Structural properties and gelatinisation characteristics of potato and cassava starches and mutants thereof

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ABSTRACT

The molecular size of amylopectin (AP) and amylose (AM), AP chain length distribution, crystallinity and granular structure (morphology and granule size distribution) of five wild type potato starches (wtps), five AM free potato starches (amfps), four high-AM potato starches (haps), one wild type cassava starch (wtcs) and one AM free cassava starch (amfcs) were investigated and related to their gelatinisation characteristics. Starches with higher levels of short chains [degree of polymerisation (DP) 6–9 and DP 10–14] had lower gelatinisation onset (T_o), peak (T_p) and conclusion (T_c) temperatures, whereas higher contents of longer chains (DP 18–25 and DP 25–80) led to higher gelatinisation temperatures. Gelatinisation enthalpies (ΔH) increased with degree of crystallinity. The granules of wtps were larger than those of amfps and haps, respectively. No differences in morphology were observed between wtps and amfps granules, but the haps granules had more irregular surfaces and showed multi-lobed granules.

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1. Introduction

Starch is the main constituent of many roots and tubers. Its variety of structures and, consequently, physicochemical properties, offers numerous possibilities for industrial applications. There is a continuous demand for novel starch properties. It is hence very important to understand the link between structural aspects and physicochemical properties (Fredriksson, Silverio, Andersson, Eliasson, & Aman, 1998; Jobling, 2004; Vandeputte, Derycke, Geeroms,

& Delcour, 2003; Vandeputte, Vermeylen, Geeroms, & Delcour, 2003a, 2003b).

Common granular starch is semi-crystalline. It consists of amylopectin [AP, a highly branched α -(1,4)-glucan with 5% α -(1,6)-bonds, MW = 1.7×10^8 for wild type potato starch (wtps), 2.0×10^8 for amylose free potato starch (amfps), 0.7×10^8 for wild type cassava starch (wtcs)] (Yoo & Jane, 2002) and amylose [AM, a nearly linear α -(1,4)-glucan, molecular weight (MW) = 1.03×10^6 for wtps, 1.08×10^6 for wtcs] (Morrison & Karkalas, 1990).

In the generally accepted AP structural model, the short AP chains form double helices which are organised in clusters. These clusters are connected by the long chains of AP which extend in two or more clusters (Fig. 1). The chain length (CL, i.e. the number of glucose residues making up one chain) distribution is polymodal (Hizukuri, 1986). A chains (outer chains) are attached to B chains. The latter are linked to other B chains or the C chain and carry one or more A chains. The C chain contains the single reducing group of AP. B1 chains are located in 1 cluster while B2, B3 and B4 chains span 2, 3 and 4 clusters, respectively (Hizukuri, 1986). Potato starch is unique in that it contains phosphate ester groups (0.2–0.4% of the glucose units) (Takeda & Hizukuri, 1982), covalently linked to AP mainly at the C-6 (61%) and C-3 (38%) positions with small levels on the C-2 position (Tabata & Hizukuri, 1971).

Abbreviations: AP, amylopectin; AM, amylose; amfcs, amylose free cassava starch; amfps, amylose free potato starches; CL, chain length; conA, concanavalinA; DSC, differential scanning calorimetry; dm, dry matter; DP, degree of polymerisation; ΔH , enthalpy; GOPOD, glucose oxidase/peroxidase; glc-6-P, glucose-6-phosphate; GBSS, granule bound starch synthase; haps, high-amylose potato starches; HPAC, high performance anion-exchange chromatography; HPSEC, high performance size exclusion chromatography; MW, molecular weight; NAD⁺, nicotinamide adenosine dinucleotide; R, Pearson correlation coefficient; PAD, pulsed amperometric detection; RC, relative crystallinity; RNA, ribonucleic acid; SEM, scanning electron microscopy; SEC, size exclusion chromatography; SBE, starch branching enzymes; SS, starch synthases; T_o , onset temperature; T_p , peak temperature; T_c , conclusion temperature; wtcs, wild type cassava starch; wtps, wild type potato starches.

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Fig. 1. Cluster structure of amylopectin with indication of A, B, and C chains. The solid lines represent α -1,4 linked glucose units, arrows indicate α -1,6 linkages. The only chain carrying a reducing end ($\circ$) is the C chain. The external chains are A chains. B1 chains expand over one cluster only, while B2 and B3 chains expand over two and three clusters, respectively. Adapted from Hizukuri (1986).

Starch itself has different levels of organisation [from granules (2–100 μ m) (Buléon, Colonna, Plancho, & Ball, 1998) to growth rings (120–500 nm) (French, 1984), blocklets (20–500 nm) (Gallant, Bouchet, & Baldwin, 1997), lamellae (9–10 nm) (Jenkins, Cameron, & Donald, 1993) and AP and AM molecules (0.1–1.0 nm)]. Granules consist of alternating semi-crystalline and amorphous growth rings. AM is deposited mainly in the amorphous growth rings and, to a small extent, in the semi-crystalline growth rings (Jenkins & Donald, 1995). In contrast, semi-crystalline growth rings are built from alternating amorphous and crystalline lamellae of the AP structure. Irrespective of the botanical origin, the repeat distance of the combination of one amorphous and one crystalline lamella is 9 nm (Oostergetel & van Bruggen, 1989). It has been suggested that the lamellae of AP behave like side-chain liquid-crystalline polymers. In this view, the double helices form the rigid units, while the spacer units contain the branch points of AP and possibly AM (Daniels & Donald, 2003). The spacer units are connected to the long, flexible backbones of AP (Waigh et al., 2000, Donald, Kato, Perry, & Weigh, 2001).

The structure of starch results from the action of different biosynthetic enzymes. Impacting biosynthetic pathways yields starches with different AM/AP ratios. Granule bound starch synthase (GBSS) primarily catalyses AM synthesis (Kuipers, Jacobsen, & Visser, 1994). In the case of potato starch, antisense ribonucleic acid (RNA) inhibition of the expression of GBSS (Visser et al., 1991) or mutation by Röntgen-irradiation (Hovenkamp-Hermelink et al., 1987) have led to amfps. Both CL distribution of short chains [degree of polymerisation (DP) < 35, as measured by HPAEC] and granule morphology are comparable for wtps and amfps (Visser, Suurs, Bruinenberg, Bleeker, & Jacobsen, 1997, Fulton et al., 2002). The starch branching enzymes (SBE) A and B catalyse the hydrolysis of an α -(1,4)-bond followed by transfer of the liberated reducing end for the formation of an α -(1,6)-bond. Repression of SBE A and SBE B activities results in an up to sixfold increased degree of phosphorylation (Schwall et al., 2000) and AM contents up to 89%, depending on the used determination method. It drastically changes the AP structure [an increase in longer chains of DP > 20] and the resultant granules show deep fissures and irregular surfaces (Schwall et al., 2000, Blennow et al., 2005).

