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Physico-chemical and degradative properties of *in-planta* re-structured potato starch

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ABSTRACT

Starch re-structured directly in potato tubers by antisense suppression of starch branching enzyme (SBE), granule bound starch synthase (GBSS) or glucan water dikinase (GWD) genes was studied with the aim at disclosing the effects on resulting physico-chemical and enzyme degradative properties. The starches were selected to provide a combined system with specific and extensive alterations in amylose and covalently esterified glucose-6-phosphate (G6P) contents. As an effect of the altered chemical composition of the starches their hydrothermal characteristics varied significantly. Despite of the extreme alterations in phosphate content, the amylose content had a major affect on swelling power, enthalpy for starch gelatinization and pasting parameters as assessed by Rapid Visco Analysis (RVA). However, a combined influence of the starch phosphate and long glucan chains as represented by high amylose or long amylopectin chain length was indicated by their positive correlation to the final viscosity and set back (RVA) demonstrating the formation of a highly hydrated and gel-forming system during re-structuring of the starch pastes. Clear inverse correlations between glucoamylase-catalyzed digestibility and amylopectin chain length and starch phosphate and lack of such correlation with amylose content indicates a combined structuring role of the phosphate groups and amylopectin chains on the starch glucan matrix.

1. Introduction

Starch phosphorylation is the only known natural modification of starch. The phosphate groups are bound as monoesters at the C-6 and C-3 positions of glucose units (Blennow, Bay-Smidt, Olsen, & Møller, 2000a; Tabata & Hizukuri, 1971). The level of starch phosphorylation varies with the botanical source and it was shown that starches from tuberous tissues are more phosphorylated than those of cereal seeds (Blennow, Engelsen, Munck. & Møller, 2000b; Tabata, Nagata, & Hizukuri, 1975).

Potato, being the hitherto most extensively studied system with respect to starch phosphorylation, is known to contain relatively large amounts of covalently bound phosphate to starch. Starch isolated from potato tubers contains in an average, one phosphorylated glucose residue out of 300 glucose residues and one-third of phosphate groups are located at the C-3 position whereas two-third are in the C-6 position of the glucosyl residue (Tabata & Hizukuri, 1971). It is also established that the majority of the phosphate is bound to amylopectin and that amylose contain insignificant amount of phosphates (Hizukuri & Tabata, 1970).

Even though starch phosphorylation is known for more than a century, its biochemical pathway and molecular mechanism have been studied very recently. It is now known that the starch phosphorylation at the C-6 and C-3 positions of glucose residues are catalyzed by two distinct dikinases; glucan water dikinase (GWD) and phosphoglucan water dikinase (PWD), respectively (Ritte et al., 2006). The level of phosphate in potato tubers can be suppressed by antisense suppression of the GWD1 protein (Lorberth, Ritte, Willmitzer, & Kossmann, 1998; Viksø-Nielsen et al., 2001). However, it has not been demonstrated that over-expression of GWD results in higher phosphate levels. An interesting phenomenon with starch phosphorylation is the changes in the starch phosphate content with altered enzyme activities involved in starch synthesis (Blennow et al., 2005; Lloyd, Landschütze, & Kossmann, 1999; Wischmann et al., 2005) and it has been demonstrated that the degree of starch phosphorylation is correlated positively to long amylopectin chains (Blennow et al., 1998). It is now well established (Blennow et al., 2005; Schwall et al., 2000; Wischmann et al., 2005) that reduced activity of starch branching enzymes (SBE) by antisense gene technology increases the content of longer amylopectin chains and increases starch phosphorylation. Transgenic potato tubers expressing the glycogen synthase gene of *Escherichia coli* has resulted in a modified starch with highly branched amylopectin and lowered phosphorylation

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(Shewmaker et al., 1994). Such results can possibly be explained by direct protein–protein interaction between starch phosphorylating enzymes and starch synthesis enzymes. However, as judged from GWD kinetic analysis it is likely that these effects are supposedly linked the substrate specificity of the GWD itself (Mikkelsen & Blennow, 2004; Mikkelsen, Baunsgaard, & Blennow, 2004) i.e. GWD has high activity towards structures formed by long amylopectin chains. However, the positioning of the phosphate groups with respect to the crystalline lattice of the starch granule is less clear, and phosphate groups have been demonstrated to be located both in crystalline and in amorphous sections of the starch granule (Blennow et al., 2000a). Suppression of the granule bound starch synthase I (GBSS I) generates a virtually amylose-free tuber starch but only results in a minor increase in phosphate levels which is an effect of the decreased amylose content, amylose not being phosphorylated (Kozlov, Blennow, Krivandin, & Yuryev, 2007).

