ELSEVIER

Carbohydrate Polymers 47 (2002) 331–340

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

Comparison of potato amylopectin starches and potato starches — influence of year and variety

K. Svegmark[a,*], K. Helmersson[a], G. Nilsson[a,b], P.-O. Nilsson[a], R. Andersson[c], E. Svensson[d]

[a]*Lyckeby Stärkelsen, S-291 91 Kristianstad, Sweden*
[b]*Department of Analytical Chemistry, Lund University, P.O. Box 124, S-221 00 Lund, Sweden*
[c]*Department of Food Science, Swedish University of Agricultural Sciences, P.O. Box 7051, S-750 07 Uppsala, Sweden*
[d]*Department of Food Technology, Lund University, P.O. Box 124, S-221 00 Lund, Sweden*

Received 2 November 2000; accepted 18 January 2001

Abstract

Starches from three potato varieties and their respective transformants producing amylopectin starch were studied over a period of 3 years. The gelatinisation, swelling and dispersion properties were studied using differential scanning calorimetry (DSC), X-ray diffraction, swelling capacity measurements and a Brabender Viscograph.

The potato amylopectin starches (PAP) exhibited higher endothermic temperatures as well as higher enthalpies than the normal potato starches (NPS). PAP samples gave rise to an exceptionally sharp viscosity peak during gelatinisation and a relatively low increase in viscosity on cooling. Swelling capacity measurements showed that PAP granules swelled more rapidly, and that the dispersion of the swollen granules occurred at a lower temperature (85°C). Analysis of variance (ANOVA) also revealed that the year influenced the DSC results, and that both year and variety affect some of the Brabender parameters. Furthermore, the PAP and NPS samples were subjected to heat–moisture treatment at three different moisture levels, and the Brabender viscosity properties were studied.

Keywords: Starch; Potato; Potato amylopectin starch; DSC; Swelling capacity; Heat–moisture treatment; Crystallinity; Brabender viscosity

1. Introduction

Starch granules are normally composed of two types of polysaccharide: amylose and amylopectin. The amylose fraction is essentially linear, whereas amylopectin is highly branched. The amylopectin chains have a tree-like arrangement of double helices forming crystals in the granule, whereas amylose is mainly amorphous. Once solubilised in hot water, the linear amylose crystallises on cooling and forms gels or other aggregated structures. Most commercial starches are chemically modified by side-chains in order to stabilise amylose and achieve storage stability. The amylopectin is water-soluble and fairly stable on storage, depending on the source of the amylopectin. The two starch components have different properties and are thus not suited for the same applications (Zobel, 1988a). Starch producers and plant breeders have therefore searched for plants that will produce starch enriched in either component. Natural mutants with low content of amylose have so far only been found in crops of cereals such as maize, rice, barley, wheat and sorghum and these high-amylopectin starches are usually referred to as 'waxy'.

Normally, potato starches from different genetic sources show limited variation in amylose content (Barichello, Yada & Coffin, 1991; Shannon & Garwood, 1984). A recessive high-amylopectin potato mutant was produced from a monohaploid plant by X-radiation (Jacobsen, Hovenkamp-Hermelink, Krijsgsheld, Nijdam, Pijnacker, Witholt et al., 1989). By genetic engineering, using anti-sense technique, it has been possible to modify the potato tuber so that it produces granular starch practically without amylose (Hofvander, Persson, Tallberg & Wikström, 1992; Tallberg, Hofvander, Persson & Wikström, 1998). We will refer to this type of starch as potato amylopectin starch or PAP. Previously, the physico-chemical and functional properties of PAP-starches have been studied by, e.g. Fredriksson, Silverio, Andersson, Eliasson and Åman (1998), Hermansson and Svegmark (1996), McPherson and Jane (1999), Nilsson, Bergquist, Nilsson and Gorton (1996), Visser, Suurs, Bruinenberg, Bleeker and Jacobsen (1997) and Visser, Suurs, Steeneken and Jacobsen (1997).

Cereal sources of high-amylopectin starches contain lipids and proteins, whereas potato starch is essentially

* Corresponding author. Fax: +46-3113-4049.
E-mail address: karin.svegmark@swipnet.se (K. Svegmark).

0144-8617/02/$ - see front matter
PII: S0144-8617(01)00174-6

free of these compounds. However, amylopectin from potatoes contains small amounts of covalently bonded phosphate groups (Swinkels, 1985). Each source of amylopectin has a characteristic chain length (CL) distribution (Fredriksson et al., 1998; Kalichevsky, Orford & Ring, 1990), which influences properties such as storage stability.

The difference in swelling behaviour between the PAP and the normal potato starch (NPS) has been examined using light microscopy (Hermansson & Svegmark, 1996). The micrographs revealed that PAP granules only transiently formed the typical structures of swollen granules. Shortly after gelatinisation the pastes transformed into a macromolecular dispersion appearing somewhat aggregated or phase-separated (Hermansson & Svegmark, 1996). The associative forces within the gel particles of the swollen granule have not been explained. Wong and Lelievre (1982) pointed out that there must be some type of structure crosslinking within the swollen gel particles. The crosslinking structure could be either due to polymer entanglement, lipid–amylose complexes, or vestiges of crystalline material. The swelling capacity of a cereal granule is believed to be a function of the content of amylose and lipids, the size of amylose and the crystallinity (Ghiasi, Hoseney & Varriano-Marston, 1982; Tester & Morrison, 1990a,b; Wong & Lelievre, 1982; Zeleznak & Hoseney, 1987; Zobel, 1984). The PAP granule, lacking both amylose and lipids, is therefore an interesting material for the study of the relation between the association and crystallisation of starch molecules in the granule and the swelling/dispersion of granule starch. The starch crystallinity and the degree of association of starch chains in granular starch can be manipulated by either annealing or heat–moisture treatment. The characteristic B-pattern, generally found for tubers, is transformed to an A-pattern, typical of cereals, by the heat–moisture treatment (Lorentz & Kulp, 1983).