The current study aimed to explore the relation between the structure of potato and cassava starches and their gelatinisation behaviour. To that end, five wtps, five amfps, four high-AM potato starches (haps), one wtcs and one amylose free cassava starch (amfcs) were studied. As these genetically modified starches have only been characterised to a limited degree, an extensive study of the structure and the physicochemical properties of these starches is necessary. In the present study, molecular (crystallinity, molecular size distribution, CL distribution) and granular (morphology and size distribution) structure of these starches was analysed. In addition, we here for the first time report on the impact of these properties on gelatinisation characteristics.

2. Materials and methods

2.1. Materials

Table 1 lists all potato and cassava starches, their genotype and the corresponding enzyme suppression. They were obtained from Wageningen University (Wageningen, The Netherlands) or BASF Plant Science Holding GmbH (Ludwigshafen, Germany). Sixteen starches were investigated: five wild type potato starches (wtps1–wtps5), five AM free potato starches (amfps1–amfps5), four high-AM potato starches (haps1–haps4), one wild type cassava starch (wtcs) and one AM free cassava starch (amfcs). Sample numbers wtps3 and amfps3 originate from a mix of different genotypes. All amfps and amfcs samples were the result of (partial) RNA inhibition of GBSS, except for amfps3 which resulted from point mutation of GBSS by X-ray irradiation. haps samples were the result of (partial) RNA inhibition of SBE A and SBE B.

All reagents, chemicals and enzymes used were of at least analytical grade and obtained from Sigma–Aldrich (Bornem, Belgium) unless indicated otherwise. Where enzymes were used, their Units (U) were as defined by the respective suppliers.

Table 1

Overview of the examined starches with their corresponding genotype and the corresponding suppressed enzymes.

	name	genotype	enzyme suppression
wild type potato starches (wtps)	wtps1	kuras	none
	wtps2	karnico	none
	wtps3	mix	none
	wtps4	dinamo	none
	wtps5	producent	none
amylose free potato starches (amfps)	amfps1	kuras	antisense RNA inhibition of GBSS
	amfps2	karnico	antisense RNA inhibition of GBSS
	amfps3	mix	point mutation in GBSS by X-ray irradiation ^a
	amfps4	karnico	antisense RNA inhibition of GBSS ^b
	amfps5	karnico	partial antisense RNA inhibition of GBSS ^b
high-amylose potato starches (haps)	haps1	kuras	partial antisense RNA inhibition of SBE A and B
	haps2	dinamo	partial antisense RNA inhibition of SBE A and B
	haps3	producent	partial antisense RNA inhibition of SBE A and B
	haps4	dinamo	antisense RNA inhibition of SBE A and SBE B
cassava starches	wtcs	tms60444	none
	amfcs	tms60444	antisense RNA inhibition of GBSS ^c

^a Visser et al. (1997).

^b Kuipers et al. (1994).

^c Raemakers et al. (2005).

Table 2

Amylose (AM) contents, starch damage, glucose-6-phosphate (glc-6-P) contents, relative crystallinity (RC) and relative levels of amylopectin of large molecular weight (region I), amylopectin with smaller molecular weight (region II) and amylose (region III) of wild type (wt), amylose free (amf) and high-amylose (ha) potato (p) and cassava (c) starches (s).

sample	AM content (%)	starch damage (%)	glc-6-P content (nmol/mg)	RC (%)	region I (%)	region II (%)	region III (%)
wtps1	18.9 (0.6)	0.8 (0.1)	16.9 (0.8) ^{ef}	30.0	42.1 (1.2)	33.4 (0.0)	24.5 (1.3)
wtps2	22.4 (0.3)	0.7 (0.0)	9.2 (0.8) ^{h,j}	30.0	41.1 (3.1)	28.7 (2.7)	30.2 (0.8)
wtps3	20.3 (0.2)	0.2 (0.0)	9.8 (0.5) ^j	33.0	48.6 (2.4)	25.8 (0.8)	25.5 (3.3)
wtps4	20.7 (0.3)	0.7 (0.0)	20.4 (0.6) ^c	36.0	46.1 (2.5)	24.7 (1.5)	29.2 (1.1)
wtps5	19.9 (0.9)	0.9 (0.0)	12.8 (1.1) ^{h,g}	35.5	40.4 (1.1)	30.3 (2.5)	29.3 (1.6)
amfps1	3.9 (0.1)	0.8 (0.1)	21.4 (1.1) ^e	36.0	50.2 (3.5)	37.0 (3.4)	12.9 (1.5)
amfps2	3.6 (0.0)	0.7 (0.0)	10.6 (1.0) ^{h,i,j}	37.5	42.4 (1.8)	41.4 (0.8)	16.2 (1.0)
amfps3	4.2 (0.2)	0.3 (0.0)	8.6 (0.6) ^j	38.5	22.2 (0.5)	50.5 (2.4)	27.3 (1.9)
amfps4	7.5 (0.2)	0.8 (0.0)	14.4 (1.0) ^{f,g}	34.0	58.7 (1.9)	31.6 (1.2)	9.7 (1.7)
amfps5	3.9 (0.1)	0.8 (0.0)	11.6 (0.8) ^{h,i}	34.0	57.5 (1.2)	29.2 (1.1)	13.4 (0.9)
hps1	27.4 (0.5)	0.8 (0.0)	64.3 (2.6) ^c	28.5	30.2 (2.9)	31.1 (2.1)	39.0 (1.6)
hps2	33.1 (1.6)	0.8 (0.0)	73.3 (1.2) ^a	29.0	10.2 (1.5)	30.3 (4.3)	59.5 (5.7)
hps3	37.3 (1.3)	0.9 (0.0)	69.5 (0.8) ^b	26.5	18.7 (1.4)	29.2 (0.5)	52.1 (1.4)
hps4	78.4 (0.9)	0.3 (0.0)	50.0 (1.8) ^d	21.0	1.6 (0.2)	10.8 (0.6)	87.6 (0.8)
wtcs	18.4 (0.1)	1.3 (0.0)	0.0 (0.0) ^k	40.0	43.8 (1.5)	28.7 (1.3)	27.6 (1.7)
amfcs	6.0 (0.1)	1.2 (0.0)	0.0 (0.0) ^k	49.0	52.9 (2.1)	33.3 (0.9)	13.8 (1.6)

Results indicated with the same letter are not significantly different ($P < 0.05$).

