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The influence of an increased degree of branching on the physico-chemical properties of starch from genetically modified potato

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Abstract

Transgenic potatoes were studied which contained starch with an increased degree of branching of the starch as a result of the expression of the glycogen branching enzyme gene (glgB) of *Anacystis nidulans* or *Escherichia coli*. These trans-genes were expressed in a normal amylose-containing wildtype and in an amylose-free (amf) potato mutant. The degree of branching of these starches had increased up to 25%. This increase in the degree of branching could be partly explained by the presence of 5–15% more short chains in the amylopectin, the so-called A chains. The influence of the altered degree of branching on the physico-chemical properties of the starches was investigated. No change in granule size or morphology could be observed for the altered starches of these transgenic plants. Regardless of the presence or absence of amylose, starches with an increased degree of branching showed a shift towards more short chains of the amylopectin, a lower peak viscosity and for the amylose-free starch a tendency to form weaker gels. These results show that increasing the degree of branching of amylopectin leads to specific changes in the physico-chemical properties of the starch.

Keywords: Potato starch; Branching degree; Physico-chemical properties; Genetic modification

1. Introduction

Starch is the most abundant storage carbohydrate in higher plants. It can be found in storage organs such as roots and tubers in a granular form. Size and shape of the storage starch granules are specific for each starch crop (Jane et al., 1994). Starch consists of two types of glucose polymers; amylose, an essentially unbranched α-1,4 linked glucose polymer and amylopectin, consisting of α-1,4 linked chains with α-1,6 branches. Common starches contain about 15–30% amylose. Native starch and starch derivatives are widely used in the manufacturing of food, paper, textiles, adhesives, pharmaceuticals and building materials. The physical properties of starches and, therefore, the applications, depend on the starch composition (amylose:amylopectin ratio, size and degree of branching of amylose and amylopectin) and are unique for each botanical source (Swinkels, 1985). Many studies were undertaken to clarify the relationship between structural characteristics and the physico-chemical properties of starch (Howling, 1980; Tester & Morrison, 1990; Sanders et al., 1990; Jane & Chen, 1992; Wang et al., 1993a,b). In these studies native starches from different botanical origins and mutants with an altered starch composition were studied. Most mutants are found in maize where they are known for almost all of the isoforms of the enzymes involved in starch biosynthesis, resulting in starches with different composition and characteristics. The best studied example of mutant starch is that of the waxy mutant of maize (Echt & Schwartz, 1981). This mutation has also been identified and studied in other crops like potato (Hovenkamp-Hermelink et al., 1987), rice, barley and wheat (Sano, 1984; Shannon & Garwood, 1994; Nakamura et al., 1995). These mutants contain amylose-free starch as a result of a defect in the gene encoding granule bound starch synthase (GBSS) and a subsequent absence of enzymatic activity (Smith et al., 1995). Another group of mutants contains a relatively high amount of

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amylose in the starch as a result of a mutation in one of the isoforms of starch branching enzyme and can be found in maize and pea: amylose-extender maize (Boyer & Preiss, 1981; Hedman & Boyer, 1992) and wrinkled pea (Smith, 1988; Edwards et al., 1988). Several examples of genetically modified potato starches are described after the introduction of bacterial genes into the plant. The production of unique carbohydrates such as cyclodextrins by introduction and expression of the cyclodextrin glycosyltransferase gene from *Klebsiella* sp. in potato was described by Oakes et al. (1991). After introduction of the *E. coli* glgA gene, encoding glycogen synthase, in potato, a change in the starch composition could be observed. An increased degree of branching (measured by HPLC analysis of isoamylase debranched starch) and a decreased amylose content were described for starch of glgA expressing potatoes (Shewmaker et al., 1994). Some physical properties of the starch like gelatinization and thermal behavior had also changed: the onset temperature (T_o) of gelatinization measured by differential scanning calorimetry (DSC) analysis was lower and rapid visco analysis (RVA) showed a higher paste temperature and a lower peak viscosity for the starch of the transgenic potatoes.

In this paper we describe the physico-chemical analysis of starch from genetically modified potatoes in both the normal amylose containing as well as in the amylose-free mutant (amf) background. The degree of branching of these starches had increased after the introduction and expression of the glycogen branching enzyme encoding gene (glgB) of *E. coli* (Kortstee et al., 1996) or *A. nidulans* (Kortstee et al., 1997). The influence of the increased branching degree of the starch on the physico-chemical proprties will be shown.

2. Experimental procedures

2.1. Plant material

Transgenic plants were obtained in previous research which showed an increased degree of branching of the starch as the result of the expression of the bacterial branching enzyme of *E. coli* or *A. nidulans*. Molecular biological and biochemical analysis of these plants has partly been described before (Kortstee et al., 1996; Kortstee et al., 1997). The individually selected clones in the diploid amylose-free (amf) background of clone 1029-31 (Jacobsen et al., 1989), transformed with the *E. coli* branching enzyme, were: EC-13, EC-17 and EC-20 with clone 1029-31 as the untransformed control. Clones with amylose containing starch and transformed with the branching enzyme of *A. nidulans* were: AN-9 and AN-14, with A16 (El-Kharbotly et al., 1995) as the untransformed control. Plants were grown in the greenhouse and in the field. Mature tubers were harvested for starch isolation.

2.2. Isolation of starch

Starch was isolated from 5 kg (fresh weight) of field grown tubers. Tubers were ground with 1 g (±200 ppm) $Na_2S_2O_5$ in a Robot Coupe model R15 (series 5, Spangenberg BV, Vianen, Holland) for 5 min. at position 1 followed by 5 min at position 2. The sludge was mixed with 5 l demineralized water (dH_2O) and sieved through a shaking sieve (PERFLUX, N.V. TEMA, Den Haag, Holland) with 122 μM pores. The sludge was washed with dH_2O two more times and sieved to extract most starch and the granules were allowed to precipitate for at least 2 h. After the granules had settled, the potato juice was decanted and the starch was resuspended in 1 l dH_2O and filtered through filter cloth with pores of size 112 μm. The starch was washed at least three more times with dH_2O to remove small dirt particles and coagulated protein. After the final wash, the starch was filtered through Whatmann paper (3 mm) on a Buchner funnel. The starch was air dried on filter paper for at least 72 h to a moisture content of about 20%. The dried starch was passed through a sieve of pore size 508–635 μm, collected and stored at room temperature. Isolation of starch from greenhouse grown tubers was as described by Kuipers et al. (1994).

