Available online at www.sciencedirect.com
ScienceDirect
Carbohydrate Polymers 67 (2007) 481–490
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Comparison of methods to determine the degree of gelatinisation for both high and low starch concentrations

Tim Baks [a,*], Ikenna S. Ngene [a], Jeroen J.G. van Soest [b,1], Anja E.M. Janssen [a], Remko M. Boom [a]

[a] Food and Bioprocess Engineering Group, Wageningen University and Research Centre, Wageningen, The Netherlands
[b] Agrotechnology and Food Sciences Group, Wageningen University and Research Centre, Wageningen, The Netherlands

Received 1 June 2006; received in revised form 13 June 2006; accepted 13 June 2006
Available online 24 July 2006

Abstract

A general procedure was developed to measure the degree of gelatinisation in samples over a broad concentration range. Measurements based on birefringence, DSC (Differential scanning calorimetry), X-ray and amylose–iodine complex formation were used. If a 10 w/w % wheat starch–water mixture was used, each method resulted in approximately the same degree of gelatinisation vs. temperature curve. In case the gelatinisation of a 60 w/w % wheat starch–water mixture was followed as a function of the temperature, each method resulted in a different degree of gelatinisation vs. temperature curve. DSC and X-ray measurements are preferred, because they can be used to determine when the final stage of the gelatinisation process has been completed. Birefringence and amylose–iodine complex formation measurements are suitable alternatives if DSC and X-ray equipment is not available, but will lead to different results. The differences between the methods can be explained by considering the phenomena that take place during the gelatinisation at limiting water conditions.

Based on the experimental data obtained with DSC and X-ray measurements, the gelatinisation of 10 w/w % and 60 w/w % wheat starch–water mixtures started at the same temperature (approximately 50 °C). However, complete gelatinisation was reached at different temperatures (approximately 75 °C and 115 °C for, respectively, 10 w/w % and 60 w/w % wheat starch–water mixtures) according to the experimental DSC and X-ray data. These results are in accordance with independent DSC measurements that were carried out.

The Flory equation was adapted to provide a quantitative explanation for the curves describing the degree of starch gelatinisation as a function of the starch–water ratio and the temperature. The gelatinisation curves that were obtained with the model are in good agreement with the experimentally determined curves. The parameters T_m^0, ΔH_u and χ_{12} that resulted in the lowest sum of the squared residuals are 291 ± 63 °C, 29.2 ± 3.9 kJ/mol and 0.53 ± 0.05 (95% confidence interval). These values agree with other values reported in literature.

Keywords: Starch gelatinisation; Flory equation; Melting temperature of starch; Wide angle X-ray scattering (WAXS); Differential scanning calorimetry (DSC); Amylose–iodine complex formation; Birefringence

1. Introduction

Starch is a biopolymer that occurs naturally as water-insoluble and birefringent granules. Each granule consists of concentric growth rings of alternating amorphous and semi-crystalline composition. The semi-crystalline growth rings contain stacks of amorphous and crystalline lamellae. The crystalline lamellae consist of chain sections of amylopectin that form double helices (type A), while other chain sections of amylopectin form connections between the helices (type B). Branching points of both A and B chains of amylopectin are usually found within the amorphous lamellae (Jenkins et al., 1994; Waigh, Gidley, Komanshek, & Donald, 2000).

When a suspension of starch is heated in the presence of excess quantities of water, an irreversible order–disorder transition called gelatinisation takes place (Cooke &

* Corresponding author. Tel.: +31 317 48 37 70; fax: +31 317 48 22 37.
E-mail address: tim.baks@wur.nl (T. Baks).
[1] Present address: CPKelco BV, P.O. Box 31, 6500 AA, Nijmegen, The Netherlands.