2.2. AM content

AM and starch contents were determined according to a Megazyme (Bray, Ireland) procedure, which itself is based on the method developed by Yun and Matheson (1990). For the AM content determination, samples were incubated with concanavalinA (conA) to precipitate AP. Next, AM, which remained in the supernatant, was measured. AM content and starch content were determined after degradation with a mixture of amyloglucosidase/ α -amylase and incubation with glucose oxidase/peroxidase (GOPOD, Megazyme). Analyses were performed in triplicate and AM content was expressed as % of total starch content.

2.3. Starch damage

Damaged starch was measured in triplicate according to the damaged starch assay (Megazyme, AACC method 76-31). The method relies on the enzymic degradation of the damaged granules by α -amylase and glucoamylase, while at the same time minimizing the degradation of intact granules, followed by quantification of the released glucose units by GOPOD.

2.4. Nitrogen content

Nitrogen was determined in triplicate using an adaptation of the AOAC official Dumas combustion method (AOAC, 1995) to an automated Dumas protein analysis system (EAS, VarioMax N/CN, Elt, Gouda, The Netherlands).

2.5. Glucose-6-phosphate content

Glucose-6-phosphate (glc-6-P) content was determined based on Bay-Smidt, Wischmann, Olsen, and Nielsen (1994) with slight modifications. Deionised water (5.0 ml) was added to 150.0 mg starch and the suspension was heated at 100 °C for 90 s under vigorous mixing followed by the addition of α -amylase solution [6 U in 20 μ l 5.0 mM 2-morpholinoethanesulfonic acid (MES)/KOH, pH 6.4 with 4 mM CaCl_2] from *Bacillus licheniformis* (Megazyme) and heating for 15 min at 100 °C. After centrifugation (3000 g, 10 min), 1.0 ml supernatant of each sample was acid hydrolysed for 240 min at 100 °C by addition of 62 μ l concentrated HCl. Next, glc-6-P and total glucose contents

of the hydrolysed samples were determined. For glc-6-P content, 10 mg activated carbon was added to the hydrolysed samples followed by centrifugation (12 000 g, 30 s). In a microplate, 250 μ l of a 270 mM solution of K_2HPO_4 containing 135 mM KOH and 10 μ l of 40 mM β -nicotinamide adenosine dinucleotide (NAD^+) [in 50 mM 3-morpholinopropane-1-sulfonic acid (MOPS)/KOH buffer, pH 7.3] were added to 100 μ l supernatant. A standard curve was constructed for a concentration range of 0–0.1 mg/ml glc-6-P. Before and after addition of glc-6-P dehydrogenase from *Leuconostoc mesenteroides* (1 U) and thorough mixing, absorbance at 340 nm was measured. Analyses were performed in sixfold.

For the determination of glucose content, each hydrolysed sample was centrifuged (13 000 g, 1 min). Next, 750 μ l supernatant was neutralized with 0.7 M KOH solution (750 μ l) and the neutralized sample was diluted 200 times. In a microtiter plate, 220 μ l MOPS/KOH buffer (50 mM, pH 7.3, 5.0 mM MgCl_2 , 1.0 mM EDTA disodium salt) was added to 100 μ l sample or 100 μ l glucose solution (standard curve, 0–0.1 mg/ml), followed by addition of 10 μ l of adenosine triphosphate solution (100 mM), 10 μ l NAD^+ solution (10 mM) and a solution of hexokinase from *Saccharomyces cerevisiae* (10 U). Before and after addition of glc-6-P dehydrogenase from *L. mesenteroides* (1 U) and thorough mixing, absorbance at 340 nm was measured. Starch contents were calculated as 0.9 times the glucose levels. Analyses were performed in sixfold. The glc-6-P content was expressed as nmol glc-6-P/mg starch.

2.6. Granular structure

The granule size distribution of the potato starches was determined with laser diffraction particle sizing (Malvern Mastersizer S, Goffin Meyvis, Bergen op Zoom, The Netherlands). The wet sample dispersion unit was used and information was obtained from 64 channels over a particle size range of 0.05–900 μ m. The results were expressed in volume percentage (%), using Mastersizer S Version 3.1 software (1996).

For the Scanning Electron Microscopy (SEM) analyses, starch was deposited on a stub and sputter coated with gold (SPI-MODULE TM Sputter Coater, SPI Supplies, West Chester, PA, USA). The samples were analysed using a JSM-6360 scanning electron microscopy (JEOL, Peabody, MA, USA) at 10 kV.

Fig. 2. Scanning electron microscopy images of the starch granules of intact wild type (wt), amylose free (amf) and high-amylose (ha) potato (p) and cassava (c) starches (s).

2.7. Relative crystallinity

Wide angle X-ray diffraction (WAXD) measurements were performed with Ni-filtered Cu $\kappa\alpha$ radiation on a Rigaku rotating anode device (Rigaku, Tokyo, Japan) operating at 40 kV and 100 mA, using a Rigaku high temperature X-ray diffractometer (Rigaku). Detection was with a scintillation counter with a powder diffractometer operating in transmission mode. Starches were first equilibrated for 48 h in a humidifier ($20 \pm 2\%$ final moisture content). Highly crystalline A- and B-type starches were prepared by acid hydrolysis

(2.2 M HCl) of waxy maize starch and high-AM maize starch, respectively, for 6 weeks at 37°C with regular gentle shaking. The suspensions were filtered and the solids washed with deionised water until the pH was neutral followed by air-drying.

An amorphous sample was prepared by freeze drying potato starch, gelatinised in excess water. Starch was enclosed in aluminium foil and scattering patterns were measured in $6.5\text{--}33^\circ$ range with a fixed time of 10 s per step of 0.05° . Data were normalised after measurements. Differences between two measurements were less than 5.0%. The relative crystallinity (RC) was

Table 3
Granule size distribution divided in four classes: 0–20 μm , 20–40 μm , 40–60 μm , >60 μm and average granule diameter of wild type (wt), amylose free (amf) and high-amylose (ha) potato (p) and cassava (c) starches (s).