2.3. Starch granule size and morphology

Average granule size and size distribution of the isolated starch were determined by a Coulter counter multisizer IIe. 10 mg of starch was dispesed in 160 ml of Isoton II, according to the manufacturers instructions. The diameter of the tube was 200 μm and 50.000 particles were counted with a coincidence of 10%. Starch granules were stained with three times diluted Lugols solution (an 1% I_2/KI solution, 1:2 w/w) and examined under the microscope (magnification 100×) to check granule morphology.

2.4. λmax and blue value

5 mg of starch was dissolved in 1 ml of Dimethylsulphoxyde (DMSO) by boiling for 20 min. and diluted to 10% DMSO. Of the 10% DMSO-starch solution, 20 μl was added to 180 μl dH_2O and complexed with iodine by adding 800 μl KI/I_2 solution (Delrue et al., 1992), the final starch concentration was 0.01%. The λmax was determined as the wavelength with maximum absorption after scanning from 700 to 400 nm. The Blue Value was determined by boiling 10 mg of starch in 1 ml of 0.1 N NaOH for 50 min. After cooling, 9 ml of dH_2O were added. Of this solution 15 μl was added to 285 μl four times diluted Lugol's solution, and scanned from 700 to 400 nm with a Beckman DU-64 spectrophotometer. The Blue Value was determined as the absorption at 655 nm.

2.5. CL2B chromatography

100 mg starch was dissolved in 1 ml 0.1 N NaOH by boiling for 60 min. The sample was diluted by adding 9 ml of dH_2O, centrifuged for 5 min at 3000 ***g*** at room temperature and loaded on a CL2B column (2.6 by 200 cm, Pharmacia). Fractions of 10 ml were collected during elution of the column with 0.01 N NaOH at a flow rate of 30 ml/h. Of each fraction 200 μl was added to 800 μl of an iodine/potassium–iodine solution (Delrue et al., 1992) and scanned from 700 to 400 nm to determine the λmax and the maximum absorbtion.

2.6. Amylose content

The apparent amylose content was determined according to the method described by Hovenkamp-Hermelink et al. (1989). For the amylose-free starches amylose percentages of ≤ 3 were found and considered to be negligible.

2.7. Determination of starch degree of branching

The degree of branching of starch was determined with the Luff–Schoorl method (Schoorl, 1925) with slight modifications as described earlier (Kortstee et al., 1996).

2.8. Chain length distribution

Isoamylase debranched starch was separated by HPLC (a Dionex Carbopac PA 100 series). The peaks in the original chromatogram were reintegrated and corrected for the response factor to obtain a number percentage chain length distribution as was described earlier (Kortstee et al., 1996).

2.9. Swelling power in H_2O

Swelling power was determined according to Leach et al. (1959) with some modifications. Amylose containing starch suspensions (0.5% w/v) in water (preboiled seralpure) were heated to 60, 65, 70, 75 and 80°C, respectively, and kept at that temperature for 15 min at slow stirring, just sufficient to keep the starch completely suspended and to minimize shearing of swollen granules. Amylose-free starch suspensions (0.5% w/v) were heated to 66–80°C (at 2°C intervals) and kept at that temperature for 30 min under slow stirring. The swollen starch suspensions were poured in a measuring glass and allowed to precipitate overnight. The volume of sedimented swollen granules was determined and a sample of the clear supernatant was removed to determine the amount of dissolved starch. The percentage of dissolved starch was determined with the Boehringer kit no. 207748 for measuring starch content, according to the manufacturers specifications, but with smaller volumes to fit in a microtiterplate. The swelling power was corrected for the amount of solubilized starch.

2.10. DSC

Differential Scanning Calorimetry was performed with a Perkin Elmer Pyris 1 equipped with a Neslab RTE-140 glyco-cooler. The instrument was calibrated with indium (mp = 156.6°C) and zinc (mp = 419.47°C). Approximately 10 mg of starch (dry weight basis) was weighed accurately into a stainless steel pan and 40 μl of dH_2O was added. The pan was hermetically sealed and allowed to equilibrate at least 1 h before analysis. The suspension was heated from 20°C to 100°C at a scanning rate of 10°C/min. An empty stainless steel cup was used as a reference. For each endotherm the onset temperature (T_o) of melting and the enthalpy ΔH (J/g) were computed automatically. The results are the average of three scans. Enthalpies were calculated on a starch dry-weight basis. The dry matter content of the starch was determined at least in duplicate by oven drying of 30 mg samples for 4 h at 120°C.

2.11. Viscosity (RVA/Bohlin)

An 8% starch suspension in dH_2O (dwb) was placed in the RVA cup. The heating profile used was as follows: 2 min. at 45°C, heating to 90°C at a rate of 5°C/min, heating at 90°C for 6 min., cooling from 90 to 30°C at 14°C/min. and kept at 30°C for 6 min. The viscosity was measured in RVA units. For both the amylose containing and the *amf* starches the pasting profile of a 5% starch:water (w:v) suspension was measured with a Bohlin VOR Rheometer (Mettler Toledo, Tiel, The Netherlands) as described by Flipse et al. (1996). The pasting profile consisted of heating to 90°C, keeping the paste at this temperature for 15 min. and cooling to 20°C at a rate of 1°C/min.