0144-8617/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2006.06.016

Gidley, 1992; Jenkins & Donald, 1998; Liu & Lelievre, 1993). During gelatinisation, starch granules take up water, swell, lose crystallinity and leach amylose (Parker & Ring, 2001). In addition, heat is taken up, according to the characteristic gelatinisation endotherm that can be measured with Differential scanning calorimetry (DSC). If the amount of water is insufficient to provide complete swelling and disruption of the starch granules, only part of the crystallinity of the starch granules is lost. The remaining crystallinity only disappears after heating to higher temperatures. A melting transition occurs giving rise to an additional DSC endotherm (Donovan, 1979; Eliasson, 1980; Randzio, Flis-Kabulska, & Grolier, 2002; Whittam, Noel, & Ring, 1990). For a brief overview of the qualitative models describing the gelatinisation of starch we refer to Jenkins and Donald (1998). A more recent qualitative model was developed by Waigh et al. (2000) using a liquid-crystalline approach to describe the gelatinisation of starch. The Flory equation has been used by several authors for a quantitative description of the gelatinisation or melting temperature as a function of the starch concentration (Burt & Russell, 1983; Donovan, 1979; Donovan & Mapes, 1980; Donovan, Lorenz, & Kulp, 1983; Parker & Ring, 2001; Russell, 1987; Whittam et al., 1990).

Several analysis techniques are used to study different aspects of the gelatinisation process. Birefringence is used to follow the ordering in the granule on the length scale of the wavelength of light (approximately 500 nm) (Lelievre, 1974; Waigh et al., 2000). Wide angle X-ray scattering (SAXS) and short angle X-ray scattering (WAXS) can be used to follow, respectively, short-range order (crystalline double helices) and long-range order (alternating crystalline and amorphous lamellae) (Jenkins et al., 1994). Where X-ray scattering probes the double helices packed in regular arrays, solid state NMR detects the double helix content at a molecular order level (Cooke & Gidley, 1992; Gidley & Bociek, 1985). IR spectroscopy can also be used to follow the gelatinisation of starch on a short-range molecular level, because the IR spectrum of starch is affected by changes in structure such as starch chain conformation, helicity and crystallinity (Van Soest, Tournois, de Wit, & Vliegenthart, 1995). Liu, Lelievre, and Ayoung-Chee (1991) have found a quantitative correlation between crystallinity loss and thermal transitions during the gelatinisation of starch, since melting is a first order transition accompanied by a heat effect that can be measured well. Therefore, DSC measurements can also be used to follow the loss of order that takes place during the gelatinisation process. Amylose chains are released during the gelatinisation process and these chains can be determined colorimetrically (Birch & Priestley, 1973), as dissolved amylose forms a blue complex with iodine (Calabrese & Khan, 1999). When a starch–water mixture gelatinises, the water distribution and manner at which water is bound to the starch matrix changes (Tang & Hills, 2001). These changes affect the dielectric properties of the starch–water system and for this reason conductance measurements can be used to monitor the gelatinisation process (Karapantsios, Sakonidou, & Raphaelides, 2000). Besides the conductance, the viscosity of the starch–water mixture also changes during gelatinisation due to swelling of the granules. Monitoring the viscosity can therefore also be used to follow the gelatinisation. After starch has been gelatinised, it is susceptible to hydrolytic enzymes (Tester & Sommerville, 2001). For this reason, enzymatic methods have also been used to investigate the gelatinisation process indirectly (Roussel, Vieille, Billet, & Cheftel, 1991).

Comparisons between the analysis methods were made to elucidate the mechanisms that take place during the order–disorder transition of the gelatinisation process (Chaiwanichsiri, Ohnishi, Suzuki, Takai, & Miyawaki, 2001; Cooke & Gidley, 1992; Liu et al., 1991; Waigh et al., 2000). Liu et al. compared birefringence and X-ray measurements during the gelatinisation of a 2 w/w % corn starch suspension. However, these authors did not compare these measurements at higher starch concentrations.

In this article, DSC, X-ray, birefringence and amylose–iodine complex formation measurements will be used to determine the degree of gelatinisation in 10 w/w % and 60 w/w % starch suspensions in water. A general procedure was developed to measure the degree of gelatinisation at both low and high starch concentrations. Furthermore, the observed differences between the analysis techniques will be discussed. In addition, the differences between the gelatinisation behaviour of diluted and concentrated starch suspensions will be explained based on the Flory equation. The Flory equation is adapted to describe the degree of gelatinisation as function of both temperature and starch concentration.