Sample	0–20 μm (%)	20–40 μm (%)	40–60 μm (%)	>60 μm (%)	average diameter (μm)
wtps1	6.3	25.6	42.8	25.4	52.4
wtps2	6.9	23.1	39.6	30.4	55.2
wtps3	8.2	33.1	40.9	17.8	47.5
wtps4	6.2	18.6	36.9	38.3	59.6
wtps5	8.5	27.9	40.8	22.8	50.3
amfps1	10.1	33.5	37.6	18.8	47.2
amfps2	11.3	28.2	34.6	25.9	51.2
amfps3	8.1	33.0	43.7	15.2	46.3
amfps4	10.0	32.3	40.8	16.6	53.7
amfps5	5.9	23.8	42.4	27.8	53.8
haps1	19.4	44.2	28.4	8.0	37.5
haps2	15.8	38.4	32.6	13.2	41.9
haps3	18.1	41.0	29.9	11.1	39.8
haps4	22.0	50.7	23.8	3.5	33.7
wtcs	81.1	18.3	0.6	0.0	18.0
amfcs	84.2	15.3	0.5	0.0	16.9

Fig. 3. Size exclusion chromatography (SEC) profiles of intact wild type (wt), amylose free (amf) and high-amylose (ha) potato (p) and cassava (c) starches (s). In the primary axis, refractive index detector (RID) signal is given and in the secondary axis, the maximum wavelength (nm) is shown.

calculated according to Wakelin, Virgin, and Crystal (1959) and is given by

$$\text{relative crystallinity} = \frac{\sum_i |(I_s - I_a)_i|}{\sum_i |(I_c - I_a)_i|}$$

with

I_s : intensity of the sample for each diffraction angle

I_a : intensity of an amorphous sample for each diffraction angle

I_c : intensity of a high crystalline A-type and B-type crystals for each diffraction angle for cassava starch and potato starch, respectively.

2.8. Molecular distribution of amylopectin and amylose

AP and AM were separated by size exclusion chromatography (SEC) after starch solubilisation. Samples (30 mg, accurately weighed) were solubilised in 1.0 ml KOH solution (0.2 M) by heating for 5 min at 95 °C, followed by diluting to 20.0 ml with

deionised water. These conditions resulted in at least 95% starch solubilisation [assessed according to Dubois, Gilles, Hamilton, Rebers, and Smith (1956)] with minimal degradation. After filtration (0.45 µm), 5.0 ml of the solubilised sample was applied on a Toyopearl HW-75F gel (600 × 26 mm) (TosoHaas, Stuttgart, Germany) at 50 °C (Blennow, Bay-Smidt, & Bauer, 2001). A Biologic Duo Flow Core System (Bio-Rad Laboratories, Hercules, CA, USA), equipped with a refractive index detector (RID-10A, Shimadzu, Kyoto, Japan), was used. Elution was with 0.01 M KOH solution (containing 0.02% sodium azide) at a flow rate of 0.75 ml/min. Fractions (5.0 ml) were collected and their iodine binding was measured by mixing 1.8 ml of the fraction with 0.1 ml HCl solution (1.0 M) and 0.1 ml KI/I₂ solution (0.90 mg KI/ml and 0.38 mg I₂/ml). After 30 min incubation at room temperature, the fractions were spectrophotometrically scanned (500–800 nm wavelength range).

2.9. Chain length distribution analysis

Enzymatically debranched starches were prepared based on Umemoto, Nakamura, Satoh, and Terashima (1999) with slight

Fig. 4. Relative levels of chains with DP 6–40 of debranched wild type (wt), amylose free (amf) and high-amylose (ha) potato (p) and cassava (c) starches (s) as measured by high performance anion-exchange chromatography (HPAEC) profiles.

modifications. Starch (10.0 mg) was suspended in 5.0 ml water and boiled (60 min) to gelatinise the starch. Sodium acetate buffer (50 μ l, 600 mM, pH 4.6) and 10 μ l of 2.0% (w/v) sodium azide were added to the samples (1.0 ml). Isoamylase from *Pseudomonas amylofermosa* (12 U, Megazyme) was used to debranch the sample for 24 h at 40 °C. The enzyme was then inactivated by heating (100 °C, 10 min). A second incubation for 24 h at 40 °C was performed after adding the same isoamylase (12 U). After heating (100 °C, 10 min), cooled samples were filtered (0.22 μ m) and analysed with high performance anion-exchange chromatography (HPAEC) with pulsed amperometric detection (PAD), using Carbo-pac PA-100 Guard and Carbo-pac PA-100 anion-exchange (250 mm $\times$ 4 mm) columns. The eluents and the eluent gradient program were as described by Jacobs, Eerlingen, Rouseu, Colonna, and Delcoul (1998). Individual peaks in the chromatograms were corrected for molar PAD detector responses (Koch, Andersson, & Aman, 1998). Analyses were performed at least in quadruplicate. The coefficient of variation for the measurements did not exceed 5%. The relative amounts of AP chains were normalised and divided into five fractions: DP 6–9, DP 10–14, DP 15–17, DP 18–25 and DP > 25.

Enzymatically debranched starches were prepared for high performance size exclusion chromatography (HPSEC) based on Klucinec and Thompson (1998) with slight modifications. Starch (5.0 mg) was dissolved in 120 μ l DMSO (90%) with thorough mixing for 10 min at 100 °C. After addition of 880 μ l sodium acetate buffer (100 mM, pH 4.5), the suspension was heated again for 10 min at 100 °C. After cooling, 50 μ l solution of the above isoamylase (1250 U), dissolved in sodium acetate buffer (100 mM, pH 4.5), was added and the samples were incubated for 12 h at 40 °C. The enzyme was inactivated by heating for 10 min at 100 °C. A second incubation for 12 h at 40 °C was performed after renewed addition of the same isoamylase (625 U). After inactivation of isoamylase (10 min, 100 °C), the sample was filtered (0.45 μ m) and injected. The HPSEC system consisted of a pump (LC-20AD/LC-20AT, Shimadzu), an injector (SIL-20A autoinjector, Shimadzu) and a RID-10A. Two Zorbax PSM 60S columns (6.2 $\times$ 250 mm), packed with 5 μ m porous silica microspheres (Agilent Technologies, Wilmington, DE, USA) connected in series, were used. These columns were maintained at 50 °C using a column oven (CTO-20AC, Shimadzu), while the detector was maintained at 40 °C. The mobile phase was on-line degassed (DGU-20 A5, Shimadzu) DMSO (90%). The flow

Table 4

Chain length distribution of wild type (wt), amylose free (amf) and high-amylose (ha) potato (p) and cassava (c) starches (s), resulting from high performance size exclusion chromatography, divided in different regions with relative levels of degree of polymerisation (DP) > 1500, 1500–600, 600–150, 150–80, 80–25, 25–12, 12–7, 7–3 with standard deviation between brackets.