2.12. Gel strength

Starch pastes (20% w:v) in water were prepared by adding 3.6 g of starch (20% moisture) to 14.4 ml of preboiled dH_2O in a straight side wide mouth polypropylene jar (Nalgene, 2118-9050). The jar was placed in a waterbath of 50°C on a plate and stirred at 300 rpm using a magnetic stirrer (dimensions of magnet: 25 × 6 mm) to disperse the starch. The sample was then heated to 80°C while stirring, until the stirrer became trapped in the gel. This usually happened at about 70°C, indicating the start of gelation. Immediately the sample was transferred to a 90°C waterbath, incubated for 1 h, cooled on ice and stored at 4°C for 5 days. Gel strength was measured using a texture analyzer (T04, Etia, Compiegne, France) with a 20 N loadcell and a punch probe with a diameter of 6 mm. The gel was compressed at a speed of 2 mm/s until rupture. For each sample, six separate gels were prepared and measured.

Table 1
Apparent amylose content (AM%), λmax and blue value (B.V.) of starches from greenhouse grown and field grown plants

Clone	Greenhouse grown tubers			Field grown tubers		
	AM%	λmax (± 2)	B.V (± 0.1)	AM%	λmax (± 2)	B.V (± 0.1)
1029-31	nd	555	nd	≤3	555	0.342
EC-13	nd	555	nd	≤3	552	0.308
EC-17	nd	nd	nd	≤3	555	0.327
EC-20	nd	550	nd	≤3	550	0.312
A16	22.0 ± 3.1	576	0.750	21.4 ± 0.5	573	0.501
AN-9	21.7 ± 1.5	579	0.910	20.3 ± 0.6	573	0.427
AN-14	21.3 ± 2.7	574	0.890	14.6 ± 0.4	573	0.470

nd = not determined.

3. Results

3.1. Granule size and morphology

Starch isolated from greenhouse- and field-grown tubers was stained with iodine and examined at the microscopic level. No changes in granule morphology could be observed for the starches of the transgenic potatoes. All transformants had red or blue staining starch according to their original background (amylose-free starch stained red, amylose containing starch granules stained blue). The median (d50) granule size by volume of the field-grown amf starches was between 22 and 32 μm. Starch of transformant EC-13 appeared to contain slightly larger granules than normal amf starches, but all values were within the range of normal variation. The d50 of the field-grown amylose containing starches was 19–27 μm by volume. Starch of transformant AN-9 consisted of more smaller granules, the median granule size for these granules was 19 μm compared to the other amylose containing starches with a d50 of 26–27 μm. Starch from greenhouse grown transgenic plants showed similar results; no change in granule size or shape compared to the control starch was observed.

3.2. Composition and fine structure

The apparent amount and structure of amylose in starch with an increased degree of branching did not seem to have changed, according to spectrophotometrical analysis as can be seen in Table 1, except for starch of tubers from field-grown transformant AN-14, which displayed a lowered amylose:amylopectin ratio of about 0.15, although the granules stained completely blue with iodine. The same clone but grown in the greenhouse, displayed an apparent amylose content of 22%, identical to the untransformed control.

The λmax of the starch of the transgenic plants had remained the same or was lowered. The latter was an indication of a higher degree of branching since the iodine binding capacity is inversely related to the degree of branching as was shown by Krisman (1962). The Blue Value for the amylose-free starches was lower than for the amylose containing starches as was expected. Within the groups no significant changes were found between the controls and the transformants.

Separation of amylose and amylopectin by size exclusion chromatography with a CL2B column is shown in Fig. 1. Amylopectin is eluted from the column as a single narrow peak, sometimes with a shoulder. The λmax of the fractions of this peak was approx. 560 nm, confirming the identity of the eluted glucan. The amylose-free starches from 1029-31 and EC-20 eluted from the column as one large peak, with a λmax of the fractions of 560 nm. Behind the amylopectin bulk a few glucan containing fractions elute from the column with a λmax of up to 600 nm. The glucan in those fractions is sometimes called the intermediate material and is believed to consist of long chained amylopectin and/or branched amylose (Whistler & Doane, 1961). Whether this intermediate fraction exists in vivo in the starch granule or if it signifies starch breakdown is still debated (Tester & Karkalas, 1996). Amylose was eluted from the column in a much broader peak, the λmax of these fractions were higher than 600 nm. In the case of starch from AN-14, however, the fractions eluted from the column immediately after amylopectin had a λmax of 580 nm. Finally fractions with a λmax ≥ 600 nm, indicating amylose, were eluted from the column. The presence of fractions with a λmax of 580 nm suggests that they consist of the so-called intermediate material. The control starch did not contain this type of glucan.

The degree of branching of the starch expressed as DE (after isoamylase digestion) is presented in Table 2. The degree of branching (DE) of greenhouse-grown

Table 2
Degree of branching (DE) and percentage of A-chains of starch from greenhouse grown plants

Clone	DE	% A chains
1029-31	3.8 ± 0.2	29
EC-13	4.4 ± 0.4	30
EC-17	5.0 ± 0.2	nd
EC-20	4.9 ± 0.1	45
A16	3.6 ± 0.2	30
AN-9	4.3 ± 0.2	35
AN-14	4.5 ± 0.2	40

nd = not determined.