2. Theory

The Flory equation is often used to relate the melting temperature T_m of a polymer in a polymer–diluent mixture to the volume fraction ϕ_1 of the diluent (Flory, 1953):

$$\frac{1}{T_m} - \frac{1}{T_m^0} = \left(\frac{R}{\Delta H_u}\frac{V_2}{V_1}\right) \cdot \left[\phi_1 - \chi_{12}\phi_1^2\right] \quad (1)$$

where T_m^0 is the melting point of the pure polymer, R is the gas constant, ΔH_u the heat of fusion per repeating unit, V_1 and V_2 are the molar volumes of the diluent and the repeating unit of the polymer and χ_{12} is the Flory–Huggins polymer–diluent interaction parameter. In addition, we have assumed that the ratio V_2/V_1 and χ_{12} are temperature independent. The Flory–Huggins interaction parameter is known to depend linearly on the reciprocal of the absolute temperature (Rudin, 1999). Therefore, χ_{12} decreases with 20% over the temperature interval of interest (50–115 °C). This variation is not too large and for this reason the assumption of a constant χ_{12} parameter seems justified. In the derivation of Eq. (1), it is assumed that the heat of fusion and the entropy of fusion do not depend on the temperature. According to Hoffman (1958), Van Krevelen

(1976) and Mandelkern (1989), this assumption is not justified anymore for large differences in T_m and T_m^0. Hoffman (1958) assumed that the enthalpy of fusion depends linearly on the temperature. In turn, this enthalpy equation can be used to derive an equation to describe the entropy of fusion as a function of the temperature. The Flory equation can now be derived by taking into account the temperature dependency of the free energy difference involved in the melting transition (composed of the enthalpy and entropy equations). Application of Hoffman's approximation is only valid when the temperature that is considered is larger than the glass transition temperature and smaller than T_m^0. Application of Hoffman's approximation leads to the following equation:

$$\frac{1}{T_m} - \frac{1}{T_m^0} = \left(\frac{R}{\Delta H_u}\frac{V_2}{V_1}\right) \cdot \left(\frac{T_m^0}{T_m}\right) \cdot \left[\phi_1 - \chi_{12}\phi_1^2\right] \tag{2a}$$

This equation can be rewritten in a more useful form leading to Eq. (2b):

$$T_m = T_m^0 - \left(\frac{R}{\Delta H_u}\frac{V_2}{V_1}\right) \cdot \left(T_m^0\right)^2 \cdot \left[\phi_1 - \chi_{12}\phi_1^2\right] \tag{2b}$$

We would like to use Eq. (2b) to describe the relation between the starch concentration, melting temperature and degree of gelatinisation of starch in starch–water mixtures. To achieve this aim, the procedure described below can be followed.

The degree of crystallinity (α_c) of the polymer (starch) is given by:

$$\alpha_c = \frac{v_2^c}{v_2^a + v_2^c} \tag{3}$$

where v_2^a is the volume of amorphous starch and v_2^c is the volume of crystalline starch. This equation can be rewritten to obtain:

$$v_2^a = \left(v_2^a + v_2^c\right)(1 - \alpha_c) \tag{4}$$

The volume fraction of the solvent in the amorphous part of the system (ϕ_1) and the overall volume fractions of the solvent (ϕ_1^T) can be calculated with:

$$\phi_1 = \frac{v_1}{v_1 + v_2^a}, \tag{5a}$$

$$\phi_1^T = \frac{v_1}{v_1 + v_2^a + v_2^c} \tag{5b}$$

where v_1 is equal to the volume occupied by the solvent.

Substitution of Eq. (4), dividing the denominator and numerator through $v_1 + v_2^a + v_2^c$, substitution of Eq. (5b), and substitution $\phi_1^T = 1 - \phi_2^T$ changes Eq. (5a) into

$$\phi_1 = \frac{1 - \phi_2^T}{1 - \alpha_c \cdot \phi_2^T} \tag{6}$$

where ϕ_2^T is the overall volume fraction (averaged over the crystalline and amorphous part of the system) of the starch. Only part of the native starch is crystalline (Cooke & Gidley, 1992) and the remaining non-crystalline fraction cannot crystallize, e.g. those chain sections close to a branching point. This non-crystalline fraction acts as an inert part of the system. For this reason, the relative crystallinity can be used instead of the degree of crystallinity. Therefore, α_c in Eq. (6) can be replaced with 1-*DG*, in which DG stands for the degree of gelatinisation. This replacement results in the following equation after substitution in Eq. (2b):

$$T_m = T_m^0 - \left(\frac{R}{\Delta H_u}\frac{V_2}{V_1}\right) \cdot \left(T_m^0\right)^2 \cdot \left[\left(\frac{1 - \phi_2^T}{1 - (1 - DG) \cdot \phi_2^T}\right) - \chi_{12}\left(\frac{1 - \phi_2^T}{1 - (1 - DG) \cdot \phi_2^T}\right)^2\right] \tag{7}$$

