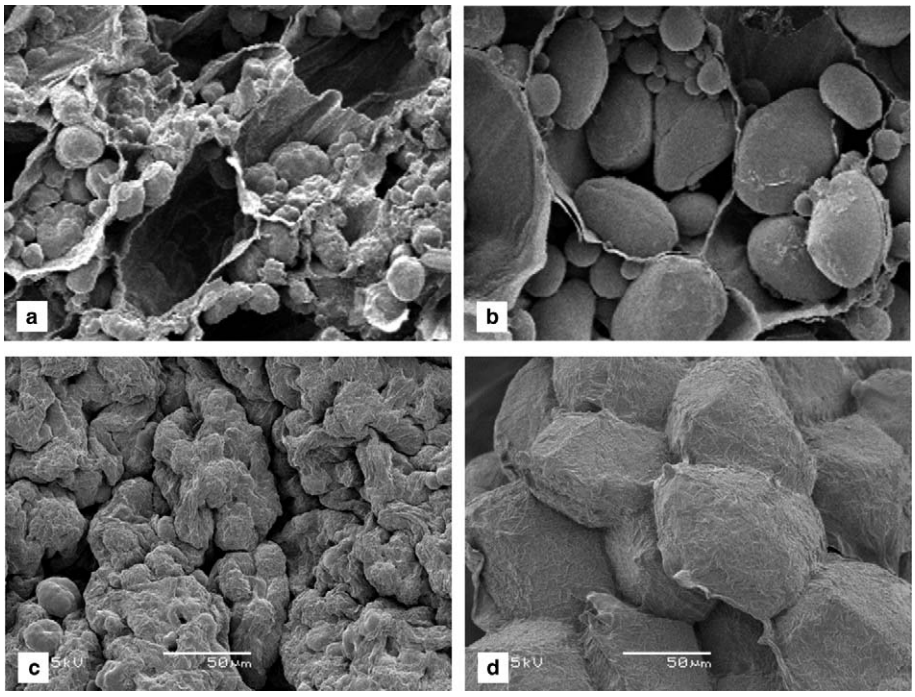

Fig. 3. SEM image of potato tissue: (a) raw line 715, (b) raw line 527-1, (c) heat treated line 715, (d) heat treated line 527-1. Magnification: 500×.

Table 2
Low molecular weight carbohydrate content (g/100 g dry matter) in heat-treated tubers harvested in 2002 and 2003

Sample	2002 Harvest				2003 Harvest			
	Glucose	Fructose	Sucrose	Total	Glucose	Fructose	Sucrose	Total
715	11.6 ± 0.2	7.4 ± 0.1	8.5 ± 0.5	27.5	8.2 ± 0.6	5.5 ± 0.5	15.2 ± 1.8	28.9
342	3.8 ± 0.1	2.5 ± 0.1	7.5 ± 0.6	13.8	na	na	na	na
418	2.8 ± 0.0	2.0 ± 0.1	8.4 ± 0.2	13.2	2.0 ± 0.5	1.8 ± 0.3	3.5 ± 0.8	7.3
2017	2.1 ± 0.1	1.4 ± 0.0	4.7 ± 0.3	8.2	1.8 ± 0.1	1.4 ± 0.3	3.5 ± 0.2	6.7
527-1	2.2 ± 0.2	1.9 ± 0.2	5.4 ± 0.2	9.5	1.7 ± 0.2	1.5 ± 0.0	3.5 ± 0.0	6.7
Din	1.2 ± 0.0	1.0 ± 0.0	3.9 ± 1.1	6.1	0.7 ± 0.1	0.7 ± 0.1	1.2 ± 0.0	2.6
Pre	1.1 ± 0.1	1.0 ± 0.0	2.9 ± 0.1	5.0	1.4 ± 0.0	1.3 ± 0.1	2.7 ± 0.0	5.4
Pro	1.9 ± 0.1	1.7 ± 0.0	4.6 ± 0.1	8.2	1.7 ± 0.0	1.5 ± 0.1	3.3 ± 0.0	6.5

Values are means ± s.d. of four replicates.
na = not analysed.