Influence on growing conditions, year and harvest date has to be considered when comparing starch from different varieties. Weisenborn, Orr, Casper and Tacke (1994) studied Brabender viscosity curves of starch samples and found a variation not only with starch variety but also a major variation with crop year. The variation over the studied 6 years showed no correlation to total precipitation or average temperature. Madsen and Christensen (1996) have shown that harvesting date and tuber size influenced the Brabender properties of starch from four potato cultivars in different ways. Variation of influence by growing condition on potato starch properties such as the gelatinisation behaviour (differential scanning calorimetry, DSC), granular size, amylose and phosphorus content of potato starch have also been demonstrated (Cottrell, Duffus, Paterson & Mackay, 1995; Haase & Plate, 1996). In order to confirm actual differences between potato starches it is therefore necessary to perform a study over several years or to study different growing conditions. Similar effects of planting dates and growing conditions have been shown also for cereal starches (Tester, South, Morrison & Ellis, 1991; Campbell et al., 1994; Shi, Seib & Bernardin, 1994).

The present study was undertaken to compare the properties of potato starches and PAP-starches and to gain insight into the relation between physico-chemical properties and the swelling behaviour. We have compared potato starches from three mother potato varieties with starches from their respective amylose-free transformants over a period of 3 years. We were thus able to compare variations between the amylose-containing and the amylose-free starches; the contribution from the genetic background; and the variation between different years. Finally the effect of heat–moisture treatment of PAP and NPS granules were compared.

2. Materials and methods

The potato cultivars studied were Producent, Prevalent and Dianella, which are used for industrial starch production in Sweden. Potato transformants producing PAP were obtained from these mother varieties using the anti-sense gene technique as described in Hofvander et al. (1992) and Tallberg et al. (1998). The NPS and the PAP were produced in 1993, 1994 and 1995. The tubers were bred, each season, at the same geographical location and with the same periods of cultivation. The plant material was supplied by Amylogene HB, a joint venture between Lyckeby Stärkelsen, Kristianstad, Sweden and Svalöf Weibull AB, Svalöv, Sweden. The starches were extracted at a pilot plant at Lyckeby Stärkelsen, according to standardised methods.

The samples studied were nine NPSs and nine PAPs. The NPS (from the mother varieties) will be referred to as: Prevalent NPS (-93, -94 and -95), Producent NPS (-93, -94 and -95) and Dianella NPS (-93, -94 and -95). The PAP (from transformants produced from the mother varieties) will be referred to as Prevalent PAP (-93, -94 and -95), Producent PAP (-93, -94 and -95) and Dianella PAP (-93, -94 and -95).

2.1. Differential scanning calorimetry

The gelatinisation properties of the starches were investigated with a Perkin–Elmer DSC-2C calorimeter in the temperature range of 17–97°C, at a heating rate of 10°C/min. The instrument was calibrated with gallium (M_p = 29.8°C) and indium (M_p = 156.6°C). Coated aluminium pans from TA Instruments (USA) were used for experiments, and an empty pan with double lids was used as a reference pan. The starch samples were mixed in preweighed DSC-pans with doubly distilled water at a starch to water ratio of 1:3, and re-weighed. The mixtures were equilibrated for 1 h before the DSC measurements to obtain an even distribution of water. The dry matter content of each individual sample was determined by drying punctured DSC-pans in a heating cabinet at 105°C for 16 h after scanning. The enthalpies are all calculated and given on the basis

of dry matter substance as the mean of duplicate measurements.

2.2. Wide-angle X-ray investigations (WAXS)

The crystallographic properties of the potato and amylopectin potato starch varieties were examined on a Guinier-camera arrangement with a quartz monochromator. A Cu-anode (Philips PW 2273/20, The Netherlands) gave an average wavelength of 1.54 Å, and was operated at 40 kV and 20 mA. All the starch samples were examined at a starch to water ratio of 1:1, and mounted in hermetically sealed cuvettes to keep their moisture during examination. The scattered patterns were recorded on Reflex 25 Medical X-ray film (CEA AB, Sweden), processed according to the recommendations of the manufacturer.

2.3. Size-exclusion chromatography (SEC)

The amylose content was determined using debranched starch that was injected into a SEC-system (Hizukuri, 1985; Salomonsson & Sundberg, 1994; Sargeant, 1982). Starch (10 mg) was dissolved by heating in water (3.75 ml) for 10 min during continuous stirring. 0.25 ml of 0.4 M acetate buffer, pH 3.8 were added prior to debranching with 125 U of isoamylase (EC 3.2.1.68) from *Pseudomonas amyloderamosa* obtained from ICN Biochemicals Inc. (Ohio, OSA, cat. no. 190106). The sample was incubated for 2.5 h at 40°C during continuous stirring. Inactivation of the enzyme was performed by heating at 100°C for 1 min and the protein was removed by centrifugation. To prevent retrogradation, 1 ml of 1 M KOH was added to the debranched sample. The SEC-system consisted of a pump (LKB, model P1, Pharmacia, Uppsala, Sweden) set at a flow rate of 0.5 ml/min, a column (1.6 cm × 70 cm) packed with Sepharose CL-6B (Pharmacia), an injection valve (V-7, Pharmacia) with a 2.0-ml injection loop, a differential refractive index (DRI) detector (RID-6A, Shimadzu, Kyoto, Japan), and a fraction collector (RediFrac, LKB, Pharmacia). The eluent was 0.1 M KOH with 0.002% Na-azide.

The amylose content was determined by comparing the peak areas of the long-chain (amylose) and short-chain (amylopectin) fractions, respectively, obtained from the DRI response. Some samples were additionally investigated by collecting fractions (3 ml) from the SEC-system in which the total carbohydrate content was determined according to the method by Dubois, Gilles, Hamilton, Rebers and Smith (1956). The resulting peak areas of the plots were compared as described for DRI detection. Results obtained showed the same amylose content with both detection procedures. There were no detectable losses during sample preparation and the recovery from the SEC column was on average 98.5%.

2.4. Brabender viscosity

In the present investigation a Brabender Viscograph (type E) (Brabender OHG, Duisberg, Germany) was used. Starch dispersions, at a concentration of 4% (dry weight basis), were heated in distilled water from 25 to 95°C at a rate of 1.5°C/min, kept at 95°C for 30 min and then cooled to 25°C. Viscosity was measured using a 350-cmg cartridge. The same procedure was used to evaluate heat–moisture-treated starches, except that the concentration was 5% and a 700-cmg cartridge was used.

2.5. Swelling capacity and dispersability (solubility) measurements

Swelling capacity measurements were performed using standard procedures as described by Leach, McCowen and Schoch (1959). Starch was suspended in distilled water at 50°C at concentrations of 0.07% w/w. The samples were then kept at different temperatures between 55 and 95°C for 30 min. After centrifugation, at 1000 g for 15 min, the weight of the sediment and the supernatant was measured. The concentration of starch in the supernatant solution was determined by enzymatic hydrolysis into glucose, followed by injection in a liquid chromatographic system for glucose analysis, according to standardised procedures (Lyckeby Stärkelsen). The samples investigated were Prevalent NPS-95 and Prevalent PAP-95.