Eq. (7) gives a relationship between the melting temperature, the starch concentration and the degree of gelatinisation of starch in starch–water mixtures.

3. Experimental

3.1. Materials

Wheat starch (S5127) was obtained from Sigma–Aldrich (Steinheim, Germany) and it had a moisture content of 9.95 ± 0.43 w/w % (based on 22 measurements, 95% confidence interval). The moisture content was determined by drying the wheat starch in a hot air oven at 105 °C or in a vacuum oven at 80 °C until the mass of the samples was constant in time. The water content of wheat starch was taken into account during all experiments.

All other chemicals (potassium hydroxide, fuming hydrochloric acid, potassium iodide, iodine crystals) were at least analytical grade and they were purchased from Merck (Darmstadt, Germany). Milli-Q water was used for all experiments.

3.2. Gelatinisation of starch in starch–water mixtures

For the gelatinisation of 10 w/w % starch–water mixtures, a temperature controlled batch reactor equipped with anchor stirrer was used. A Philips HR 7638 kitchen machine (Amsterdam, The Netherlands) was used to prepare 60 w/w % starch slurries (also for DSC measurements). These slurries were gelatinised or melted in a compression molder from PHI (City of Industry, USA). The mould consisted of three stainless steel plates with dimensions 300×350 mm. The upper and lower plate had a thickness of 4 mm. The middle plate had a rectangular hole with dimensions $180 \times 120 \times 5$ mm. To facilitate the removal of the sample from the mould, the sample was covered with printable plastic overhead slides on both sides of the middle plate. The pressure applied to the mould was 6 ton, which was sufficient to keep water loss due to evaporation negligible.

During all gelatinisation experiments, the starch solutions or slurries were first heated to the desired temperature and held at this temperature for 45 min to equilibrate the starch–water mixture.

3.3. Handling of samples

To reduce recrystallization, samples taken from the batch reactor or compression molder were immediately transferred to a vessel with liquid nitrogen. After the samples were completely frozen, they were stored in an −80 °C freezer until further use.

After storage, the samples were freeze-dried in a Christ Epsilon 2-6D freeze dryer (Osterode am Harz, Germany) prior to analysis. Freeze-drying was started at −20 °C and 1.030 mbar for 7 h followed by a second drying stage at −5 °C and 0.01 mbar for 12 h. The freeze-dried samples were grinded in an analytical mill (type A10) from IKA (Staufen, Germany) and sieved to obtain a fine powder that could be dissolved easily. The powder obtained after sieving was stored in a desiccator before further use.

3.4. Birefringence measurements

A 1 w/w % solution of the freeze-dried starch powder was stirred for 1 h. After mixing, approximately 20 μl of this solution was transferred to a microscopic slide and viewed under normal and polarized light using a Zeiss Axioscop 50 (Jena, Germany) equipped with a CCD camera module. Pictures of different locations on the slide were taken randomly both under normal and polarized light. The total number of granules (n_t) and the number of granules that had not lost their polarization or Maltese crosses (n_m) was determined. The ratio n_m/n_t can be used as a measure for the degree of gelatinisation of the sample (Bauer & Knorr, 2005; Burt & Russell, 1983; Liu et al., 1991). Granules or parts of granules with part of the Maltese cross still present were taken as ungelatinised granules during the analysis.