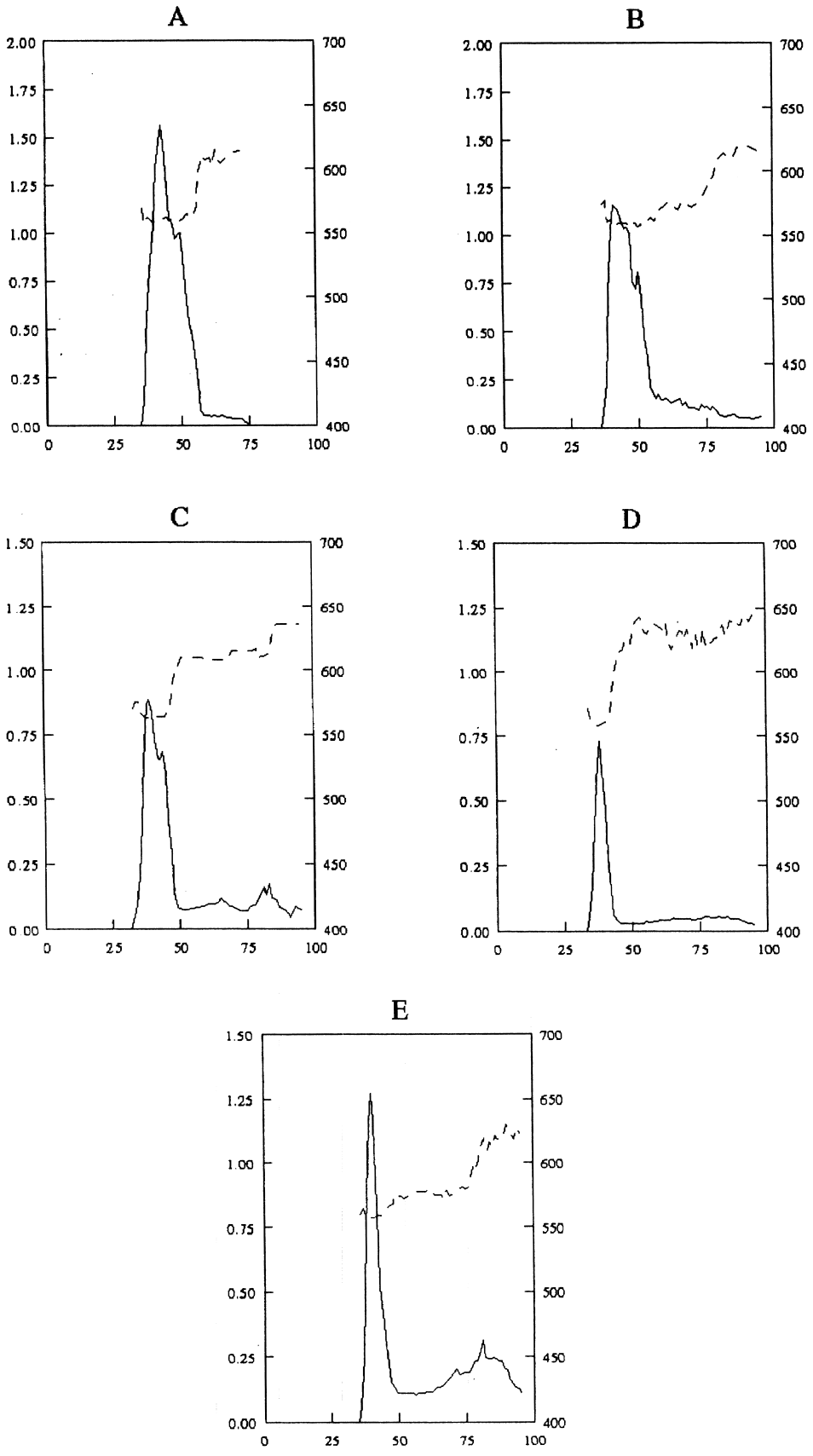

Fig. 1. Gel permeation chromatogram of *amf* control 1029-31 and amylose containing control A16 starch and their transformants. The optical density of the iodine-polysaccharide complex was measured for each 10 ml fraction at λmax(unbroken line), where λmax is displayed as a broken line. Starch from A: untransformed control 1029-31, B: EC-20, C: untransformed control A16, D: AN-9 and E: AN-14.

Table 3
Differential scanning calorimetry measurements of starch:water suspensions from greenhouse-grown plants, 20% (w:w); temperature of onset of gelatinization (T_o), temperature of gelatinization peak (T_p) and enthalpy (ΔH) released

Transformant	T_o (°C)	T_p (°C)	ΔH kJ/g starch
1029-31	68.3	72.2	18.7
EC-20	69.2	73.9	18.6
A16	62.5 ± 0.3	66.0 ± 0.3	11.4 ± 0.2
AN-9	61.5 ± 0.4	65.1 ± 0.2	10.5 ± 0.3
AN-14	64.1 ± 0.2	67.5 ± 0.4	12.0 ± 0.2

untransformed amf tuber starch was 3.8 ± 0.2. Starch from transgenic amf plants expressing the *E. coli* branching enzyme had a degree of branching varying from 3.8 to maximally DE 5.0 ± 0.2. For field grown tubers of these transformants similar values were observed. The degree of branching of starch of the untransformed wildtype clone A16 was 3.6 ± 0.2. Transgenic plants carrying the pB$_{19}$13AN construct, containing the glgB gene of *A. nidulans*, showed a degree of branching expressed in DE in the range from 3.6 to 4.5. Starch from greenhouse-grown and field-grown tubers showed a similar degree of branching. For the amf as well as for the wildtype based altered starches the degree of branching (DE) had increased maximally up to approximately 25%. Starch from the greenhouse-grown amf