Covalently bound phosphate in starch affects its physico-chemical properties and thereby its usability for different industrial processes. The phosphate presumably influences the stability of the crystalline structures in the amylopectin molecules of the starch granules, and thereby the physico-chemical properties of starch (Blennow et al., 2005; Kortstee et al., 1998; Kozlov et al., 2007; Muhrbeck & Eliasson, 1991; Wischmann et al., 2005).

The dietary and enzymatic degradability of starch is affected by several factors including starch granule integrity. Among them, the granule structure; granule shape and size (MacGregor & Ballance, 1980; Noda et al., 2008; Valetudie, Colonna, Boucht, & Gallant, 1993), crystalline structure (Zhang & Oates, 1999; Zhou, Hoover, & Liu, 2004) are well known factors controlling the rate and extent of enzymatic hydrolysis. Such factors are in turn directed by the molecular constituents of the starch, especially the presence of amylose (Fuwa, Nakajima, & Hamada, 1977; Kitahara et al., 2007; Noda et al., 2002; Sandstedt, Strahan, Ueda, & Abbot, 1962), amylopectin chain length (Srichuwong et al., 2005), and starch phosphate content (Abe, Takeda, & Hizukuri, 1982; Absar et al., 2009; Noda et al., 2008). However, it is unlikely that such structures per se are responsible for these correlations. Only for raw starch, the relationships of median granule size and phosphorus content with digestibility can be established and it has been demonstrated that after gelatinization, such definite relationships do not exist (Noda et al., 2008). Hence, for the highly dense and aggregated starch granule, the nature of granule surface are determining efficiency of enzymatic degradation and relations to molecular structures are supposedly effects of the higher structures that can generate at the nano and micro scales. However, it is problematic to analyze such structures. The presence of different types of blocklets has been proposed to control the extent of hydrolysis (Gallant, Bouchet, Buléon, & Pérez, 1992; Tang, Mitsunaga, & Kawamura, 2006).

Transgenic potato lines with re-structured starch generated by suppressed expression of genes coding for SBE, GWD and GBSSI are now available (Blennow et al., 2005; Kozlov et al., 2007; Viksø-Nielsen et al., 2001) providing an interesting system to study the combined effects of specific branching patterns and phosphate on starch functionality. This study aims at addressing such effects focusing on starch hydrothermal and degradative properties.

2. Materials and methods

2.1. Plant materials

Twelve transgenic potato lines together with their parent control lines were used in the study. Transgenic lines belonging to the H944 series were developed by antisense suppression of the starch branching enzyme SBEI and SBEII isoforms (Blennow et al., 2005) in the mother cultivar Dianella. The transgenic potato lines H924, H926, A4-9.6 and S11-51 were generated by antisense RNA technology as described elsewhere for the glucan water dikinase (GWD) and granule bound starch synthase (GBSS), respectively (Kozlov et al., 2007). The S11-51 was generated in the cultivar Kuras background and all others in the cultivar Dianella.

Transgenic plants together with their control mother cultivars Dianella and Kuras were grown outdoors until mature in Styrofoam boxes with well-drained and fully fertilised soil April–September in Denmark. Fresh tubers were harvested and starch was immediately prepared as described (Blennow et al., 1998).