3.5. Amylose–iodine complex formation

The amylose–iodine method used in this article is an adapted version of the method developed by Birch and Priestley (1973). A freeze-dried sample (0.04 g) was dissolved in 50 ml of 0.15 M KOH and the solution was mixed for 15 min. The resulting solution is centrifuged to remove the insoluble part of the sample. After centrifugation, 1 ml of the supernatant is removed and neutralized with 9 ml 0.017 M HCl. Subsequently, 0.1 ml iodine reagent (1 g iodine and 4 g potassium iodide in 100 ml water) was added to form a blue complex with the dissolved amylose present in the sample. The absorbance was measured at 25 °C and 600 nm (A_1). The procedure was repeated, however, in this case 0.40 M KOH was used to ensure complete solubilisation of all the amylose present in the sample. The supernatant was neutralized with 0.045 M HCl. After adding 0.1 ml iodine reagent, the absorbance at 25 °C and 600 nm was measured (A_2). The ratio A_1(0.15 M KOH)/A_2(0.40 M KOH) can be used as a measure for the degree of gelatinisation of the sample.

3.6. Wide angle X-ray diffraction

Diffractograms were recorded with a Philips PC-APD diffractometer. The scattered X-ray radiation was recorded by a proportional moving detector over a 4° and 40° (2θ) angular range with an angular scanning velocity of 1.2°/min and a measurement frequency of 1 s^{-1}. The Cu Kα radiation from the anode operating at 40 kV and 50 mA was monochromated with use of a 15-mm thick Ni foil. The diffractograms were smoothened with the computer program 'Table Curve 2D' (Jandel Scientific, 1994, version 2.0, San Rafael, USA) by applying Savitsky–Golay data smoothening (5% smoothening). The procedure of Van Soest et al. (1995) was used for the determination of the relative crystallinity. For wheat starch, the characteristic peak at $2\theta = 22.9°$ was selected. A straight line was used to approach the baseline below the characteristic peak. A Matlab script was used to calculate the slope and y-intercept of the baseline. In addition, this script was used to calculate the total area below the characteristic peak (A_t), the total area below the characteristic peak minus the area below the baseline (A_c), the height of the characteristic peak (H_t), and the total height of the characteristic peak minus the height of the baseline at the diffraction angle at $2\theta = 22.9°$ (H_c). The ratios $R_A = A_c/A_t$ and $R_H = H_c/H_t$ were obtained for native wheat starch (100% relative crystallinity) and for completely gelatinised or molten wheat starch (0% relative crystallinity) The relative crystallinity of a sample based on peak areas (X_{rA}) or peak heights (X_{rH}) can now be determined with the following equations:

$$X_{rA} = \frac{(R_A)_s}{(R_A)_n};\ X_{rH} = \frac{(R_H)_s}{(R_H)_n} \quad (8a, 8b)$$

where the subscript n stands for native wheat starch and the subscript s stands for sample. The relative crystallinity can be used as a measure for the degree of gelatinisation by applying the following equation:

$$DG = 1 - X_r \quad (9)$$

where X_r can be either X_{rA} or X_{rH}. The difference between the degree of gelatinisation calculated with X_{rA} and the degree of gelatinisation calculated with X_{rH} is equal to 5.9 ± 2.7% (95% confidence interval based on 26 measurements). This difference seems negligible. We have used the calculation based upon X_{rA} to determine the degree of gelatinisation.

3.7. Differential scanning calorimetry (DSC)

First, freeze-dried starch sample was transferred to a stainless steel DSC cup, dissolved in milli-Q water to obtain a 10 w/w % starch solution (approximately) and hermetically sealed. Thermograms of the resulting solu-

tions were made using a Perkin-Elmer DSC-7 equipped with a PE model TAC7/DX Thermal Analysis Controller (Boston, USA). An empty pan was used as reference. Before the actual measurement, samples were held at 0 °C for 5 min in the DSC measuring cell. Subsequently, the sample was heated at 10 °C/min from 0 °C to 150 °C. The raw data was processed with Pyris 6 (Perkin-Elmer) to obtain the enthalpy needed to gelatinise the remaining crystalline part of the starch in the sample (ΔH_s). The characteristic gelatinisation endotherm occurs in the thermogram between 51 °C and 76 °C. With use of the gelatinisation enthalpy of native wheat starch (ΔH_n), the degree of gelatinisation of the sample can be determined (Qu & Wang, 1994; Wang & Sastry, 1997):

$$DG = 1 - \frac{\Delta H_s}{\Delta H_n} \tag{10}$$

DSC measurements were also used to determine the temperature at which the first crystallites start to gelatinise (T_{oc}) and the temperature at which the most perfect crystallites have just been gelatinised or melted (T_{cc}) for 10 w/w % and 60 w/w % wheat starch–water mixtures. In case of the 10 w/w % wheat starch–water mixtures, wheat starch and water were added to the DSC cup before the measurement. In case of the 60 w/w % wheat starch–water mixtures, wheat starch and water were mixed as mentioned above and left to rest for at least 36 h. This wheat starch–water mixture was used for the DSC measurements.