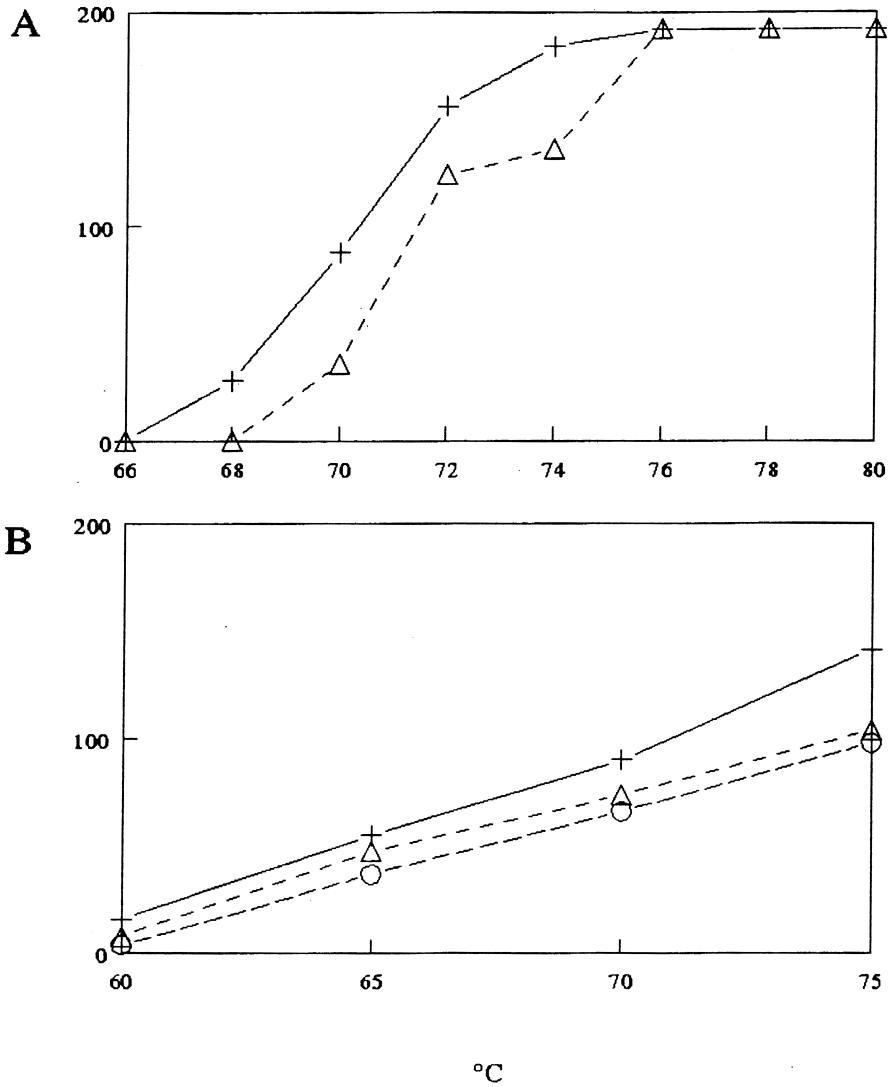

Fig. 2. Swelling power of starch from *amf* (A) and wildtype (B) greenhouse-grown transgenic plants. The swelling power of a 0.5% (w:w) starch suspension was measured in water. The swelling power was corrected for the amount of solubilized starch. A: +, 1029-31; Δ, EC-20. B: +, A16; O, AN-14; Δ, AN-9.

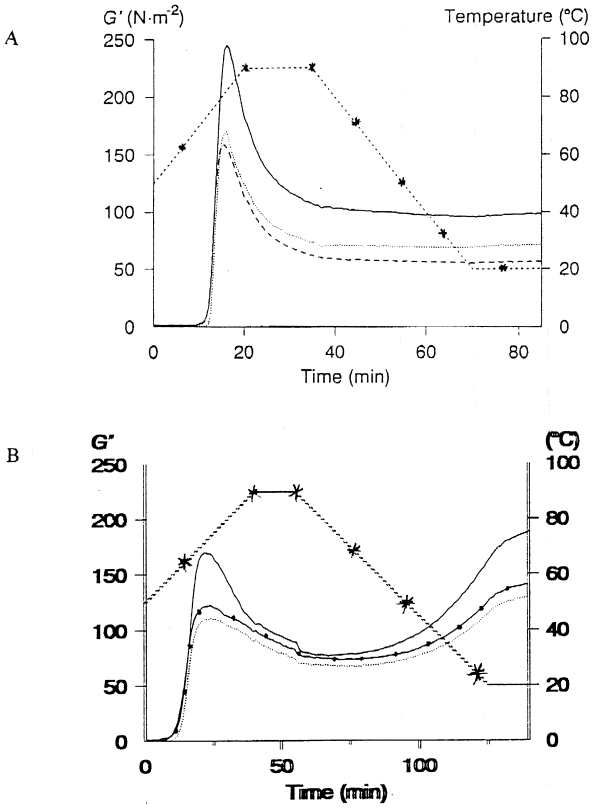

Fig. 3. Bohlin gelation profile of 5% starch suspensions. (a) Changes in the storage moduli of 5% (w:w) amylose-free starch suspensions during heating and subsequent cooling. - -*- -, temperature against time; -, amylose free control starch; ....., transformant EC-13; - - - -, transformant EC-20. (b) Changes in the storage moduli (G') of 5% starch suspensions during heating and subsequent cooling. -*-, temperature against time; - control starch of A16; ....., transformant AN-9; - - - - -, transformant AN-14.

tubers from transformant EC-20 displayed a shift in chain length distribution compared to the (un)transformed control. About 10–20% more short chains were present, shifting the ratio short to long chains from 3:1 to 9:1 as reported before (Kortstee et al., 1996). The wildtype based starches of transgenic plants were analyzed in the same way. Starch from greenhouse grown tubers of transformant AN-14 contained about 10% more short chains (dp $\leq$ 16) compared to the controls (Table 2). In the same series starch from transformant AN-9 contained about 5% more short chains. Similar results were obtained with starch from field grown tubers which showed also a shift towards more short chains, but to a lesser degree.

3.3. Gelatinization characteristics

The thermal properties of the amylose-free and wildtype based starches of transgenic potatoes were measured in starch: H_2O suspensions (20% starch w/v, 80% H_2O v/v) using differential scanning calorimetry. The results are presented in Table 3. From the greenhouse grown amf tubers only the untransformed control and transformant EC-20 were analyzed. EC-20 appeared to have a higher temperature of onset of gelatinization compared to the control (T_o 69.2 and 68.3°C, respectively). The peak temperature (T_p) had likewise shifted to a higher temperature, but a similar enthalpy (ΔH) and gelatinization range ($T_p - T_o$) were found for both the transformant and the control. Results from field-grown tuber starch revealed no significant differences in temperature of onset of gelatinization between the control starch and starch from transgenic plants (data not shown).