2.6. Heat–moisture treatment

Starches (Prevalent NPS-95 and Prevalent PAP-95) were heated in sealed glass bottles, initially in a boiling water bath and then transferred to an autoclave. The samples were autoclaved at 125°C for 2 h (2.5 bar).

Before treatment, starch was conditioned to different moisture content in a climate chamber for 16 h. A moisture content of around 18% was obtained at a relative humidity (RH) of 85% and $T = 40$°C; 11% moisture content was obtained at 20% RH and at 20°C. In order to reach the highest moisture content (approximately 24%), water was sprayed on to starch powder in a starch blender, which was then was kept overnight to obtain a homogeneous moisture distribution.

The moisture level was measured before and after the heat treatment in order to detect any leakage of water into or out of the sealed bottles. The samples lost 2% of water during the treatment. The moisture levels discussed are the average of the levels before and after treatment.

2.7. Statistical analysis — ANOVA

Analysis of variance (ANOVA) was carried out with the statistical package MINITAB (Minitab Inc., State Collage, PA, USA). The results are reported in the text as P-values on levels of $P < 0.001$, $P < 0.01$, $P < 0.05$ and $P < 0.1$.

Table 1
Results from DSC analysis

		ΔH (J/g)		T_m (°C)		T_o (°C)	
		NPS	PAP	NPS	PAP	NPS	PAP
Prevalent	−93	15.1	16.7	58.8	64.5	53.3	55.3
	−94	16.2	17.8	61.8	67.8	53.1	58.8
	−95	16.5	17.3	62.1	66.3	55.6	58.5
Producent	−93	15.4	17.6	59.1	63.8	53.5	54.8
	−94	16.9	17.7	61.3	67.8	52.1	60.1
	−95	15.9	17.2	62.4	68.0	56.1	59.8
Dianella	−93	15.4	16.1	60.8	62.8	53.5	54.8
	−94	16.2	18.0	62.8	66.1	54.3	57.3
	−95	15.6	16.7	62.3	64.8	54.8	56.8
	Average	15.9	17.2	61.2	65.7	54.0	57.3
	St. dev.	0.6	0.6	1.4	1.9	1.3	2.1
	Conf. int 0.95	15.5–16.3	16.8–17.6	60.3–62.2	64.5–67.0	53.2–54.9	56.0–58.7

3. Results and discussion

3.1. Crystalline properties of PAP granule

3.1.1. Gelatinisation properties studied by DSC

Starches from three different potato varieties (Prevalent, Producent and Dianella) and their respective transformants producing amylopectin starch were studied by DSC. The DSC investigation was performed on material from three different harvest years, 1993, 1994 and 1995, and the results (enthalpy (ΔH), onset temperature (T_o), and the peak temperature (T_m), are shown in Table 1. The peak temperature (T_m) represents the most distinct parameter of the endothermic DSC transitions. In Fig. 1 one can see the difference in the T_m of granules from NPS and PAP over these 3 years. The PAP showed significantly higher T_m than the NPS for each pair of mother and transformant, each year ($P < 0.001$). The same patterns were also seen with ΔH and T_o (Table 1). The PAP generally had higher enthalpies (ΔH) than the NPS of each variety ($P < 0.001$) and the average increase was 8%. Visser, Suurs, Steeneken et al. (1997) investigated the gelatinisation properties of amylose-containing and amylose-free potato starches with DSC. However, they did not detect any differences in enthalpies.

ANOVA revealed a statistically significant influence of year over the DSC results (ΔH, $P = 0.001$; T_m, $P < 0.001$; T_o, $P < 0.01$). The varieties (Prevalent, Producent and Dianella) did not show any influence on ΔH and T_o ($P > 0.16$).

The DSC results, showing higher enthalpies and temperatures of PAP compared to NPS indicate a higher degree of crystallinity within the PAP granules. This could simply be due to the fact that the amorphous amylose in the NPS decreases the relative amount of crystalline material in the granule. PAP consists only of amylopectin and therefore gives a higher degree of crystallinity. The glass transition that precedes gelatinisation may also be affected by the absence of amylose in the PAP granules. The existence of an effective glass transition has been observed for both wheat and waxy maize granular starches by DSC (Slade & Levine, 1988). A glass transition will determine the temperature of starch gelatinisation with water acting as a plasticiser.

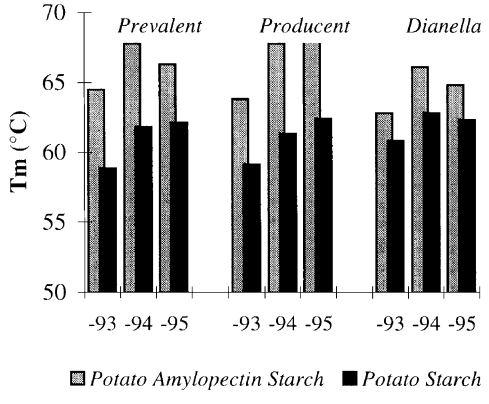

Fig. 1. The peak temperatures (T_m) of the endothermic DSC transition of normal potato starch and of amylopectin potato starch from three different mother varieties (Prevalent, Producent and Dianella) over a period of 3 years.

3.1.2. X-ray diffraction

The X-ray diffraction patterns of NPS and PAP all showed identical *d*-spacings with a single spacing at 16.08 Å (strong), a double at 9.00 and 7.84 Å (very weak), another double at 6.51 and 6.11 Å (weak), and single spacings at 5.34 (strong), 4.72 (weak), 4.16 (medium), 3.80 (medium) and finally 3.52 Å (weak). The composition of these spacings is characteristic of the B-pattern being well recognised from most kinds of tuber starches, in contrast to the A-pattern of cereals and the C-pattern of legumes, with other characteristic *d*-spacings (Zobel, 1988b). The B-pattern has been