2.2. Amylose content

The amylose content of starch was determined by iodine colorimetry using a method modified from Hovenkamp-Hermelink et al. (1988). Ten micrograms of starch was dissolved in 5 ml of 1 M NaOH with vigorous stirring overnight. Ten microliters of this sample was added to 200 μl of the diluted iodine solution (0.26 g I_2 and 2.6 g KI in 10 ml water, diluted 1000 times in 100 mM HCl) in an Eliza plate, and absorbance was measured at 550 and 620 nm after 2 min in an Eliza plate reader. Amylose levels were calculated by plotting the A620/A550 ratio against a standard curve of starches with amylose content quantified with size exclusion chromatography (Blennow, Bay-Smidt, & Bauer, 2001).

2.3. Glucose-6-phosphate (G6P) content

The concentration of phosphate monoesterified to the starch was determined as the content of G6P after acid hydrolysis using an enzyme-linked assay as described (Bay-Smidt, Wischmann, Olsen, & Nielsen, 1994).

2.4. Amylopectin chain length distribution

The amylopectin chain length distribution was determined following enzymatic hydrolysis of the α-1,6-branch linkages with isoamylase. Linear oligosaccharides released by the isoamylase were separated using high-performance anion-exchange chromatography (HPAEC) with pulsed amperometric detection (PAD) using a Dionex DX500 system (Dionex Corp., Sunnyvale, CA) as previously described (Blennow et al., 1998).

2.5. Thermal properties using differential scanning calorimetry (DSC)

DSC analysis was conducted using a DSC 6100 (Seiko Instruments, Tokyo), according to Noda et al. (2004). Ten milligrams of a sample (dry weight basis) was weighed in a silver pan and distilled water was then added to make a suspension of 30% (dry weight basis, w/w). A sealed pan with distilled water was used as a reference. Scans were run at a heating rate of 2 °C/min from 25 to 130 °C. The peak gelatinization temperature, onset gelatinization temperature and the enthalpy for starch gelatinization were recorded. The analysis was repeated twice.

2.6. Swelling power

Starch slurry (20 mg of starch in dry weight basis in 5 ml of distilled water) was gelatinized at both 70 °C and 80 °C for 20 min with frequent mixing to avoid forming of starch clots. Gels were cooled at 20 °C for 5 min and then centrifuged at 9000*g* for 15 min at 10 °C. Swelling power was calculated and it was expressed as the weight of swelled starch residue per 1 g of starch (dry weight). The analysis was performed in duplicate.

2.7. Pasting properties by rapid visco analyzer

The pasting properties of potato starches as analyzed using a Rapid Visco Analyzer (RVA-4) (Newport Scientific Pvt., Ltd., Australia) were determined with two replicates according to Noda et al. (2004). Each sample of potato starch was added to 25 ml of distilled water to prepare a 4% suspension (dry weight basis, w/w). The suspension was kept at 50 °C for 1 min and then heated up to 95 °C at 12.2 °C/min and held for 2.5 min at 95 °C. It was then cooled at 50 °C (cooling rate of 11.8 °C/min) and kept for 2 min. Peak viscosity, breakdown (difference between the peak and holding viscosity), final viscosity, setback (difference between final and holding viscosity), peak time were measured from the pasting curve using Thermocline for Windows software (Newport Scientific Pvt., Ltd., Australia). The viscosity parameters were recorded in rapid visco units (RVU) and peak time was given in min.

2.8. Digestibility of raw starch

One milliliter of a 2% raw starch suspension was digested with 4.15 U of crystalline glucoamylase from *Rhizopus* sp. (Oriental Yeast Co., Ltd., Tokyo, Japan) for 4 h at 40 °C according to the modified method of Noda, Takahata, and Nagata (1992). The amount of glucose released during enzyme digestion was estimated by the phenol–sulfuric method (Dubois et al., 1956), and enzyme digestibility was calculated as the percentage of glucose released during incubation with the enzyme to the total amount of sugar in the starch on a weight basis.

2.9. Data analysis

The analysis of variance for each parameter measured were performed using Microsoft Excel 2003 and then means were compared by least significant difference (LSD) for each property using SAS v. 9.1. The average DP of amylopectin was not subjected to statistical analysis due to insufficient number of replicate for some samples.