Starch from wild-type based transformants from greenhouse grown tubers did not display a change in temperature of onset of gelatinization, enthalpy or gelatinization range. Starch from the fieldgrown tubers of the same group of transformants was also analyzed by DSC. Transformant AN-14 appeared to have a higher temperature of onset of gelatinization, a higher T_p, but the same enthalpy and gelatinization range compared to the control. In general only the starch from the amf and wildtype transformants with the greatest change in degree of branching showed a shift in the temperature of onset of gelatinization and peak temperature. In those cases both the T_o and the T_p had increased approximately 1°C compared to their respective controls.

3.4. Swelling

The swelling power of starch from greenhouse grown tubers was measured in excess water [Fig. 2(a,b)]. The swelling curve of the amf starches differed radically from that of the amylose containing starches. The amf starches swell very fast, within a temperature rise of only a few degrees Celsius they are completely swollen instead of over an extended temperature range like the amylose-containing starches. Starch of transformant EC-20 displayed a shift in the swelling curve, the whole swelling curve was shifted to a higher temperature (+2°C) as can be seen in Fig. 2(a).

For the amylose containing starches [Fig. 2(b)] a lowered swelling power could be detected for the transformants with an increased starch degree of branching compared to the control. In a temperature range from 60 to 75°C the volume of swollen granules increased linearly. The swelling curve of the starch with the increased degree of branching (AN-9 and AN-14) was parallel, but lower than that of the control. For the starches from field-grown plants no differences could be detected in swelling power between the starches from transformants and their respective controls.

3.5. Viscosity (Bohlin/RVA)

Because of limited sample size, starches from greenhouse-grown tubers were subjected to Bohlin analysis to determine changes in storage modulus (G') during heating and subsequent cooling. Starch from field-grown tubers was analyzed by RVA to measure the pasting properties. The difference between both methods lies in the applied shearing forces. In the Bohlin assay starch granules remain intact during heating and cooling, whereas they are degraded by shearing forces during RVA.

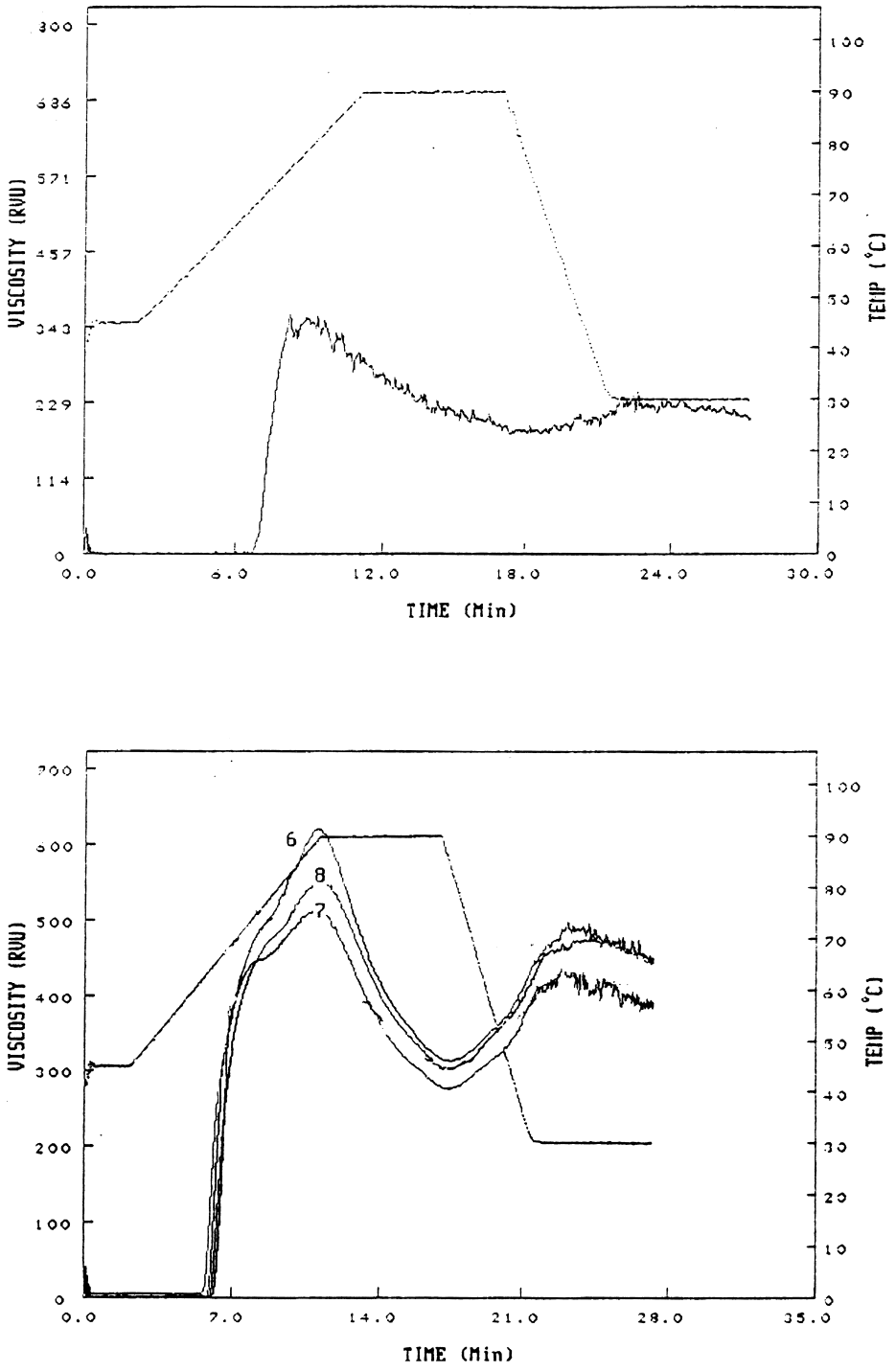

Fig. 4. Rapid visco analysis of 8% starch suspensions. (a) Changes in viscosity of 8% (w:v) amylose-free starch suspensions during heating and subsequent cooling measured by RVA. B. Changes in viscosity of 8% (w:w) amylose containing starch suspensions during heating and subsequnt cooling measured by RVA. - - - - - -, temperature against time; –7–, transformant AN-9; –8–, transformant AN-14; –6–, untransformed control A16.