Sample		amylose chains + long B chains			B4 chains	B2 + B3 chains	A + B1 chains		
		>1500	1500–600	600–150	150–80	80–25	25–12	12–7	7–3
wtps1	DP _w ¹	2.7 (0.5)	4.4 (0.4)	13.5 (1.2)	5.3 (0.6)	18.1 (0.5)	27.7 (1.4)	20.9 (0.8)	2.4 (0.3)
	DP _n ²	0.0 (0.0)	0.1 (0.0)	1.0 (0.1)	0.9 (0.1)	8.6 (0.4)	37.1 (1.2)	44.7 (0.6)	7.6 (1.2)
wtps2	DP _w ¹	1.9 (0.1)	2.6 (0.6)	10.5 (1.6)	5.7 (0.2)	21.7 (0.0)	28.9 (0.7)	20.2 (0.0)	2.1 (0.3)
	DP _n ²	0.0 (0.0)	0.1 (0.0)	0.9 (0.3)	1.1 (0.2)	10.7 (0.3)	38.3 (1.0)	42.7 (0.6)	6.3 (1.1)
wtps3	DP _w ¹	3.2 (0.3)	3.0 (0.4)	13.5 (0.6)	5.7 (0.5)	19.8 (0.5)	26.6 (1.0)	19.0 (1.6)	2.2 (0.1)
	DP _n ²	0.0 (0.0)	0.1 (0.0)	1.0 (0.1)	1.0 (0.1)	9.8 (0.6)	37.6 (0.4)	43.5 (1.0)	7.2 (0.2)
wtps4	DP _w ¹	4.0 (0.6)	5.0 (1.0)	15.1 (2.1)	5.3 (0.6)	18.2 (1.0)	26.5 (2.1)	18.0 (1.8)	1.7 (0.1)
	DP _n ²	0.0 (0.0)	0.1 (0.0)	1.2 (0.5)	0.9 (0.5)	9.0 (0.5)	40.3 (0.8)	43.4 (2.8)	5.1 (1.2)
wtps5	DP _w ¹	2.1 (0.3)	2.3 (0.3)	8.6 (0.5)	3.6 (0.5)	18.7 (0.3)	33.3 (1.8)	23.9 (1.1)	2.4 (0.2)
	DP _n ²	0.0 (0.0)	0.1 (0.0)	0.7 (0.2)	0.8 (0.3)	9.9 (0.6)	39.5 (0.6)	42.8 (0.5)	6.2 (0.9)
amfps1	DP _w ¹	2.2 (0.3)	2.9 (0.4)	9.3 (0.1)	7.0 (0.2)	22.0 (1.1)	27.4 (1.4)	19.3 (0.5)	2.1 (0.0)
	DP _n ²	0.0 (0.0)	0.1 (0.0)	0.6 (0.1)	1.2 (0.1)	10.5 (0.7)	37.7 (0.9)	43.3 (1.5)	6.7 (0.5)
amfps2	DP _w ¹	1.5 (0.1)	1.8 (0.4)	7.1 (0.9)	8.0 (1.7)	25.3 (1.6)	28.4 (0.7)	20.3 (0.9)	2.3 (0.4)
	DP _n ²	0.0 (0.0)	0.0 (0.0)	0.5 (0.1)	1.5 (0.4)	11.9 (1.0)	36.7 (0.4)	42.4 (0.6)	7.0 (1.3)
amfps3	DP _w ¹	2.5 (0.5)	1.7 (0.2)	11.7 (0.2)	12.3 (0.8)	20.8 (1.3)	22.2 (0.4)	17.8 (1.2)	1.9 (0.0)
	DP _n ²	0.0 (0.0)	0.0 (0.0)	1.1 (0.0)	2.9 (0.1)	11.1 (0.4)	34.6 (0.3)	43.9 (0.8)	6.3 (0.2)
amfps4	DP _w ¹	2.2 (0.3)	2.0 (0.4)	8.7 (0.9)	9.5 (0.8)	23.3 (1.9)	25.9 (1.7)	19.7 (1.9)	2.0 (0.2)
	DP _n ²	0.0 (0.0)	0.0 (0.0)	0.6 (0.1)	1.9 (0.3)	10.3 (0.2)	34.7 (1.3)	45.7 (0.3)	6.7 (0.8)
amfps5	DP _w ¹	2.2 (0.4)	2.2 (0.8)	8.0 (1.6)	5.6 (0.8)	22.6 (0.8)	29.6 (2.0)	21.6 (1.5)	2.4 (0.3)
	DP _n ²	0.0 (0.0)	0.0 (0.0)	0.5 (0.1)	0.9 (0.2)	10.4 (0.3)	37.2 (0.9)	43.8 (1.0)	7.2 (1.3)
hps1	DP _w ¹	24.7 (1.2)	10.9 (0.3)	16.9 (1.0)	5.7 (0.1)	12.1 (0.5)	21.1 (1.4)	7.9 (0.4)	0.8 (0.1)
	DP _n ²	0.2 (0.0)	0.4 (0.1)	2.8 (0.2)	1.9 (0.2)	13.7 (1.6)	48.1 (1.7)	32.9 (2.9)	0.0 (0.0)
hps2	DP _w ¹	26.9 (0.9)	13.5 (0.2)	23.9 (0.1)	5.6 (0.1)	10.0 (0.1)	14.4 (0.2)	5.7 (0.9)	0.1 (0.0)
	DP _n ²	0.3 (0.0)	0.7 (0.1)	4.7 (0.3)	2.7 (0.1)	13.4 (0.7)	47.8 (2.1)	30.3 (0.0)	0.0 (0.0)
hps3	DP _w ¹	29.4 (1.3)	12.7 (0.6)	25.2 (3.4)	6.3 (0.3)	8.9 (0.8)	11.7 (0.9)	4.3 (0.9)	0.0 (0.0)
	DP _n ²	0.4 (0.0)	0.8 (0.0)	5.9 (1.3)	3.5 (0.1)	13.0 (0.3)	47.1 (1.3)	29.4 (1.2)	0.0 (0.0)
hps4	DP _w ¹	24.4 (1.1)	10.2 (0.1)	44.1 (0.0)	11.5 (0.4)	6.1 (0.5)	3.2 (0.2)	0.7 (0.0)	0.0 (0.0)
	DP _n ²	0.6 (0.0)	1.6 (0.0)	26.4 (1.0)	14.7 (0.1)	20.1 (0.9)	27.4 (0.4)	9.3 (0.2)	0.0 (0.0)
wtcs	DP _w ¹	2.4 (1.0)	2.8 (0.4)	7.1 (1.1)	3.4 (0.2)	20.3 (0.5)	30.1 (1.2)	27.7 (0.9)	3.5 (0.0)
	DP _n ²	0.0 (0.0)	0.0 (0.0)	0.4 (0.1)	0.5 (0.1)	8.6 (0.2)	32.0 (0.4)	49.1 (0.6)	9.4 (0.5)
amfcs	DP _w ¹	0.6 (0.1)	0.8 (0.2)	3.6 (0.1)	4.8 (0.2)	24.5 (0.1)	33.1 (0.1)	28.9 (0.5)	3.3 (0.2)
	DP _n ²	0.0 (0.0)	0.0 (0.0)	0.2 (0.0)	0.7 (0.0)	9.8 (0.0)	33.1 (0.0)	47.6 (0.7)	8.6 (0.7)