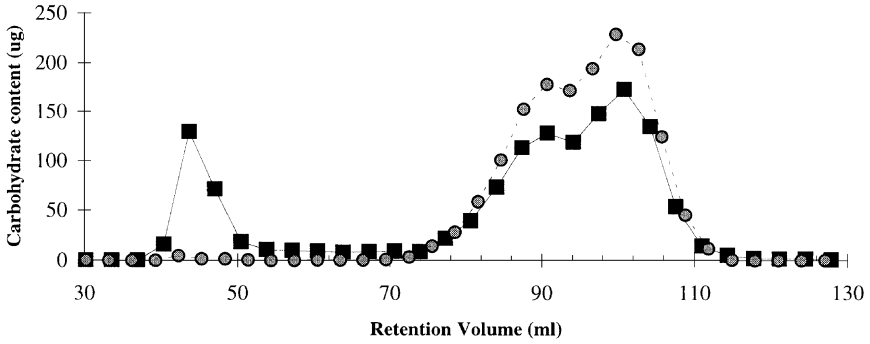

Fig. 2. Chain profiles of debranched starches obtained by size-exclusion chromatography and analysis of starch contents in each fraction using phenol sulphuric method. (■, potato starch; ●, potato amylopectin starch).

interpreted in terms of a hexagonal symmetry, and packing of the double helices of amylopectin (Imberty & Pérez, 1988; Wu & Sarko, 1978). McPherson and Jane (1999) also found that PAP displayed a typical B-type of diffraction pattern. Strictly speaking, the identical B-patterns of both NPS and PAP samples indicate that the fine structure of the amylopectin remains unaffected by the genetic modification using the anti-sense technique.

3.1.3. Influence of weather conditions

An interesting observation is the significant difference in gelatinisation properties over the 3 years of 1993, 1994 and 1995 discussed above. Generally, the samples from 1994 have higher enthalpy and temperature values than the other two years, see Fig. 1 and Table 1. Amylopectin is not a perfectly crystalline material, and dislocations seem to be introduced into the clusters already during synthesis since crystallinity can be increased by annealing (Knutson, 1990). The content of phosphorous in tubers is known to vary with growth condition (Haase & Plate, 1996) and may be one source of the crystalline imperfection (Muhrbeck & Svensson, 1996). The DSC results of the present investigation show that the synthesis mechanism is most likely influenced by variations in the growth conditions.

During the years of this study, the weather conditions varied substantially (SMHI, 1993–1995). In Southern Sweden, the summer of 1993 was cold and rainy. The summer of 1994 was the opposite, being hot and sunny. The following summer of 1995 was more of what could be expected in Southern Sweden. The temperature never exceeded 25°C in July 1993, whereas it rose above 25°C for 20 days in July 1994 and for 7 days in July 1995. The recorded average July temperature at the local meteorological observation station was 14.8°C in 1993; 19.6°C in 1994 and 17.3°C in 1995. Owing to irrigation, it is difficult to discuss the effects of the natural precipitation. However, the differences are large between July 1993 with 173.3 mm, and July 1994 with 0 (zero!) mm. In July 1995 the natural precipitation was recorded as 50 mm.

3.2. Amylose content

The CL distribution of PAP is polymodal with a main peak maximum of about CL 20 and 60 glucose units (GU) (Hizukuri, 1985; Kalichevsky et al., 1990). By contrast, potato amylose has a much higher average CL, e.g. CL = 700–900 (Takeda et al., 1984; Hizukuri, 1991). Fig. 2 shows the CL distributions of Prevalent NPS and Prevalent PAP, 1994. The first peak was assumed to be the amylose fraction (peak at 44 ml), whereas the debranched amylopectin fraction was eluted later as a bimodal peak (at 90 and 100 ml. respectively). The amylose content of the Prevalent NPS shown in Fig. 2 was determined to be 18.3% by post-column carbohydrate determination; in general, the mother varieties had an amylose content of around 19%. These results correspond well with the amylose content of other potato starches as reported in the literature. It can be clearly seen that the PAP starch is essentially free of amylose. The content of amylose in the Prevalent PAP, 1994, in Fig. 2 was $0.8 \pm 0.3\%$**. Prevalent PAP gave amylose peaks near or below the detection limit all years. The highest content of amylose was found in Dianella PAP, 1994 ($3.5 \pm 0.9\%$**) and Producent PAP, 1994 ($3.2 \pm 1.0\%$**) in 1994.

No significant differences in the distributions of the debranched amylopectin chains were observed between the NPS and the PAP with separation in an SEC column, implying that the fine structure of amylopectin is basically unaffected by the transformation. Corresponding CL distribution of amylopectin from PAP and NPS were also shown by Fredriksson et al. (1998) and by McPherson and Jane (1999). However, some minor but not consistent differences were found. Nilsson et al. (1996) also demonstrated that the PAP had the same degree of branching as purified amylopectin from potato starch using NMR.

3.3. The swelling and dispersion/dissolution of the granule

A standard method for following the swelling and dispersion/dissolution of swollen granules, and leaching of starch was developed by Leach et al. (1959). Starch samples were heated in excess of water at different temperatures. After

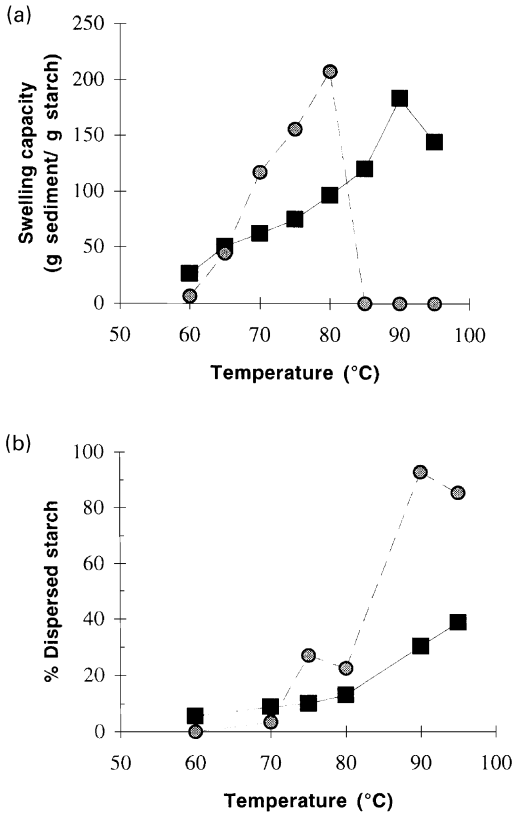

Fig. 3. (a) Swelling capacity of (■) potato starch (Prevalent NPS-95) and (●) potato amylopectin starch (Prevalent PAP-95). (b) Amount of starch in the supernatant of (■) potato starch (Prevalent NPS-95) and (●) potato amylopectin starch (Prevalent PAP-95).

centrifugation, the swelling capacity (sediment weight) and the amount of starch in the supernatant were measured. Each type of starch gives a typical swelling pattern. Potato starches give a high gel volume. The swollen potato granules are fragile structures and easily break down by shear. The PAP granules are even more sensitive (Hermansson & Svegmark, 1996), so that shearing was kept to an absolute minimum in this study.