3. Results and discussion

3.1. Amylose content

The amylose content among transgenic potato lines and their parents was significantly different (Table 1). The highest amylose content of 36.6% was found in line H944-14.5, a transgenic potato with antisense suppressed activity of SBE (asSBE). All transgenic lines with suppressed SBE had much higher amylose content than the parent lines. The amylose content of the transgenic potatoes with antisense suppression of GWD (asGWD) was not different from that of the parental lines but was very low for S11-51.1 and A4-9.6 where GBSS activity was suppressed (asGBSS).

3.2. G6P content

A tremendous variation in G6P content was accomplished in the starch material (Table 1). Increased G6P was achieved by antisense suppression of SBE and a decrease in the G6P was accomplished by reducing the activity of GWD as demonstrated in previous studies (Blennow et al., 2005; Kitahara et al., 2007). Only a slight increase in the G6P was made due to the reduction of activity of GBSS.

3.3. Average degree of polymerization (DP) of amylopectin

The average DP as determined by HPAEC-PAD and calculated for chains from DP 6 to DP 60 was DP 26–27 for the control, asGWD and asGBSS starches. For the asSBE these numbers were considerably higher (DP 29–30) (Table 1). These data are well in accordance with previous results (Blennow et al., 2005; Kozlov et al., 2007) and hence, the amylopectin chain length distribution was only significantly affected by the asSBE approach.

Table 1
Genetic modification and starch molecular composition of potato samples.

Line	Mother line	Deficiency	Amylose, content (%)	Average DP of amylopectin	G6P, nmol/mg starch
Dianella control 1	Dianella	None	20.7e	26.8*	15.6[f]
Kuras control	Kuras	None	23.1[d]	27.4	14.0[f]
Dianella control 2	Dianella	None	20.9[e]	26.8	15.1[f]
Dianella H944-3.1	Dianella	↓SBE	34.3[b]	30.0	51.6[a]
Dianella H944-3.1B	Dianella	↓SBE	32.6[b]	30.1	52.4[a]
Dianella H944-3.3	Dianella	↓SBE	27.9[c]	29.0	42.5[c]
Dianella H944-6.1	Dianella	↓SBE	27.7[c]	28.7	36.9[d]
Dianella H944-14.5	Dianella	↓SBE	36.6[a]	29.7	53.8[a]
Dianella H944-14.5B	Dianella	↓SBE	36.4[a]	29.5	47.1[b]
Dianella H944-15.1	Dianella	↓SBE	28.2[c]	29.1	41.9[c]
Dianella H926-28.4A	Dianella	↓GWD	23.9[d]	25.1	2.2[g]
Dianella H924-14.3	Dianella	↓GWD	23.0[d]	25.8	2.2[g]
Dianella H926-28.4B	Dianella	↓GWD	23.5[d]	25.4	2.1[g]
Kuras S11-51.1	Kuras	↓GBSS	1.3[g]	26.6	21.2[e]
Dianella A4-9.6	Dianella	↓GBSS	9.0[f]	27.7	17.2[f]

Values followed by the same letters in the same column are not significantly different at $p < 0.05$ level.
* Mean separation was not performed due to insufficient number of replications for some samples.

3.4. Thermodynamic properties of gelatinization

The peak gelatinization temperature, onset gelatinization temperature and the enthalpy for starch gelatinization varied significantly for the different starch types (Table 2). The peak gelatinization temperature of control starch samples varied from 65.6 to 68.1 °C. The transgenic lines H926-28.4A, H926-28.4B and H926-14.3 having suppressed GWD expression and low phosphate content gave peak gelatinization temperatures within the same range as the control starches. Therefore, it can be postulated that the reduction in phosphate content has no major influence on the starch crystalline arrangement. High peak gelatinization temperature in transgenic lines, S11-51.1 and A4-9.6, having low amylose content would be due to the increase in starch crystallinity in agreement with previous data (Huang et al., 2007; Kozlov et al., 2007). Likewise, the peak gelatinization temperatures of the transgenic potato in the H944 series was at least high 5 °C higher than the control lines (average 74.7 °C) again in agreement with the previous observation (Blennow et al., 2005). Shewmaker et al. (1994) supposed an effect of the increased amylose content. The effect of the marked increase in starch phosphate on gelatinization temperature would be much high to overcome the effect of high amylose in lowering gelatinization temperature. This was very clearly shown by the absence of significant correlation of amylose content with either peak or onset gelatinization temperatures in contrast to the very high correlation found to the G6P content (Table 3). However, minor enthalpic effects attributed to the presence of phosphate have been indicated recently (Kozlov et al., 2007) indicating a minor introduction of amorphous sections induced by the phosphate groups.