DP_w¹: chain length distribution levels expressed on weight basis in percent.

DP_n²: chain length distribution levels expressed on number basis in percent.

rate was 0.5 ml/min and the injection volume was 50 µl. Separate injections of the following standards (50 µl) were performed: glucose [(MW) = 180, DP 1], maltose (MW = 342, DP 2), maltotriose (MW = 504, DP 3), maltopentaose (MW = 829, DP 5), maltoheptaose (MW = 1153, DP 7) and Shodex pullulan standards (Showa Denko, Munich, Germany) with MWs of 5.9×10^3 (DP 5), 11.8×10^3 (DP 10), 22.8×10^3 (DP 20) and 47.3×10^3 (DP 50). In order to obtain molar-based chromatograms, the differential refractive index response at each time was divided by the MW of the carbohydrates eluting at that time. The chromatograms were divided into 8 regions based on the retention times of the minima (I–VII) or the inflection point (VII–VIII). DP limits of the different regions were: I (DP > 1500), II (DP 1500–600), III (DP 600–150), IV (DP 150–80), V (DP 80–25), VI (DP 25–12), VII (DP 12–7), VIII (DP 7–3). Regions I, II and III correspond to the AM molecules and very long B chains of AP, regions IV and V to long B chains and regions VI–VIII to A and B1 chains (Hizukuri, 1986).

The total carbohydrate content was measured by the phenol sulfuric acid method (Dubois et al., 1956) and the concentration of reducing groups was determined using the method described by Waffenschmidt and Jaenicke (1987).

2.10. Differential Scanning Calorimetry

Differential Scanning Calorimetry (DSC) was carried out with a Q1000 DSC (TA instruments, New Castle, DE, USA). Starch (4.00–6.00 mg) was accurately weighed in an aluminium pan (Perkin Elmer, Waltham, MA, USA) and deionised water was added [1:3 w/w starch dry matter (dm):water]. The pans were hermetically sealed and equilibrated for at least 20 min. The pans were heated from 20 °C to 130 °C at 4 °C/min and an empty pan was used as reference. Calibration was with indium. Analyses were performed at least in triplicate. The onset (T_o), peak (T_p), conclusion temperature (T_c) and enthalpy (ΔH) were determined using TA Universal Analysis software. Temperature was expressed in °C and enthalpy in J/g. Gelatinisation temperature range is the difference between T_c and T_o .

2.11. Statistical analyses

Pearson's correlation coefficient analyses were performed (significance level $P < 0.05$). Statistical analyses were conducted using the Statistical Analysis System software 8.1 (SAS Institute, Cary, NC, USA). The statistical analyses were performed for wtps

Fig. 5. Schematic overview of the gelatinisation characteristics of intact wild type (wt), amylose free (amf) and high-amylose (ha) potato (p) and cassava (c) starches (s). Start and end point of the bars represent onset (T_o) and conclusion (T_c) temperatures of gelatinisation. The vertical line inside the bar is the peak temperature (T_p). Gelatinisation enthalpies (ΔH) are given with standard deviations between brackets.

and amfps samples (further referred as $n = 10$), wtps, amfps and haps samples ($n = 14$) and all starches ($n = 16$). haps samples were excluded in specific cases because they were bad leverage points. Cassava starches were excluded in the statistical analyses of RC because of their different crystal type.

3. Results and discussion

3.1. Starch structure

3.1.1. Starch characterisation

Table 2 shows AM and glc-6-P contents and the levels of damaged starch for all starches. The potato and cassava starches had a wide range of AM contents (3.2–78.4%; 6.0–18.4%, respectively). Low levels of AM were also detected in amfps samples. These low levels were probably overestimated because of the interference by poorly branched low-MW AP (Gerard, Barron, Colonna, & Planchot, 2001). All starches had a low damaged starch levels (<1.3%), and were of high purity (nitrogen content < 0.01% dm, results not shown). Glc-6-P contents are largely linearly correlated with total starch phosphorylation level (Bay-Smidt, Blennow, Bojko, & Moller, 1999). The different wild type samples (wtps1–wtps5) had a broad range of glc-6-P contents (9.2–20.4 nmol/mg starch), in agreement with Bay-Smidt et al. (1994). Apart from amfps3, amfps had slightly higher glc-6-P contents than their parent starches. The small increase in glc-6-P content is

probably due to the increased AP content. However, haps samples had the lowest AP levels and showed the highest degree of starch glucose-6-phosphorylation. This may be explained by their higher levels of longer AP chains than in wtps AP (cf. infra). In this respect, Mikkelsen, Baunsgaard, and Blennow (2004) reported a higher degree of *in vitro* phosphorylation of longer (DP 30–100) than of shorter (DP < 30) AP chains. Linear AM is a poor substrate for α -glucan water dikinase, the enzyme responsible for phosphorylation, due to the absence of α -(1,6) bonds (Mikkelsen et al., 2004). Schwall et al. (2000) also found higher levels of AP chains with DP > 20 in high-AM starches. As expected, no glc-6-P ester groups were detected in cassava starches.