Table 3
Starch contents in heat treated tubers and isolated starches (g/100 g dry matter)

Sample	Total starch		Potentially available starch		
	2003 Harvest	Isolated starch	2002 Harvest	2003 Harvest	Isolated starch
715	64.9 ± 1.6	98.9 ± 1.2	36.9 ± 2.2	54.2 ± 5.8	75.0 ± 0.2
342	na	98.1 ± 0.8	53.5 ± 0.7	na	87.4 ± 0.5
418	70.9 ± 2.0	97.5 ± 0.1	57.1 ± 3.1	69.3 ± 5.6	86.2 ± 0.3
2017	78.4 ± 9.5	101.3 ± 2.4	72.1 ± 1.6	81.6 ± 2.1	100.0 ± 0.0
527-1	76.8 ± 4.1	100.3 ± 0.7	70.1 ± 13.8	81.5 ± 1.6	96.7 ± 0.8
Din	76.1 ± 3.2	95.2 ± 0.4	68.4 ± 6.4	72.2 ± 3.5	99.2 ± 1.2
Pre	81.3 ± 4.2	102.1 ± 1.8	68.8 ± 10.0	79.5 ± 4.6	98.7 ± 1.3
Pro	81.9 ± 2.1	105.3 ± 0.5	71.5 ± 9.3	80.6 ± 5.3	100.4 ± 0.3

Values are means ± s.d. of two to six replicates.
na, not analysed.

Eberstein, Höpcke, Koniecny-Janda, & Stute, 1980; Shamai, Bianco-Peled, & Shimoni, 2003; Sievert & Pomeranz, 1989). One reason for the discrepancies could be the preceding time–temperature treatments, another reason may be the structure of the amylose in the specific samples. For amylomaize starch (70% amylose) autoclaved once, Sievert and Pomeranz (1989) reported a transition enthalpy of 2.7 J/g.

Table 4
Gelatinisation transition enthalpies (ΔH_{gel}, J/g dry matter), and onset, peak and conclusion temperatures (T_o, T_p, T_c, °C) of tissue and isolated starch samples

Sample	Potato				Starch			
	ΔH_{gel}	T_o	T_p	T_c	ΔH_{gel}	T_o	T_p	T_c
715	n.d.	–	–	–	0.6 ± 0.6	60.7 ± 1.1	n.d.	80.8 ± 3.8
342	6.4 ± 1.6	66.4 ± 1.1	75.6 ± 1.0	85.8 ± 1.6	6.7 ± 1.6	64.5 ± 1.1	80.2 ± 1.0	94.6 ± 1.6
418	6.2 ± 0.6	62.8 ± 2.5	78.7 ± 1.3	91.1 ± 2.8	6.9 ± 0.6	61.3 ± 2.5	76.4 ± 1.3	90.0 ± 2.8
2017	14.6 ± 0.4	59.6 ± 1.1	70.4 ± 0.7	81.4 ± 1.1	18.3 ± 0.4	58.3 ± 1.3	67.6 ± 0.8	81.3 ± 1.3
527-1	14.7 ± 0.2	66.1 ± 0.5	71.4 ± 0.6	83.4 ± 1.2	19.0 ± 2.3	60.5 ± 1.2	69.5 ± 0.5	83.3 ± 1.3
Din	15.2 ± 0.6	61.9 ± 1.9	71.2 ± 0.6	83.4 ± 1.9	18.8 ± 2.2	58.1 ± 1.6	67.4 ± 1.3	81.2 ± 2.2
Pre	15.5 ± 0.6	57.1 ± 0.9	67.3 ± 0.5	79.4 ± 0.7	16.4 ± 1.6	54.8 ± 0.7	64.6 ± 0.4	75.8 ± 0.7
Pro	15.1 ± 0.7	57.2 ± 0.5	67.5 ± 0.4	79.5 ± 2.3	17.6 ± 0.7	54.2 ± 0.5	65.2 ± 0.4	77.8 ± 2.3

Values are means ± s.d. of three replicates.
n.d., not detected.