Fig. 3a compares the swelling capacities (representing the phase of the swollen granules) of NPS and of PAP from 1995. The swelling capacity of the NPS corresponded well with previously reported findings (Eliasson, 1986; Leach et al., 1959). The PAP started to swell at a somewhat higher temperature. The initial swelling of the PAP was rapid and the swelling capacity reached an even higher level than the NPS. The maximum swelling capacity of PAP was reached at 80°C. Heating at higher temperatures rapidly destroyed the structures maintaining the integrity of the swollen granules of the PAP samples, and no gel sediment was found at 85°C and above (Fig. 3a).

The starch found in the supernatant represents both starch that leached out of the granules and starch originating from disruption of the swollen granules. Soluble starch is a standard term used in starch science for this fraction (Leach et al., 1959), we are using the term dispersed starch to describe the fraction of the starch found in the supernatant. The amount of dispersed material, from the PAP increased rapidly, and reached 100% already at 85°C, as can be seen in Fig. 3b. The complete dispersion of the PAP granule confirms results from previous studies using light microscopy (Hermansson & Svegmark, 1996). The state of the molecules in this macromolecular dispersion is still not fully known and is currently the subject of further investigation.

The endothermic peak of Prevalent PAP-95 ended at 74.3°C, at a heating rate of 10°C/min. Even though temperatures from DSC measurements cannot be directly compared with dispersion temperatures, it is interesting to note that the dissolution or dispersion of the granule seems to occur when the thermal disordering of the granules is complete. The

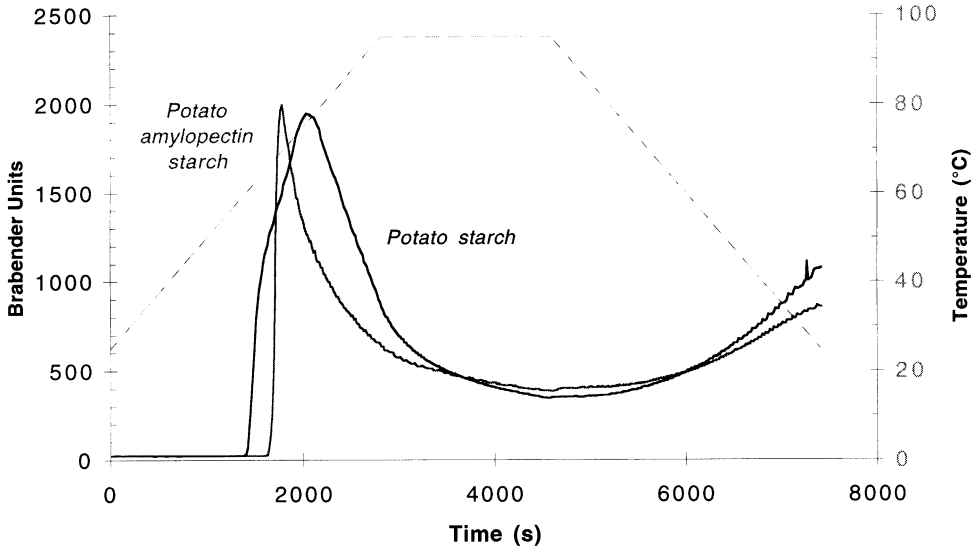

Fig. 4. Brabender viscosity curves of normal potato starch and potato amylopectin starch. The concentration was 4% and the 350-cmg Brabender cartridge was used.

Table 2
Paste characteristics from the Brabender Viscograph. The concentration was 4% and the 350-cmg cartridge was used ($n = 2$)

	Onset of viscosity temperature (°C)		Peak viscosity (BU)		Peak temperature (°C)		Viscosity 95°C, 30 min (BU)		Viscosity 25°C (BU)	
	NPS	PAP	NPS	PAP	NPS	PAP	NPS	PAP	NPS	PAP
Prevalent -93	58.8	65.9	1942	1997	77.1	69.6	311	384	980	857
-94	62.5	67	2104	2111	80	71.9	491	389	1318	822
-95	63.3	66.6	1818	2215	89.1	71.1	616	330	1560	642
Producent-93	59.5	65.9	1543	2504	84	69	340	366	993	673
-94	63.3	68.1	1687	2327	88	71.9	482	423	1324	811
-95	62.9	65.5	1405	2481	93	69.3	534	393	1423	752
Dianella-93	62.9	64.8	1520	2045	90	68.5	545	296	1381	614
-94	63.3	65.5	1730	1877	88	70	564	424	1477	878
-95	62.5	64.8	1603	2226	90.6	68.9	588	448	1485	930
Average	62	66	1706	2198	87	70	497	384	1327	775
St. dev.	2	1	221	214	5	1	106	48	208	111
Conf. int. ∓ 0.95	61–63	65–67	1560–1850	2060–2340	83–90	69–71	430–570	350–420	1190–1460	700–850

higher degree of order (ΔH) in the PAP granule does not seem to restrict the swelling or dissolution except for the slight delay in the onset of swelling, as can be seen in Fig. 3a.

3.4. Brabender viscosity, changes during heating and shearing

The Brabender Viscograph is used world-wide to characterise starch pastes. The viscosity is measured in arbitrary units (BU) during heating, shearing and cooling of the starch paste. In Fig. 4 typical Brabender Viscograph curves of potato starch and of PAP-starch are shown. There were two main characteristics of the Brabender curves of the PAP-samples that differed from those of NPS-samples. First, the PAP produced a very sharp viscosity peak. The other main characteristic of the PAP, was a lower increase in viscosity on cooling below 60°C. Data from the Brabender tests of all samples are shown in Table 2.