3.1.2. Granular structure

Fig. 2 shows SEM pictures of the starch granules. wtps and amfps granules had oval to spherical shapes and a smooth surface with only few irregularities. The size distribution of the granules was heterogeneous for all starches. No pores were observed at the surfaces. haps samples (especially haps3 and haps4) had granule structures differing from those of wtps and amfps, as also observed by Blennow et al. (2005). These granules had irregular surfaces and fissures. These phenomena were more pronounced for the starches of high-AM content. The granules of haps4 were multi-lobed. The haps granules were also smaller than those of the other potato starches. The cassava starch granules were much smaller than potato starch granules. Most of these granules were oval to round and some granules showed a truncated end, in line with Moorthy (2002).

Table 3 lists the granule size distributions as determined using laser light scattering. The granule size distribution is unimodal for potato and cassava starches. In general, potato starch granules are large (10–110 μm). For each genotype, wtps had the largest granules (mean diameter: 47.5–59.6 μm), followed by amfps (mean diameter: 46.3–53.8 μm) and haps (mean diameter: 33.7–41.9 μm). The granules of cassava starches were much smaller (mean diameter: 16.9–18.0 μm). These differences were also reflected in the SEM images.

3.1.3. Molecular structure

Fig. 3 shows the molecular size distribution of intact AP and AM together with their maximum wavelength (λ_{max}) after iodine binding. The iodine binding capacity of AM is ca. 20 times higher than that of AP. The wavelength for maximum extinction exceeds 620 nm for AM and is lower than 600 nm for AP (Hizukuri, 1996). Based on differences in iodine binding capacity, the profiles were divided into three sections and the relative amounts of high-MW AP (110–150 ml, region I), low-MW AP (150–190 ml, region II) and AM (190–245 ml, region III) were estimated (Table 2) as described by Schwall et al. (2000).

In contrast to wts (wtps and wtcs together), amfs (amfps and amfcs together) and haps samples showed a shoulder (elution volume of 150–170 ml) in their elution profiles, suggesting the presence of AP with a slightly lower average molecular size. All amfps samples except for amfps3 contained more high-MW than low-MW AP, while the opposite was observed for haps samples (except for haps1). In this respect, haps4 with the highest AM content (78.4%), did contain little if any high-MW AP. Shi, Capitani, Trzasko, and Jeffcoat (1998) described similar observations for very high-AM maize starch. AM contents measured by ConA were strongly correlated with those obtained by SEC ($R^2 = 0.94$), even though AM levels of amfps and wtps were slightly higher when measured by SEC. This is probably due to the presence of relatively small AP molecules with extended A and B chains as earlier described by Morrison and Karkalas (1990).

The maximum wavelength for iodine bound starch ranged for the region I and II AP fractions from 558 to 570 nm and from 578 to 590 nm for amfps and wtps, and haps respectively. This indicated

Fig. 6. Plot of correlation coefficients between the level of chains with degree of polymerisation (DP) 6–30 and the onset (T_o), peak (T_p) and conclusion (T_c) temperature of gelatinisation as a function of DP.

that haps samples had higher average AP CL than did wtps and amfps. For the AM fractions (region III), the maximum wavelength of amfps was lower than 620 nm, confirming the absence of AM. The maximum wavelength of haps AM fractions was lower (626–640 nm) than that for wtps (642–648 nm), indicating slight differences in the AM structures.

The CL distributions of debranched starches were analysed using HPAEC (Fig. 4) and HPSEC (Table 4). HPAEC analysis showed for all potato starches a dip for side chains with DP 8. Peak DP of wtps and amfps was DP 12–13. haps, in contrast, had a peak DP of 14–15 and the profiles showed a shoulder at DP 24–28. CL distribution of wtps and amfps hardly differed. Different amfps had slightly lower levels of short chains (DP < 14) and slightly higher levels of the longer chains (DP > 18) than their corresponding wtps. CL distribution varied more with genotype than as a result of mutation. Chain length distributions of cassava starches were clearly different with a peak DP at 11 and a shoulder at DP 19–21. In addition, the cassava starches showed no dip in their CL profiles at DP 8.

The debranched starches were also analysed using HPSEC. HPAEC analyses provide only detailed information on the short chains (DP < 50) with a high resolution where HPSEC gives complementary information on the longer chains. The HPSEC chromatograms were divided in regions based on the observed minima and inflections. Table 4 shows the mass-based and molar-based percentages of the fractions. AP of normal starches had a polymodal CL distribution with A chains (DP < 16), B1 chains (16 < DP < 25), B2 and B3 chains (25 < DP < 80), and B4 chains (DP > 80) (Hizukuri, 1986). Extremely long B chains of AP were described in literature (Bertoft, 2004). The longer chains of AM have a DP > 150.

The amounts of chains that span only 1 cluster (A and B1 chains) were comparable for wtps (81.1–89.4 mol%) and amfps (84.8–88.2 mol%) and were slightly higher for the cassava starches (89.2–90.5 mol%). The lamellar periodicity, i.e. 9 nm, corresponds to the size of one cluster (Jenkins et al., 1993): double helices of 25 glucose residues, the maximum CL of chains that span only one cluster, had a length of 8.8 nm. The other chains (B2, B3 and B4) span 2, 3 or 4 clusters. amfps had decreased or unchanged levels of chains with DP < 12 when compared to wtps of the same genotype. These short chains interfered with the perfect packing of double helices.

Extremely long B chains of AP were observed for all amfps (± 1000 glucose units). These results are in agreement with observations for starches of different botanical sources (Bertoft, 2004). amfps1–4 had higher levels of long B chains with DP 25–150 (11.3–14.0 mol%) than wtps (9.5–11.8 mol%). haps had higher levels of long chains with DP > 150 (3.4–28.6 mol%) than wtps. Comparison of AM contents (Table 2; 27.4–37.3 weight%) and levels of chains of DP > 150 (52.5–67.3 weight%) of haps1–3 indicated that these long chains did not only originate from AM. Probably, the polymodal CL distribution is slightly different. The suppression of SBE A and SBE B in haps samples is probably responsible for a decreased level of α -(1,6) bonds which is in line with the increased AP CL. As observed earlier, cassava starches had shorter chains than potato starches.