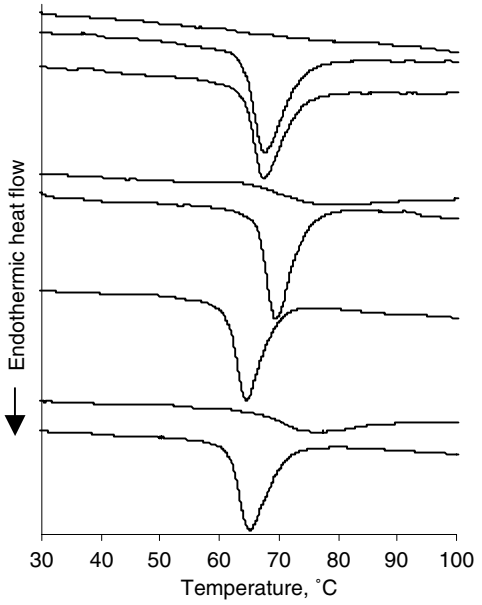

Fig. 4. DSC thermograms showing gelatinisation of isolated starch samples. Order from top to bottom: 715, 2017, Din, 342, 527-1, Pre, 418 and Pro.

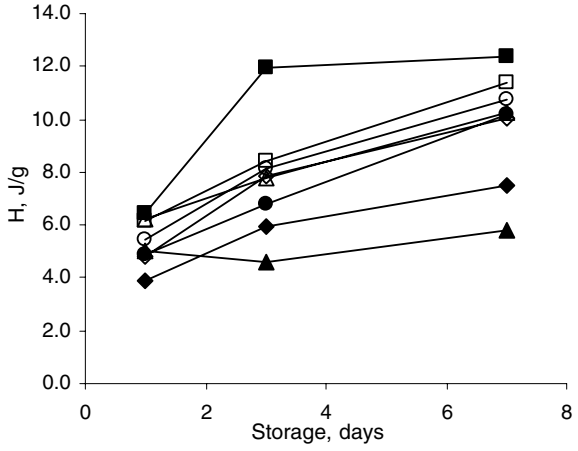

Fig. 5. Enthalpy of melting of recrystallised amylopectin in isolated starch samples as a function of storage time, ΔH is given as J/g amylopectin. ◆ 715, ▲ 342, ■ 418, ○ 2017, ◇ 527-1, □ Din, ● Pre, D Pro.

Table 5
Melting of recrystallised amylose: transition enthalpies (ΔH_{Am}, J/g dry matter), onset and conclusion temperatures (T_o and T_c, °C) of isolated starch samples

Sample	ΔH_{Am}	T_o	T_c
715	2.5 ± 0.7	111.8 ± 3.2	142.5 ± 3.9
342	1.4 ± 0.8	107.0 ± 2.2	134.1 ± 1.0
418	1.9 ± 0.5	109.9 ± 2.1	145.7 ± 3.2
2017	n.d.	n.d.	n.d.
527-1	1.7 ± 0.3	118.1 ± 3.8	144.8 ± 6.2
Din	n.d.	n.d.	n.d.
Pre	n.d.	n.d.	n.d.
Pro	n.d.	n.d.	n.d.

Values are means ± s.d. of at least three replicates.
n.d., not detected.