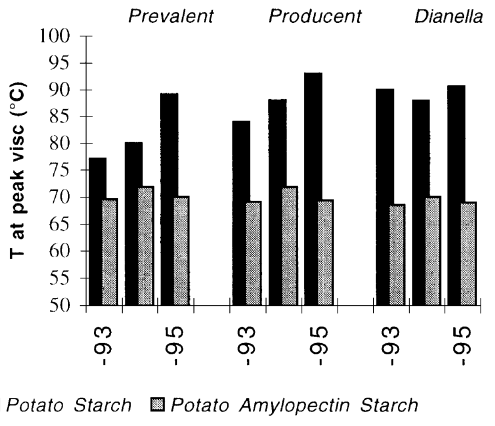

Fig. 5. Temperatures at Brabender peak viscosity (T_{peak}) for NPS and PAP samples from Prevalent, Producent and Dianella over a period of 3 years.

The temperatures at which the peak viscosity (T_{peak}) occurred in PAP-samples were low due to the exceptionally sharp viscosity peak, see Fig. 4. The PAP samples gave clearly lower T_{peak}-values than their corresponding NPS samples ($P < 0.001$), see Fig. 5 and Table 2. Both the more rapid swelling and the quicker subsequent dispersion of PAP granules shown previously in Fig. 3a and b would lead to lower T_{peak}-values. Furthermore, the stirring during the heating in the Brabender enhances disintegration of swollen granules. The difference in temperatures between the onset of swelling ($T_{o,visc}$) and the peak (T_{peak}) was on average 4.1°C for the PAP samples and 24°C for the NPS samples. Weisenborn et al. (1994) studied the Brabender viscosity curves of 44 potato starch samples representing 34 genotypes and the minimum difference in temperature ($T_{peak} - T_{o,visc}$) was 12°C for pastes at 3.25% concentration. The sharp peak of the PAP, indicated by the low values of T_{peak}, seems to be an exceptional feature of PAP.

The values of the peak viscosities (see Table 2) are not interpreted easily in structural terms because the swelling increases the viscosity and the disintegration of swollen granules decreases the viscosity. This is in contrast to the T_{peak} where both rapid swelling and rapid dispersion lead to decreased values. However, the peak viscosity values also displayed a highly significant difference; the PAP samples showing higher peak viscosities than their respective NPS-equivalent, see Table 2 ($P < 0.001$). Previously, McPherson and Jane (1999), reported that the peak viscosity of one NPS sample were higher than that of one PAP sample (using a Rapid ViscoAnalyzer). The different outcome could be due to higher shear forces in their investigation, leading to more rapid dispersions of swollen starch granules or there could be differences in starch variety and growth conditions between the NPS and PAP used.

The viscosities of the NPS increased more on cooling than those of the PAP-samples, one example can be seen

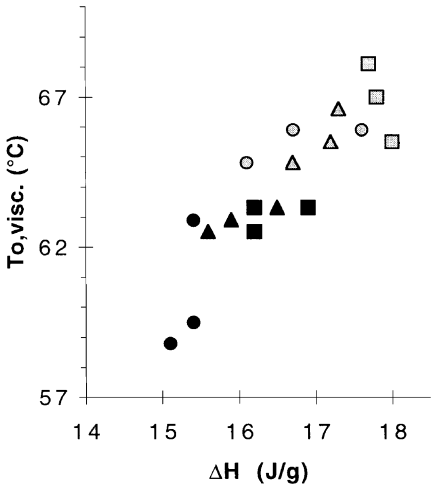

Fig. 6. Temperatures of onset of viscosity in the Brabender curves versus gelatinisation enthalpies (ΔH). Black symbols represent normal potato starches and grey symbols represent potato amylopectin starches; circles, 93; squares, 94; triangles, 95.

in Fig. 4. This can be attributed to retrogradation/aggregation of amylose in the NPS on cooling below 60°C. Table 2 shows that each year and for each variety the amylose-deficient PAP had lower viscosities at 25°C than their mother varieties ($P < 0.001$). ANOVA of the comparison of the Brabender Viscograph results in Table 2 also showed other significant differences. The viscosities after heating at 95°C for 30 min were lower for the PAP than for the NPS ($P < 0.01$) and the temperatures where the viscosities start to increase ($T_{o,visc}$) were significantly higher for the PAP than for NPS ($P < 0.001$).

Year as well as variety showed significant influence on some Brabender parameters (Table 2 and Fig. 5). The influence of crop years revealed by AVOVA was noticeable ($T_{o,visc}$: $P = 0.06$; T_{peak}, $P < 0.05$; viscosity at 95°C, $P < 0.05$; viscosity at 25°C, $P < 0.05$). In contrast to DSC results (varying with year but not with variety), some Brabender parameters also revealed differences between the varieties studied. Peak viscosity was significantly influenced by variety ($P < 0.01$) and the T_{peak} was influenced by variety ($P < 0.1$).

There were interesting positive correlations between the DSC results (T_m, ΔH) and two Brabender characteristics: $T_{o,visc}$ and T_{peak}. The connection between the $T_{o,visc}$ and the ΔH is shown in Fig. 6. PAP samples are shown in grey and NPS samples in black. A higher temperature for the onset of viscosity could be expected from a sample with higher ΔH, since ΔH indicates a higher degree of order within the granule and the $T_{o,visc}$ indicates the onset of swelling at gelatinisation. The effect of harvest year (ΔH, $P < 0.01$; $T_{o,visc}$, $P = 0.06$) can also be seen in Fig. 6. The results from 1993, represented by circles, are generally low. Results from 1994, represented by squares, were generally high. The data on the PAPs were more evenly distributed than the NPS data. Two NPS samples deviated from the others, and they can be seen at the lower left in Fig. 6. These are Prevalent and Producent NPS from 1993. These two samples also had significantly lower levels of $T_{o,visc}$ and viscosities at 25°C than the other NPS samples, as can be seen in Table 2.

The minor influence of variety is in line with the broad investigation performed by Weisenborn et al. (1994). Basically, they observed deviant Brabender profiles only for varieties related to Red Pontiac. Neither of the varieties studied here (Prevalent, Producent, Dianella) are related to Red Pontiac.

3.5. Heat–moisture treatment

Heat–moisture treatment is performed on starch at a temperature above 100°C and with low moisture content, in order to avoid gelatinisation. The result is a starch with more restricted swelling and higher gelatinisation temperatures (Banks & Greenwood, 1975). The changes are more pronounced with potato starch, which undergoes a transformation in crystalline structure, than with cereal starches. The characteristic B-pattern, generally found for tubers, is transformed to the A-pattern, typical of cereals, by the heat–moisture treatment (Lorentz & Kulp, 1983).