The enthalpy for starch gelatinization showed negative correlation to amylose content ($r = -0.75$, $p = 0.001$) and to G6P ($r = -0.55$, $p = 0.03$). Previous studies also stated that high amylose reduces the enthalpy for starch gelatinization (Blennow et al., 2005; Inouchi, Glover, Sugimoto, & Fuwa, 1984; Mangalika, Miura, Yamauchi, & Noda, 2003; McPherson & Jane, 1999). The effect of amylose on the gelatinization enthalpy is apparently stronger than that of phosphate.

Table 2
Thermal properties of starches from transgenic potatoes and their parent control lines determined by DSC.

Potato line	Peak gelatinization temperature (°C)	SD	Onset gelatinization temperature (°C)	SD	Gelatinization Enthalpy (J/g)	SD
Dianella control 1	66.45^{g}	0.07	64.30^{fgh}	0.14	21.18^{bcd}	0.31
Kuras control	65.55^{h}	0.07	63.20^{i}	0.57	19.93^{efg}	1.23
Dianella control 2	68.10^{f}	0.14	64.95^{fg}	0.21	20.48^{def}	0.22
Dianella H944-3.1	75.85^{b}	0.07	72.35^{a}	0.21	19.70^{efg}	0.57
Dianella H944-3.1B	73.80^{c}	0.42	70.85^{bc}	0.49	17.05^{h}	0.13
Dianella H944-3.3	74.25^{c}	0.07	71.60^{ab}	0.00	19.40^{fg}	0.57
Dianella H944-6.1	73.85^{c}	0.49	70.50^{c}	0.57	20.66^{cde}	0.04
Dianella H944-14.5	75.30^{b}	0.14	70.50^{c}	0.14	17.05^{h}	0.21
Dianella H944-14.5B	76.50^{a}	0.42	71.70^{ab}	0.71	20.28^{defg}	0.81
Dianella H944-15.1	73.15^{d}	0.07	68.90^{d}	0.14	18.12^{h}	0.82
Dianella H926-28.4A	65.85^{h}	0.07	63.95^{ghi}	0.35	19.35^{g}	0.07
Dianella H924-14.3	65.60^{h}	0.28	63.25^{hi}	0.49	21.90^{b}	0.14
Dianella H926-28.4B	66.90^{g}	0.00	65.35^{f}	1.34	19.55^{fg}	0.56
Kuras S11-51.1	70.45^{e}	0.07	68.70^{d}	0.42	23.14^{a}	0.18
Dianella A4-9.6	72.95^{d}	0.49	66.65^{e}	0.21	21.69^{bc}	0.01

Values followed by the same letters in the same column are not significantly different at $p < 0.05$ level.

Table 3
Coefficient of variance for different starch properties as calculated for amylose content, G6P content and amylopectin average degree of polymerization.

Starch property	Amylose content	G6P	Average DP
Amylose content	–	0.61*	0.60*
G6P	–	–	0.97***
Enzyme digestibility of raw starch after 4 h	−0.22^{NS}	−0.75**	−0.77***
Swelling power at 70 °C	−0.78***	−0.51*	−0.43^{NS}
Swelling power at 80 °C	−0.61*	−0.07^{NS}	−0.01^{NS}
Peak viscosity	0.21^{NS}	0.67**	0.70**
Breakdown viscosity	−0.37^{NS}	0.20^{NS}	0.28^{NS}
Final viscosity	0.60*	0.82***	0.80***
Setback	0.89***	0.72**	0.67**
Peak time	0.68**	0.10^{NS}	0.04^{NS}
Peak gelatinization temperature	0.45^{NS}	0.91***	0.89***
Onset gelatinization temperature	0.45^{NS}	0.94***	0.88***
Enthalpy for starch gelatinization	−0.75***	−0.55*	−0.53*

* Significant at $p \leqslant 0.05$.
** Significant at $p \leqslant 0.01$.
*** Significant at $p \leqslant 0.001$.
^{NS} Not significant.