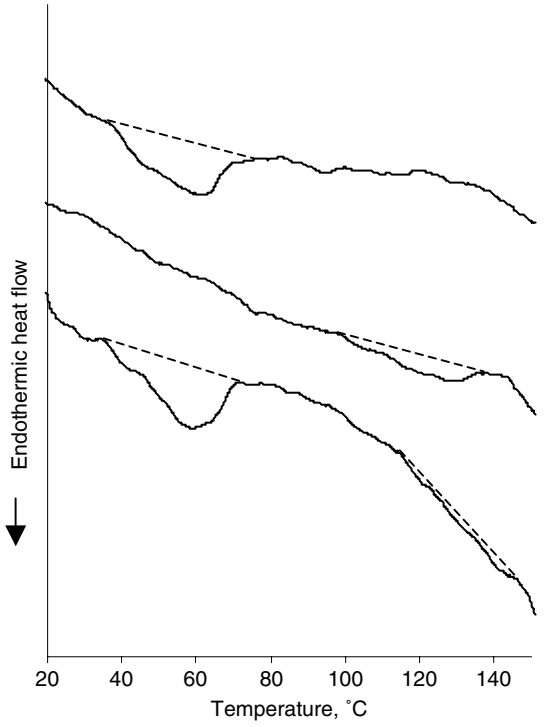

Fig. 6. DSC thermograms showing melting of recrystallised starch in isolated starch samples from one mother line, one high-amylose line, and the amylopectin line, all stored 7 days after gelatinisation. Order from top to bottom: Pre, 715 and 527-1.

3.4. Nutritional characteristics of starch

Elevated RS contents (18–34%, total starch basis) were observed in the high amylose lines (Table 6). On the contrary, starches containing normal or high proportions of amylopectin displayed RS contents below 0.7%, total starch basis. Consequently the high amylopectin line 527-1 contained negligible amounts of RS. Although the total starch contents in tubers from the high amylose lines were lower than in the mother lines, high amylose tubers contained more RS/g fresh-cooked weight (fw). One example is line 418, which contained approximately 2.5 g RS/100 g fw which can be compared with around 1 g RS/100 g fw in the mother line (Pro). As a comparison, the RS content of consumer potatoes is approximately 0.5–1 g RS/100 g fw (Åkerberg et al., 1998b; Leeman et al., 2005).

Results from measurements of in vitro starch hydrolysis rate (HI) and predicted GI's are presented in Table 7. The high amylose lines 715 and 342 gave significantly lower HI's for the starch moiety compared with 2017, 527-1 and the control line Pre. HI's for freeze-dried tubers from the high amylose lines 715 and 418 from 2003 harvest were obtained in a similar manner and were almost identical with the values obtained for 2002 harvest (not shown). When comparing GI_{HI} of the transformed lines in % of the mother lines, two of the high amylose lines displayed about 70% of the GI of their respective mother line. The ratio of different low molecular weight carbohydrates affect the GI of a carbohydrate mixture; a high proportion of fructose decrease the GI whereas a high proportion of glucose will have the opposite effect (Foster-Powell & Miller, 1995).When taking into account not only the hydrolysis features of the starch moiety, but also the contents of low molecular weight carbohydrates, the GI_c's were in the same range as the GI_{HI}'s, except for line 715. That is, the effect of the different low molecular weight carbohydrate patterns on the predicted GI_c values was essentially negligible. This may be explained by the proportions of the sugars making them compensate for each other. However, in the tubers with the highest amylose content (line 715), associated with the most lente features of the starch moiety, the over-all effects on glycaemic features are likely to be marginal since the higher amylose content was accompanied by higher contents of low molecular weight carbohydrates with glycaemic potential.

Generally, the HI values and the predicted GI's for the isolated starches were higher than for the starch moiety in heat-treated tubers. Still, a significant difference was seen between the high amylose lines and the control lines. Further, the GI's of the transformed lines as expressed in % of the GI value of the respective mother lines were lower for the high amylose lines.

Table 6
Available starch and resistant starch (RS) contents (g/100 g dry matter) in heat treated isolated starches

Sample	Available starch	RS
715	59.7 ± 0.7^{a}	34.4 ± 0.3^{a}
342	74.5 ± 1.4^{b}	18.3 ± 1.3^{b}
418	74.0 ± 1.4^{b}	17.9 ± 1.1^{b}
2017	95.5 ± 0.3^{d}	0.7 ± 0.0^{c}
527-1	98.1 ± 0.9^{c}	0.1 ± 0.0^{e}
Din	90.3 ± 0.5^{e}	0.4 ± 0.0^{d}
Pre	93.2 ± 0.4^{d}	0.6 ± 0.0^{c}
Pro	99.3 ± 0.2^{c}	0.5 ± 0.0^{cd}

Values are means ± s.e.m. (total starch basis) of five replicates.
Values within a column not sharing the same superscript are significantly different ($p < 0.05$).