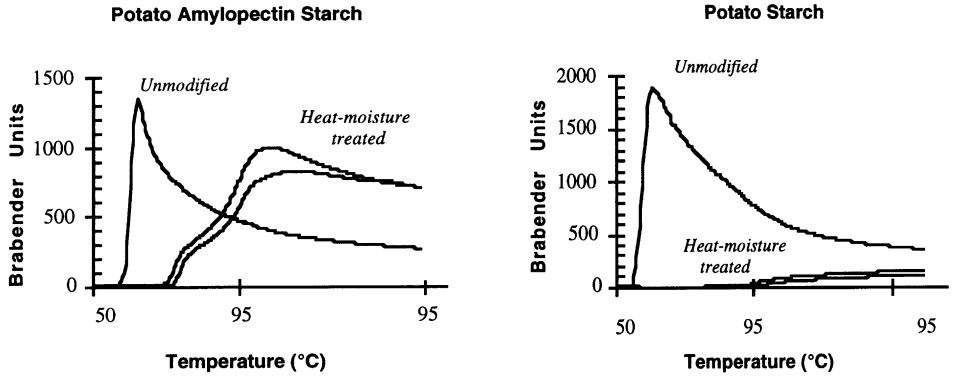

Fig. 7. Brabender viscosity curves before and after heat–moisture treatment. The concentration was 5% (DS) and the 700-cmg Brabender cartridge was used. The samples had a moisture content during treatment of approximately 17%.

Table 3
Properties before and after heat–moisture treatment at different moisture levels. The Brabender viscosity is expressed in Brabender units (BU) at 5% and the 700-cmg Brabender cartridge was used

Material	*n*	Moisture content during treatment (%)	Brabender curve	
			Onset of viscosity temperature (°C)	Peak viscosity (BU)
Potato starch: untreated		–	62	1887
Potato starch: 125°C, 2 h	2	11.0	77	520
Potato starch: 125°C, 2 h	7	17.5	92	137
Potato starch: 125°C, 2 h	2	21.8	90	141
Potato amylopectin: untreated		–	64	1450
Potato amylopectin: 125°C, 2 h	2	10.5	63	1465
Potato amylopectin: 125°C, 2 h	6	16.8	73	923
Potato amylopectin: 125°C, 2 h	2	22.8	83	510

In this study, X-ray examinations showed that the PAP crystallites were transformed from a B- to an A-pattern by the heat–moisture treatment, in the same manner as the NPS. The samples studied had a moisture content of about 23% during the treatment at 125° for 2 h.

Fig. 7 shows the Brabender characteristics of samples with and without heat–moisture treatment. The PAP samples were apparently less affected by the heat–moisture treatment than the NPS, as can be seen in Fig. 7. The PAP samples decreased much less in viscosity and the gelatinisation was less delayed than for the NPS. The results of the NPS are in agreement with those of previous studies (Hoover & Vasanthan, 1994). The Brabender curves of the heat–moisture-treated PAP samples showed, however, a much slower swelling and dispersion behaviour, compared with the unmodified PAP. The heat–moisture treatment must have introduced a structure crosslinking within the swollen PAP granule. Most probably crosslinking is due to the formation of A-type of crystals. The pastes formed from heat–moisture-treated PAP samples were cloudy or slightly opaque. Unmodified PAP gave clear solutions. The fact that the treatment restrained swelling of the amylose-containing NPS to a much higher extent than the amylose-deficient PAP implies that the amylose in NPS was involved in, or promoted the recrystallisation process.

The influence of moisture content in the starches during the heat–moisture treatments was studied, see Table 3. The effect of the heat–moisture treatment gradually decreased with decreasing moisture content, though NPS were always more affected at each moisture level. The treatment of the most dry samples (11% moisture contents) did not effect the properties of PAP, whereas the peak viscosity of NPS was decreased by 72% (Table 3). The results in Table 3 indicate differences in the glass-transition point (T_g, W_g) between the PAP and NPS granules. The role of amylose in increasing the effect of heat–moisture treatment in potato starch should be an interesting field for further studies. The amorphous amylose may enhance recrystallisation by decreasing the glass-transition temperature or by co-crystallisation with amylopectin.

Acknowledgements

The authors are grateful to Per T. Persson and Olle Wikström at Lyckeby Stärkelsen, Per Hofvander at Svalöv-Weibull AB, Profs. Ann-Charlotte Eliasson, Lund University and Anne-Marie Hermansson, SIK Göteborg, Sweden for valuable discussions. Elin Widing, Carina Ward, Sara Persson, Peter Eriksson and Annika Wessman, Lyckeby Stärkelsen are thanked for technical assistance.

References

Banks, W., & Greenwood, C. T. (1975). *Starch and its components*, Edinburgh: Edinburgh University Press.

Barichello, V., Yada, R. Y., & Coffin, R. H. (1991). Starch properties of various potato (*Solanum tuberosum* L) cultivars susceptible and resistant to low-temperature sweetening. *Journal of the Science of Food and Agriculture*, *56*, 385.

Campbell, M.R., Pollak, L.M., & White, P.J. (1994). Effect of planting date on maize starch thermal properties. Cereal Chemistry, 71 (6), 556–559.

Cottrell, J. E., Duffus, C. M., Paterson, L., & Mackay, G. R. (1995). Properties of potato starch: effects of genotype and growing conditions. *Phytochemistry*, *40* (4), 1057–1064.

Dubois, M. K., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350–356.

Eliasson, A. -C. (1986). Viscoelastic behaviour during the gelatinization of starch. I. Comparison of wheat, maize, potato and waxy-barley starches. *Journal of Texture Studies*, *17*, 253–265.

Fredriksson, H., Silverio, J., Andersson, R., Eliasson, A. -C., & Åman, P. (1998). The influence of amylose and amylopectin characteristics on gelatinisation and retrogradation properties of different starches. *Carbohydrate Polymers*, *35*, 119–134.

Ghiasi, K., Hoseney, R. C., & Varriano-Marston, E. (1982). Gelatinisation of wheat starch. I. Excess-water systems. *Cereal Chemistry*, *59* (2), 81–85.

Haase, N. U., & Plate, J. (1996). Properties of potato starch in relation to varieties and environmental factors. *Starch/Stärke*, *48*, 167–171.

Hermansson, A. -M., & Svegmark, K. (1996). Developments in the understanding of starch functionality. *Trends in Food Science and Technology*, *7*, 345–353.

Hizukuri, S. (1985). Relationship between the distribution of amylopectin and the crystalline structure of starch granules. *Carbohydrate Research*, *141*, 295–306.