3.5. Swelling power

The swelling power at both 70 and 80 °C too was significantly different among the samples (Fig. 1). There was only little difference in the swelling power within the control mother lines and S11-51.1 and A4-9.6 having lower amylose content. However the asSBE or asGWD lines had significantly lower swelling power than the controls or the asGBSS lines demonstrating strong effects of amylose and phosphate, respectively. The swelling power at 80°C was always higher than that at 70 °C but the percentage increase in swelling power due to temperature changes (Swelling power at 80 °C – swelling power at 70 °C as a percentage to swelling power at 70 °C) was higher than 140% for all lines in H944 series than the others. All other lines showed a maximal 70% increase from 70 to 80 °C.

The swelling power can be associated with the structure of amylopectin as well as the amylose content (Sasaki & Matsuki, 1998). No significant correlation between the swelling power and average DP of amylopectin chains could be observed suggesting that the amylose contributed most to the decreased swelling

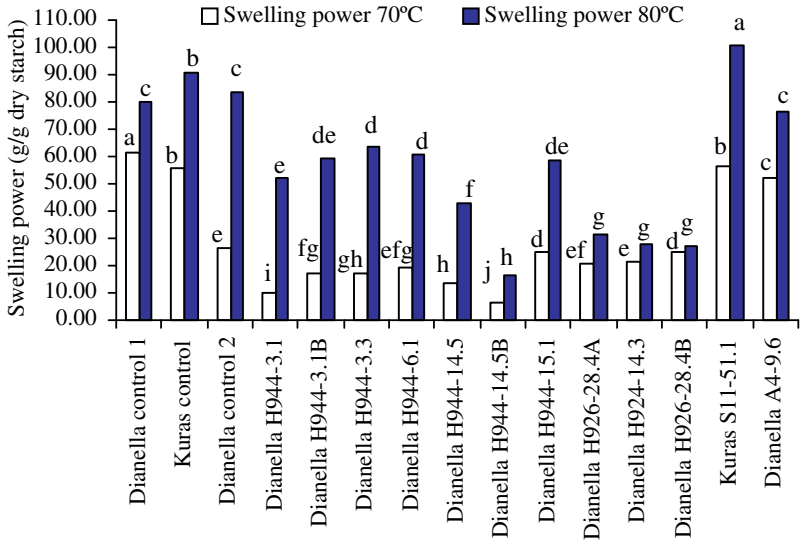

Fig. 1. Swelling power of starches from transgenic potatoes at 70 and 80 °C. Mean separation was done for swelling power at 70 and 80 °C separately. Thus values followed by the same letters in the bars representing swelling power at same temperature are not significantly different at $p < 0.05$ level.

power. It has bee demonstrated that the swelling power of starch is hindered by a high amylose content (Mangalika et al., 2003). Such effects were confirmed by our study as demonstrated by the negative correlation found between amylose content and the swelling power (Table 3). Also the phosphate content showed a negative relationship with the swelling power at 70 °C. However, when the temperature was increased to 80 °C, no such relationship was found. The starch gelatinization temperature of transgenic lines in H944 series was higher than 70 °C whereas all other lines showed approximately 5 °C lower gelatinization temperature. These data would immediately suggest that the phosphate groups inhibit swelling before complete starch gelatinization but not after reaching to optimum temperature for starch gelatinization at 80 °C. Phosphate is usually expected to increase the hydration capacity of starch and thus resulting in higher swelling in starch (Blennow et al., 2005; Kim, Wiesenborn, Lorenzen, & Berglund, 1996). In our starch types it is not possible to exclusively discriminate the effects between phosphate and amylose since these structures are related in the high amylose lines. However, it was confirmed that the effect of amylose on swelling power of starch was much stronger than that of starch phosphate content. Moreover, for the low phosphate starches having similar starch chemistry as the control starches a clear effect of phosphate on swelling power was seen and the fact that the high amylose starch types did show swelling which is not the case for non-phosphorylated high amylose starches (Colonna & Mercier, 1985) clearly demonstrates a hydrating effect of phosphate.