The onset temperature (T_o) and conclusion temperature (T_c) of gelatinisation or melting peaks often encountered in literature differ from T_{oc} and T_{cc}. The differences between T_{oc} and T_o and between T_{cc} and T_c are shown in the DSC thermograms in Fig. 1. The onset and conclusion

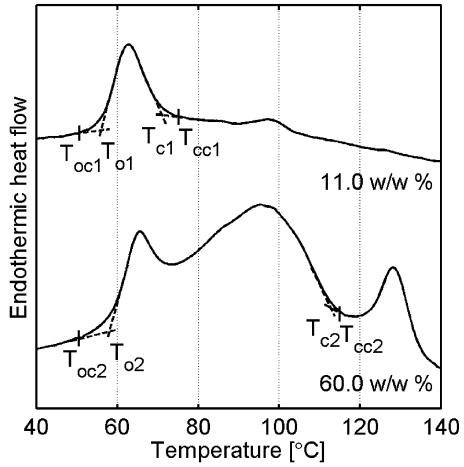

Fig. 1. DSC thermograms of different wheat starch–water mixtures. Symbols used: T_{oc} = temperature at which the first crystallites gelatinise or melt, T_o = onset temperature of gelatinisation or melting peak, T_{cc} = temperature at which the most perfect crystallites gelatinise or melt, T_c = conclusion temperature of gelatinisation or melting peak, 1 = index for 11.0 w/w % wheat starch–water mixture, and 2 = index for 60.0 w/w % wheat starch–water mixture. Note: the peak at 95 °C in the upper thermogram and the peak at 130 °C in the lower thermogram are caused by amylose–lipid complexes that are melting.

temperatures were obtained with the procedure that has been used by Eliasson and Karlsson (1983).

3.8. Curve fitting procedure

Eq. (7) can be used to calculate the melting temperature as a function of the degree of gelatinisation and starch concentration. These model values were fitted to experimental data in the temperature range between T_{oc} and T_{cc} (51–75 °C in case of 10 w/w % starch and 50–114 °C in case of 60 w/w % starch) with Mathcad (Mathsoft Engineering & Education, version 11.2, Cambridge, USA). These temperature ranges were determined by means of DSC. We have used 18.1 $cm^3\,mol^{-1}$ for V_1 (Lepori & Gianni, 2000) and 97.5 $cm^3\,mol^{-1}$ for V_2 (Shahidi, Farrell, & Edward, 1976) at 25 °C for all calculations and the ratio V_2/V_1 was assumed to be independent of temperature. To calculate the volume fractions of water during our experiments, we have used 1.65 g/cm^3 and 0.997 g/cm^3 for, respectively, the density of wheat starch (Mark, 1999) and water (Atkins, 1997). It was assumed that these densities do not depend on temperature. In addition, it was assumed that the densities of crystalline and amorphous wheat starch are equal. Three fit parameters were used (T_m^0, ΔH_u and χ_{12}) and the values of these parameters were determined by minimizing the sum of the squared residuals.

4. Results

4.1. Handling of samples

Throughout the sample handling process, care was taken that the samples remain frozen to make sure that recrystallization did not take place. X-ray measurements can be used to determine whether recrystallization has occurred (Karim, Norziah, & Seow, 2000; Ottenhof, Hill, & Farhat, 2005). Our X-ray diffractograms showed that recrystallization had not taken place prior to analysis (results not shown).

4.2. Gelatinisation of 10 w/w % and 60 w/w % wheat starch–water mixtures

Birefringence, DSC, wide angle X-ray diffraction and amylose–iodine complex formation measurements were applied for the measurement of the degree of gelatinisation of both a diluted (10 w/w %) and a concentrated (60 w/w %) starch–water mixture.