Hizukuri, S. (1991). Properties of hot-water-extractable amylose, Carbohydrate Research, 217, 251–253.

Hofvander, P., Persson, P. T., Tallberg, A. & Wikström, O. (1992). Swedish patent SE 9004096-5.

Hoover, R., & Vasanthan, T. (1994). The effect of heat moisture treatment on the structure and physicochemical properties of cereal legume and tuber starches. *Carbohydrate Research*, *252*, 33–53.

Imberty, A., & Pérez, S. (1988). A revisit to the three-dimensional structure of B-type starch. *Biopolymers*, *27*, 1205–1221.

Jacobsen, E., Hovenkamp-Hermelink, J. H. M., Krijsgsheld, H. T., Nijdam, H., Pijnacker, L. P., Witholt, B., & Feenstra, W. J. (1989). Phenotypic and genotypic characterization of an amylose-free starch mutant of the potato. *Euphytica*, *44*, 43–48.

Kalichevsky, M. T., Orford, P. D., & Ring, S. G. (1990). The retrogradation and gelation of amylopectins from various botanical sources. *Carbohydrate Research*, *198*, 49–55.

Knutson, C. A. (1990). Annealing of maize starches at elevated temperatures. *Cereal Chemistry*, *67*, 376–384.

Leach, H. W., McCowen, L. D., & Schoch, T. J. (1959). Structure of the starch granule. I. Swelling solubility patterns of various starches. *Cereal Chemistry*, *36*, 534–544.

Lorentz, K., & Kulp, K. (1983). Physico-chemical properties of defatted heat–moisture treated starches. *Starch/Stärke*, *35*, 123–129.

Madsen, M. H., & Christensen, D. H. (1996). Potato starch during growth. *Starch/Stärke*, *48*, 245–249.

McPherson, A. M., & Jane, J. (1999). Comparison of waxy potato with other root and tuber starches. *Carbohydrate Polymers*, *40*, 57–70.

Muhrbeck, P., & Svensson, E. (1996). Annealing properties of potato starches with different degrees of phosphorylation. *Carbohydrate Polymers*, *31*, 263–267.

Nilsson, G. S., Bergquist, K. -E., Nilsson, U., & Gorton, L. (1996). Determination of the degree of branching in normal and amylpectin type potato starch with ^{1}H-NMR spectroscopy: improved resolution and two-dimensional spectroscopy. *Starch/Stärke*, *48*, 352–357.

Salomonsson, A. -C., & Sundberg, B. (1994). Amylose content and chain profile of amylopectin from normal, high amylose and waxy barleys. *Starch/Stärke*, *46*, 325–328.

Sargeant, J. G. (1982). Determination of amylose:amylopectin ratios of starches. *Starch/Stärke*, *34*, 89–92.

Shannon, J. C., & Garwood, D. L. (1984). Genetics and physiology of starch development. In R. L. Whistler, E. F. Bemiller & E. F. Paschall, *Starch chemistry and technology* (pp. 25–86). , Vol. I. New York: Academic Press.

Shi, Y. -C., Seib, P. A., & Bernardin, J. E. (1994). Effects of temperature during grain-filling on starches from six wheat cultivars. *Cereal Chemistry*, *71*, 369–383.

Slade, L., & Levine, H. (1988). Non-equilibrium melting of native granular starch: Part 1. Temperature location of the glass transition associated with gelatinization of A-type cereals starches. *Carbohydrate Polymers*, *8*, 183–208.

SMHI (1993, 1994, 1995). *Väder och Vatten.* Annual reports from SMHI (Swedish Meteorological and Hydrological Institute), SMHI mätstation Kristianstad-Everöd.

Swinkels, J. J. M. (1985). Composition and properties of commercial native starches. *Starch/Stärke*, *37*, 1–5.

Takeda, Y., Shirasaka, K., & Hizikuri, S. (1984). Examination of the purity and structure of amylose by gel-permeation chromatography, Carbohydrate Research, 132, 83–92.

Tallberg, A., Hofvander, P., Persson, P. T. & Wikström, O. (1998). *Genetically engineered modification of potato to obtain amylopectin-type starch.* US patent 5,824,798.

Tester, R. F., & Morrison, W. R. (1990a). Swelling and gelatinization of cereal starches. I. Effects of amylopectin, amylose and lipids. *Cereal Chemistry*, *67* (6), 551–557.

Tester, R. F., & Morrison, W. R. (1990b). Swelling and gelatinization of cereal starches. II. Waxy rice starches. *Cereal Chemistry*, *67* (6), 558–563.

Tester, R. F., South, J. B., Morrison, W. R., & Ellis, R. P. (1991). The effects of ambient temperature during the grain-filling period on the composition and properties of starch from four barley genotypes. *Journal of Cereal Science*, *13*, 113–127.

Visser, R. G. F., Suurs, L. C. M. J., Bruinenberg, P. M., Bleeker, I., & Jacobsen, E. (1997a). Comparison between amylose-free and amylose containing potato starches. *Starch/Stärke*, *49*, 438–443.

Visser, R. G. F., Suurs, L. C. M. J., Steeneken, P. A. M., & Jacobsen, E. (1997b). Some physicochemical properties of amylose-free potato starch. *Starch/Stärke*, *49*, 443–448.

Weisenborn, D. P., Orr, P. H., Casper, H. H., & Tacke, B. T. (1994). Potato starch paste behaviour as related to some physical/chemical properties. *Journal of Food Science*, *59* (3), 648–664.

Wong, R. B. K., & Lelievre, J. (1982). Comparison of the crystallinities of wheat starches with different swelling capacities. *Starch/Stärke*, *34*, 159–161.

Wu, H. -C. H., & Sarko, A. (1978). The double helical molecular structure of crystalline B-amylose. *Carbohydrate Research*, *61*, 7–25.

Zeleznak, K. J., & Hoseney, R. C. (1987). The glass transition in starch. *Cereal Chemistry*, *64* (2), 121–124.

Zobel, H. F. (1984). Gelatinisation of starch and mechanical properties of starch pastes. In R. L. Whistler, E. F. Bemiller & E. F. Paschall, *Starch chemistry and technology* (pp. 285–309). , Vol. I. New York: Academic Press.

Zobel, H. F. (1988a). Molecules to granules: a comprehensive starch review. *Starch/Stärke*, *40*, 44–50.

Zobel, H. F. (1988b). Starch crystal transformations and their industrial importance. *Starch/Stärke*, *40*, 1–7.