3.6. Pasting characteristics

A tremendous variation in pasting properties was found among the tested starches (Table 4). Except for the line H944-14.5B, all transgenic potato having suppressed SBE expression showed very high peak viscosity, final viscosity and set back but tended to exhibit low breakdown. The low viscosity of the H944-14.5B starch is supposedly an effect of the high amylose content not followed by the same high phosphate content as the H944-14.5 (Table 1). On the other hand, potato lines with suppressed GWD activity having low phosphate content had much lower peak viscosity, breakdown, and final viscosity and a slight increase in peak time and set back, respectively as compared to the control starches. These data demonstrate a clear effect of starch phosphate on starch hydration and pasting. The relationship was confirmed by a significant positive correlation between G6P with peak and final viscosities and set back (Table 3).

In contrast to transgenic lines either suppressed SBE activity increasing starch phosphate and amylose or GWD activity reducing starch phosphate, lines with reduced activity of GBSS having low amylose levels demonstrated no significant difference in peak viscosity and breakdown from those of their control lines but a significant decrease in final viscosity, set back and peak time giving a positive correlation to amylose content.

Shewmaker et al. (1994) observed a decrease in paste viscosity due to reduced phosphate and amylose content and increased branching of amylopectin in transgenic potato. Our samples do not have high branching but much larger variation in starch phosphate and amylose and the less branching in amylopectin due to suppression of SBE is expected to have large impact on starch pasting.

Hemar et al. (2007) demonstrated a very strong correlation of peak viscosity to phosphorus content. Furthermore, they also found that the final viscosity correlated to phosphorus content at low (1%) starch concentration. Karim et al. (2007) and Noda et al. (2004) confirmed these data. Usually a negative effect of amylose content on peak and breakdown viscosites and its positive effect on set back is seen (Zaidul et al., 2007; Zeng, Morris, Batey, & Wrigley, 1997). In our study, the average DP of amylopectin and G6P positively correlated to peak viscosity, final viscosity and set back by RVA (Table 3). Though the amylose content did not significantly correlate to the peak viscosity, it positively correlated to final and set back viscosities. Therefore, it seemed that positive effects of starch phosphate and long chains of amylopectin on peak viscosity are much stronger than the negative effect of amylose. However, the final viscosity and set back are increased in all high amylose starch types.

3.7. Enzyme digestibility of raw starch

The enzyme digestibility of raw starch showed a significant variation among the tested potato samples. The highest enzyme digestibility by *Rhizopus* glucoamylase was found in the transgenic potato H924-14.3, having very low phosphate content (2.1 nmol G6P/mg in starch) due to suppression of the starch phosphorylating enzyme GWD. That was followed by the other two transgenic potato lines having the same modification. On the other hand, the least digestibility was observed in potato lines with asSBE which showed the highest G6P and amylose contents among the tested samples. This relationship suggests that the in vitro digestion of raw starch is hindered by the presence of phosphate in the starch. Thus a very clear negative correlation ($r = -0.75$ at

Table 4
RVA pasting parameters of starches from transgenic potatoes.