3.5.1. Bohlin analysis

Starch from greenhouse grown tubers of the amf transformants was analyzed by Bohlin. During heating an increase in storage modulus (G') was seen for control and transformants at the same temperature. The transformants however, reached a lower peak viscosity before the storage modulus (G') dropped and G' remained lower during cooling as can be seen in Fig. 3(a). The amylose-containing starches from the greenhouse-grown tubers were also tested by Bohlin and were found to have the same temperature at

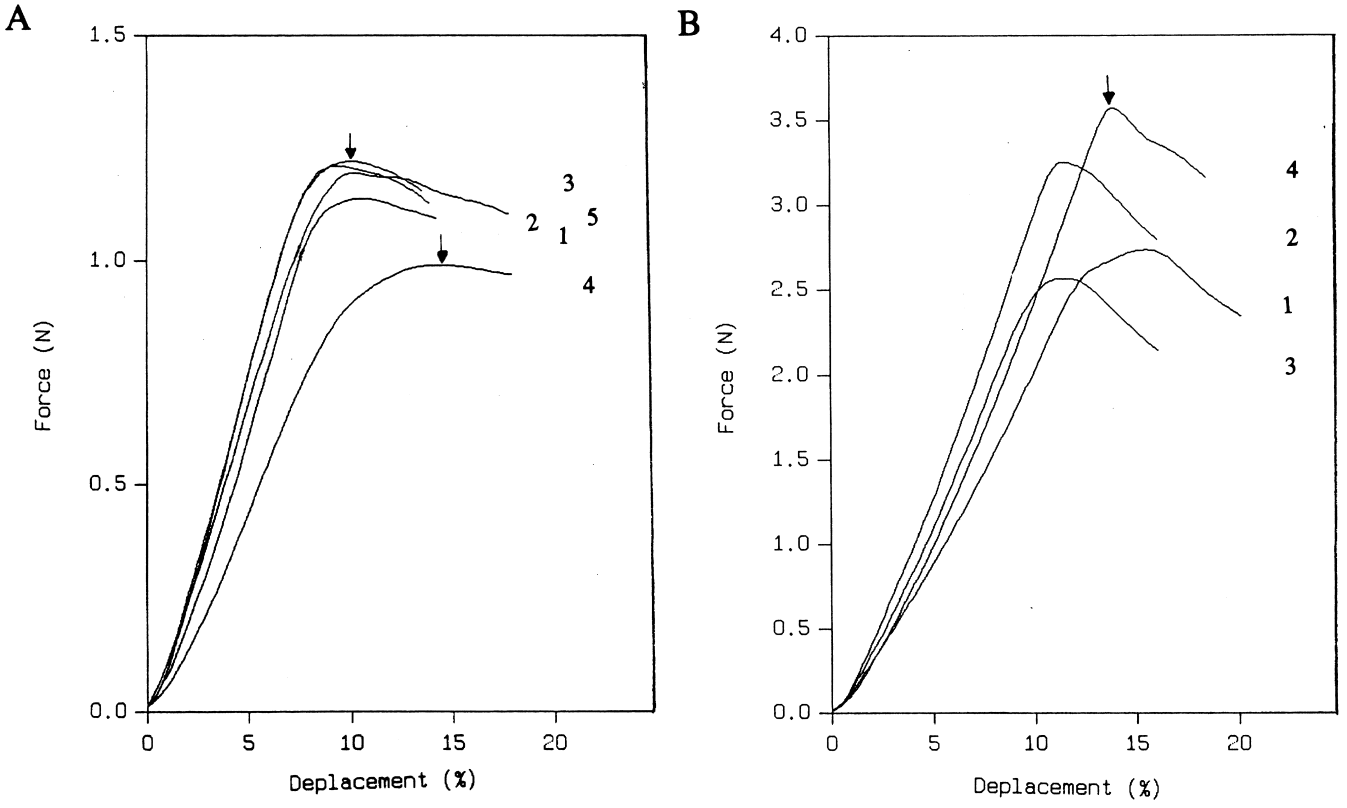

Fig. 5. Relative compression of 20% (w:v) starch gels, stored at 4°C for 5 days. (a) Gels from amylose-free starches, 1029-31 is the untransformed control (1), with transformants EC-3 (2), EC-13 (3), EC-20 (4) and EC-17 (5). (b) Gels from amylose containing starches, A16 is the untransformed control (1) and the transformants AN-9 (2), AN-14 (3) and a transformed control (4).

which G' increased for transformants and control starch. The transformants with a higher degree of branching of the starch however, had a lowered peak viscosity compared to the (un)transformed control, and after cooling, G' stayed lower for the starch from the transformants as can be seen in Fig. 3(b).

3.5.2. RVA analysis

The results from the RVA analysis of starch from field-grown tubers are displayed in Fig. 4. The amf starches all showed the same RVA profile like the one displayed in Fig. 4(a). The starches showed an increase in viscosity at the same time/temperature, reached a maximum which was much lower than that for the amylose containing starches and hardly displayed any setback viscosity after cooling. This RVA profile of the amf starches with the irregular line, may possibly be due to a higher sensitivity of the amf starch granules to shear.

From Fig. 4(b) can be seen that transformant AN-9 and AN-14 displayed a lower peak viscosity compared to the control. The rest of the pasting profile is similar for transformants and control. All amylose-containing starches showed a high peak viscosity followed by a rapid decrease in viscosity which can be attributed to the thinning effect caused by mechanical shearing. The setback viscosity after cooling and an increase in viscosity as a consequence of retrogradation could be observed for all amylose-containing starches.