WAXD measurements revealed B-type diffraction patterns for all potato starches while the cassava starches displayed an A-type pattern. Literature data revealed that cassava starches have an A- or C-type pattern (Hoover, 2001). RCs of potato and cassava starches were calculated using highly crystalline B- and A-type standards, respectively (Table 2). The RC of the different wtps ranged from 30.0% to 36.0%, in line with Zobel (1988). amfps showed the highest RC (34.0–38.5%), while haps had the lowest RC (21.0–29.0%). RCs of cassava starches were slightly higher (wtcs: 40.0% and amfcs: 49.0%) which was also observed by Zobel (1988). amfcs had also a higher RC than wtcs.

3.2. Relation between molecular structure aspects

In our dataset, no linear relation was observed between the degree of phosphorylation and other structural parameters. RC was negatively correlated with AM content ($n = 14$, $R^2 = 0.79$, $P < 0.05$). It is generally accepted that starch crystallinity can be ascribed to AP structural elements (French, 1984). Levels of chains with DP 6–9 led to lower RC ($n = 10$, $R^2 = 0.51$, $P < 0.05$) while chains with DP 18–25 ($n = 10$, $R^2 = 0.50$, $P < 0.05$) increased RC. Chains with DP 6–9 are too short to build double helices and disturb the order (Van-deputte, Vermeylen, et al., 2003b). The pitch height (6 glucose molecules/turn) of the double helices is about 2.1 nm (Buléon et al., 1998). Double helices formed by chains with DP 18–25 (6.3–8.8 nm) fit well in the lamellae of AP, explaining their contribution to RC. The cassava starches had shorter chains than potato starches in general. This is typically observed when comparing A-type and B-type starches (Vermeylen, Goderis, Reynaers, & Delcour, 2004).

3.3. Gelatinisation characteristics

Fig. 5 presents a schematic overview of the starch gelatinisation temperatures. T_p of wtps ranged from 61.7 to 65.7 °C. It was higher for amfps (67.1–70.1 °C) and highest for haps (73.2–98.9 °C). In line with observations for potato starches, amfcs had higher T_p (63.6 °C) than wtcs (60.7 °C). Their gelatinisation temperatures were lower than those of potato starches. Gelatinisation temperature ranges ($T_c - T_o$) of amfps (7.9–11.1 °C) and amfcs (14.8 °C) were smaller than or similar to those of wtps (8.4–11.2 °C) and wtcs (15.3 °C), respectively. In addition, haps had a broad gelatinisation temperature range (25.0–46.7 °C) presumably due to a heterogeneous starch population. ΔH was respectively higher and lower for amfps (23.1–25.9 J/g) and haps (6.1–19.2 J/g) than for their parent starch. Cassava starches (A-type starch) had lower enthalpies (19.5–21.5 J/g) than potato starches (B-type starch). This was already earlier observed by Fredriksson et al. (1998) for A- (wheat, rye and barley starches) and B-type starches (potato starch).

3.4. Structure related to gelatinisation characteristics

No linear correlation was observed between the gelatinisation temperatures and RC or AM content. In contrast, parameters describing CL distributions correlated with gelatinisation temperature. Levels of short chains, i.e. DP 6–9 ($n = 15$ with haps4 excluded, $R^2 = 0.66$, 0.77 and 0.63, $P < 0.05$) and DP 10–14 ($n = 16$, $R^2 = 0.66$, 0.88 and 0.91, $P < 0.05$), were negatively correlated with T_o , T_p and T_c , respectively. Levels of chains with DP 18–25 ($n = 16$, $R^2 = 0.64$, 0.80 and 0.76, $P < 0.05$) were positively correlated with T_o , T_p and T_c , respectively. Also, levels of chains with DP > 25 ($n = 16$, $R^2 = 0.91$ and 0.95, $P < 0.05$) were positively correlated with T_p and T_c , respectively. The Pearson correlation coefficients (R) for the relation between the relative levels of chains of each DP and T_o , T_p and T_c were plotted as a function of DP (Fig. 6). Gelatinisation was delayed with higher relative amounts of DP 18–30 and lower relative proportions of DP 8–14 chains. These observations are in line with results for waxy and normal rice starches (Vandeputte, Vermeylen, et al., 2003b). However, we observed an increase of the gelatinisation temperatures at slightly higher DP (DP 18–25 instead of DP 12–22). Again, this can be explained by the longer chains of B-type starches (potato) than A-type starches (cassava) (Vermeylen et al., 2004).

Levels of longer chains with DP 25–80 ($n = 16$, $R^2 = 0.65$, 0.96 and 0.79, $P < 0.05$) and with DP 80–150 ($n = 16$, $R^2 = 0.65$, 0.87 and 0.71, $P < 0.05$), measured by HPSEC, were positively correlated with T_o , T_p and T_c , respectively. These chains are suggested to contribute to more stable crystals and hence delayed gelatinisation. Especially T_p showed strong correlations with CL distribution.

A positive relation was observed between ΔH and RC ($n = 14$, $R^2 = 0.81$, $P < 0.05$), indicating that the gelatinisation enthalpy increased with crystallinity. Although RC is suggested in literature to be affected by CL distribution, no relation was found between ΔH and CL distribution. In line with Karlsson and Eliasson (2003), no influence of granule size on gelatinisation behaviour was observed.

4. Conclusions

The granules of potato starches are large and oval to spherical (10–110 μm) and those of cassava starches small and oval to round with a small population having a truncated end. Potato starches have a B-type diffraction pattern and a limited degree of phosphorylation whereas cassava starches show an A-type diffraction pattern and contain little if any phosphate ester groups. AM content, RC and AP CL distribution affect the gelatinisation behaviour of potato and cassava starches. AP CLs of the potato starches are longer than those of cassava starches. This results in lower gelatinisation temperatures for the cassava starches. Short chains ($\text{DP} < 14$) prevent crystalline order, whereas longer chains ($\text{DP} > 18$) yield more stable crystals. Consequently, increased levels of longer chains shift gelatinisation to higher temperatures, whereas short chains decrease the gelatinisation temperatures. Increased crystallinity and consequently increased AM content results in higher ΔH -values. No relation was observed between the granular structure and the gelatinisation properties.

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