Line	Peak viscosity (RVU)	Breakdown (RVU)	Final viscosity (RVU)	Setback (RVU)	Peak time (min)
Dianella control 1	275.93^{g}	156.04b^{c}	138.46^{g}	18.58^{ij}	3.67^{ghi}
Kuras control	251.50^{ij}	117.30^{d}	156.17^{f}	21.96^{ghi}	4.40^{efg}
Dianella control 2	262.21^{hi}	126.76^{d}	156.46^{f}	21.00^{hi}	4.10^{fgh}
Dianella H944-3.1	433.00^{b}	95.38^{e}	397.00^{a}	60.89^{b}	5.07^{cde}
Dianella H944-3.1B	526.38^{a}	198.34^{a}	376.63^{b}	48.59^{c}	4.70^{def}
Dianella H944-3.3	418.73^{c}	116.67^{d}	345.50^{c}	43.58^{dc}	5.04^{cde}
Dianella H944-6.1	400.34^{d}	120.50^{d}	308.25^{e}	28.42^{fg}	4.94^{cdef}
Dianella H944-14.5	315.96^{f}	11.84^{f}	380.00^{b}	75.86^{a}	5.50^{bcd}
Dianella H944-14.5B	52.75^{m}	6.50^{f}	94.86^{ij}	48.63^{c}	7.00^{a}
Dianella H944-15.1	385.42^{e}	102.63^{e}	320.96^{d}	38.17^{de}	4.90^{cdef}
Dianella H926-28.4A	92.38^{k}	5.80^{f}	115.80^{h}	29.21^{fg}	6.33^{ab}
Dianella H924-14.3	75.58^{l}	4.92^{f}	103.00^{i}	32.34^{ef}	5.77^{bc}
Dianella H926-28.4B	66.09^{l}	3.21^{f}	91.79^{j}	28.92^{fg}	6.03^{b}
Kuras S11-51.1	244.38^{j}	148.09^{c}	97.42^{ij}	1.23^{k}	3.20^{i}
Dianella A4-9.6	272.67^{gh}	159.25^{b}	124.42^{h}	11.00^{j}	3.47^{hi}

Values followed by the same letters in the same column are not significantly different at $p < 0.05$ level.

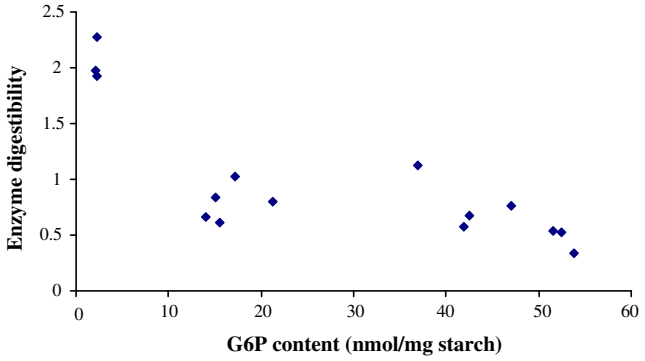

Fig. 2. Relationship between G6P content and enzyme digestibility of raw starch by *Rhizopus* glucoamylase in transgenic potatoes and their parents.

$p = 0.002$) was observed between G6P content and the raw starch digestibility among our samples (Fig. 2).

The SBE suppressor lines had simultaneous increase in the amylose content and amylopectin chain length as well as G6P content. Interestingly, the amylose content did not correlate to enzyme digestibility in our tested samples (Table 3) demonstrating that amylopectin structure including its phosphorylation plays a major role for starch granule digestibility. All of our samples with high starch phosphate concentration previously showed changes in amylopectin structure (Blennow et al., 2005). Thus, negative and strong correlation coefficients between enzyme digestibility of raw starch and G6P content and the average DP of amylopectin implies a combined effect of enzyme efficiency of the phosphate groups and amylopectin chains on the starch granule matrix (Table 3).

4. Conclusion

Compared to normal potatoes, the transgenic potatoes with suppressed GBSS, GWD and SBE activities has resulted in drastic changes in the starch molecular structure followed by significant variations in pasting and thermodynamic properties. As a result, the digestibility with glucoamylase was altered. Amylose content was the predominant factor in controlling swelling power of starch at temperatures of 70–80 °C and its effect on the swelling power was strong enough to compensate for the possible effect of phosphate groups in the starch on hydration and starch swelling. Furthermore, amylose influenced set back of gelatinized starch upon cooling and peak time in starch gelatinization and enthalpy required for starch gelatinization. Phosphate content and the amylopectin chain length controlled the peak and final viscosities and set back of starch. Interestingly, the content of phosphate had tremendously higher correlation than amylose to peak and onset gelatinization temperatures of starch. The important role of phosphate and the amylopectin chain length was further evidenced by their distinct control over enzyme digestibility of raw starch providing evidence for a major structuring role of the phosphate groups and long amylopectin chains in native starch.

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