3.6. Gel strength

In Fig. 5 the results of the relative compression studies can be seen. The curves that are displayed are representatives of the six measurements made for each sample. For the amylose-free starches it is noted that starch gels from transformant EC-13 and EC-17 are as strong as the gel of the untransformed control 1029-31. They all break at the same applied force of approximately 1 N, and showed similar displacement (about 10%) of gel material at the breaking point. The gel prepared from EC-20 starch breaks at the applied force of less then 1 N, and the displacement of gel by the probe is higher (about 15%) than that for the control. Starch from transformant EC-20 forms much weaker gels compared to the untransformed control. Amylose-free gels with a starch percentage of lower than 20% were too weak to be measured. From the amylose containing starches gels were prepared and measured. The results displayed in Fig. 5(b) show no clear differences between the control starches and the starches with the increased degree of branching with respect to their gel strength. This could be due to the large variation between the different gels of the same starch sample.

4. Discussion

Some physico-chemical properties of starches with an increased degree of branching of the amylopectin were described. Both amylose containing and amylose-free mutant starches with an increased degree of branching were used. For starches with an increased degree of branching the same tendencies were found in the physico-chemical characteristics, whether amylose was present or not. The same clones were grown in the greenhouse and on the field. The isolated starch was iodine stained and examined under the microscope for morphology of the starch granules. No change in granule morphology was seen for the transgenic starches. Granule size and size distribution remained similar for transformants and their controls except for AN-9 which had slightly smaller granules. The starch composition as far as amylose content was concerned had not changed for most of the transformants except for AN-14, which had a lowered amylose content, but a similar lmax and Blue Value compared to the control. From the CL2B profile of AN-14 starch it was observed that the starch contained an additional fraction to amylose and amylopectin, the so-called intermediate fraction. Whether this fraction consisted of highly branched amylose or long chained amylopectin is not evident from our results. The degree of branching of the starch of transgenic tubers expressed in DE had increased by 15–25% compared to the controls. The difference in DE between amf and wildtype control starch could probably be attributed to the fact that the DE was expressed as reducing power per dry weight starch. Because of the unbranched nature of amylose the overall degree of branching would have been lower in amylose containing starches. The increased degree of branching of the amylopectin of the starches could be, at least partly, explained by the presence of more short chains, the so-called A-chains. The ratio short to long chains had increased as a result of the presence of more short chains. To investigate the influence of the higher branched starches on the physical properties of the starches, thermal behavior and gelatinization were measured. For the amf and amylose containing starches the Bohlin pasting profile showed a lower peak viscosity and a lower storage modulus (G') after cooling of starches with an increased degree of branching. These results were in accordance with results obtained by Flipse et al. (1996) on their work on plants with an inhibited expression of the endogenous branching enzyme. Inhibition of potato branching enzyme in the amf mutant background lead (after iodine staining) to red staining granules with a small blue core. The Bohlin pasting profile of this type of starch showed an increased storage modulus (G') and a higher peak viscosity compared to the untransformed control. So the addition of a heterologous branching enzyme increased the degree of branching and the ratio short to long chains and lowered the peak viscosity. In addition to this the inhibition of the endogenous branching enzyme lead to a type of starch with presumably longer chains, blueish staining starch and a higher peak viscosity. The ratio short:longer chains apparently influences the physical properties of starch as was also shown by Wang et al. (1993a); Wang et al. (1993b). They described the influence of structural properties of 17 mutant maize genotypes on the physico-chemical behavior of those starches. It was concluded in their study that the relationship between starch structure and physical properties was not always clear. However, it was found that the amylose content had a large effect on swelling and gelatinization. The amylose content was negatively correlated to the swelling power, %T and peak viscosity and positively correlated to Blue Value and lmax. Other structural properties such as intermediate size content and the ratio of short to long chains were found to be negatively correlated with peak viscosity.

So we conclude that the lowered peak viscosity of the starches from our transgenic plants can be attributed to the increased degree of branching as a result of the shift in chain length distribution towards the presence of more short chains. In contrast to this were the observations of Jane & Chen (1992). They found rice amylopectin (with average shorter chain length) to have greater viscosity compared to both high amylose and waxy maize. However, the observed higher phosphorus content in rice amylopectin could have resulted in the higher viscosity.

The other characteristics of the transgenic potato starches with an increased degree of branching were not always found to be the same for both the greenhouse-grown and field-grown starches of both the amylose-free and amylose containing altered starches. Among the field-grown tuber starches less differences were found between controls and transformants, which may be due to the less favorable circumstances in the field for the introduced gene.

Generally, the temperature of onset of gelatinization seemed to have increased. This could be the result of more entanglement between the more branched amylopectin molecules. Sanders et al. (1990) studied the relationship between amylopectin structure and thermal behavior in four maize inbred lines. They found a higher *To* for aewx starch compared to the other studied starches (wx, duwx and aeduwx) and an increased high molecular weight peak in HPLC chromatograms of isoamylase debranched starch. They concluded that variation in the proportions of short and longer chains could explain the difference in thermal behavior. Their findings are directly opposite to ours in the fact that they found a higher *To* of gelatinization as a result of more longer chains, whereas we found an increased *To* with an increase of the shorter chains. Perhaps the influence of the genetic background or the presence of other substances besides the starch, like protein and phosphate is stronger here. Starches with an increased DE (after debranching) also showed a shift in swelling pattern. The swelling curves were similar to those of their respective controls, but at a lower rate. Work by Tester & Morrison

(1990) showed swelling to be a property exclusively of the amylopectin; amylose and lipids actively inhibit swelling or sometimes only act as a diluent. The uptake of water at higher temperatures, or, a decreased swelling power for potato starches with an increased degree of branching would have to be due to changes in the structure of the amylopectin, possibly the result of more entanglement between amylopectin molecules.

In this paper we have described a (first) step in the direction of the production of naturally (granule based) starches with properties analogous to chemically modified starches now in use. In the future it may even be possible to produce 'tailor made' starches for each thinkable application or even totally new applications, by the use of biotechnology. From the data we showed it also becomes clear how biotechnology can be of use in helping to clarify the relationship between starch fine structure and starch properties.

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