4. General discussion

It can be concluded that the genetically modified lines, especially those with high amylose content, behave differently compared with the control lines. All starches formed granules, although in high amylose lines the granule structures were altered. The distributions of starch within the tubers were generally normal. Cell structures in raw potatoes appeared unaffected, whereas in cooked high amylose potatoes cell structures collapsed. The different microscopic techniques used in this study together with the DSC results, show the incomplete swelling of the starch granules in high amylose lines at normal cooking temperatures. The present study confirmed earlier published

Table 7
In vitro hydrolysis (HI), predicted GI's from obtained HI values (GI_{HI}), GI's calculated from the carbohydrate content (GI_c) and GI_{HI} in % of mother variety ($GI_{HI\%}$) in heat treated tubers and isolated starches

Sample	Potato				Starch		
	HI	GI_{HI}	GI_c	$GI_{HI\%}$	HI	GI_{HI}	$GI_{HI\%}$
White wheat bread	100^{bcd}	100	–	–	100^{e}	100	–
715	69.4 ± 11.4^{e}	70	83	73	108.2 ± 3.1^{de}	105	80
342	78.4 ± 4.5^{de}	78	81	65	117.7 ± 6.7^{cd}	114	90
418	96.6 ± 4.8^{bcd}	94	94	100	117.1 ± 4.4^{cd}	113	87
2017	102.5 ± 2.9^{abc}	100	99	104	136.3 ± 5.9^{ab}	131	99
527-1	120.5 ± 3.6^{ab}	116	113	97	126.2 ± 5.1^{bc}	121	95
Din	98.2 ± 6.3^{bcd}	96	95	–	138.1 ± 5.0^{a}	132	–
Pre	124.4 ± 5.6^{a}	120	118	–	132.0 ± 4.8^{ab}	127	–
Pro	95.7 ± 4.7^{cd}	94	93	–	135.5 ± 4.9^{ab}	130	–

Values are means ± s.e.m. of six replicates. Values within the HI columns not sharing the same superscript are significantly different ($p < 0.05$).

results regarding the lower starch contents and higher contents of sugars in high amylose potato lines (Hofvander et al., 2004). Levels of glucose, fructose and sucrose were elevated in all transformed potato lines compared to the mother lines. Sugars have been reported to affect gelatinisation and recrystallisation properties as studied by DSC (Kohyama & Nishinari, 1991; Prokopowich & Biliaderis, 1995). Comparing results from tissue samples and isolated starch, no effects of sugar were detected. However, such influence could be concealed by impacts from other cell structures. A strong relation was found between ΔH_{gel} and potentially available starch, whereas no correlation was found with the total starch content. Recrystallisation of amylopectin was altered in high amylose lines, either to a higher or lower ΔH_{AP} depending on mother variety. A DSC melting peak in the region for recrystallised amylose was detected for high amylose and amylopectin starches. The fact that higher amylose contents were associated with substantially elevated RS contents in boiled isolated starches implicates the involvement of amylose in the recrystallisation and formation of amylase resistant starch. As judged from DSC measurements the amounts of recrystallised amylose correlated with RS contents with the exception of amylopectin starch. Although the RS contents in high amylose starches were substantial and accompanied by decreased GI's, the over-all predicted GI's were comparatively high.

The possibilities to influence glycaemic properties and RS contents in high amylose potato tubers are interesting from a nutritional point of view. However, some improvements of the modified tubers are necessary to obtain an increased quality both from a technological and nutritional perspective. The most important task should be to decrease the high contents of low molecular weight carbohydrates. These are likely to influence not only the glycaemic profile, but also the storage properties and the taste of the potato. Within this work, no studies have been performed on the dietary fibre content. If the transformations should result in increased contents of cell wall material, and thereby elevate the dietary fibre content, this may provide a further nutritional potential of the high amylose material.

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