The enthalpy of gelatinisation of a 11.0 ± 1.0 w/w % (95% confidence interval) wheat starch–water mixture is equal to 13.4 ± 2.0 J/g (95% confidence interval) based on 4 DSC measurements (Table 1). This gelatinisation enthalpy is in good agreement with the value reported in literature (see Table 1).

Fig. 2 shows the degree of gelatinisation of 10 w/w % wheat starch–water mixtures that have been kept at a constant temperature for 45 min obtained with microscopy

Table 1
Temperature at which the first crystallites gelatinise or melt (T_{oc}), temperature at which the most perfect crystallites gelatinise or melt (T_{cc}) and enthalpy (ΔH) of the gelatinisation or melting for different wheat starch–water mixtures determined with DSC measurements with a heating rate of 10 °C/min

C_{starch} (w/w %)	T_{oc} (°C)	T_{cc} (°C)	ΔH[a] (J/g)	Reference
11	51	75	13.4 ± 2.0[b]	This article
16	51	63	17.9	Douzals et al. (1996)
60	50	114	–	This article
55	56	101	–	Eliasson (1980)
57	45	103	–	Svensson and Eliasson (1995)
58	53	104	–	Rolee and Le Meste (1997)
65	49	116	–	Eliasson (1980)

[a] Gelatinisation enthalpy in J/g dry starch.
[b] 95% confidence interval.

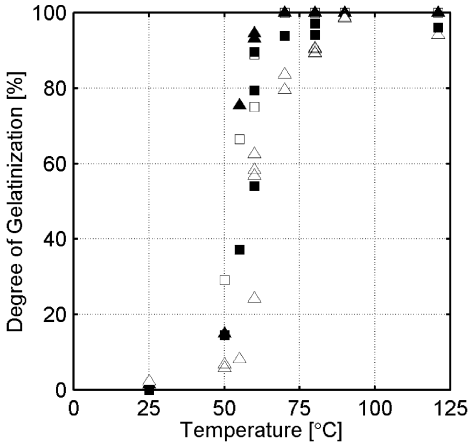

Fig. 2. Degree of gelatinisation of starch in 10 w/w % wheat starch–water mixtures as function of the treatment temperature (treatment time 45 min). Experimental data determined with birefringence (▲), DSC (□), X-ray (■), and amylose–iodine complex formation (△)

(birefringence), DSC, wide angle X-ray diffraction and amylose–iodine complexiметry. The values found with these methods give comparable degree of gelatinisation vs. treatment temperature curves. The temperature at which the gelatinisation of a 10 w/w % starch–water mixture starts, is equal to approximately 50 °C and the maximum degree of gelatinisation is reached at approximately 75 °C.

Fig. 3 shows the degree of gelatinisation of a 60 w/w % wheat starch–water mixture that has been measured with microscopy (birefringence), DSC, wide angle X-ray diffraction and amylose–iodine complexiметry after various treatments at constant temperature have been applied for 45 min. The degree of gelatinisation of the 60 w/w % starch–water mixture that was found, depended on the method used. Measurement of the degree of gelatinisation with birefringence leads to a high estimate, while measurement with amylose–iodine complexiметry provides a low estimate. DSC and wide angle X-ray measurements provide values of the degree of gelatinisation that are in between the ones obtained with birefringence measurements and amylose–iodine complexiметry. Gelatinisation of starch in a 60 w/w % starch–water mixture starts before 60 °C and the maximum degree of gelatinisation is obtained at approximately 110 °C.

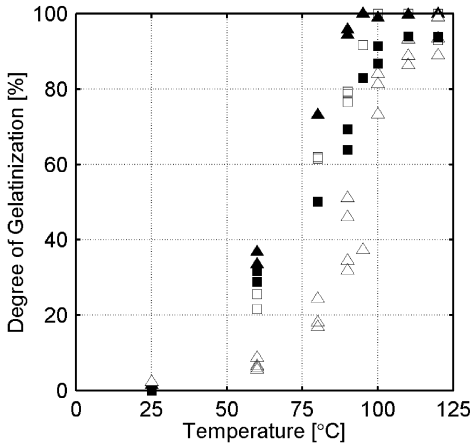

Fig. 3. Degree of gelatinisation of starch in 60 w/w % wheat starch–water mixtures as function of the treatment temperature (treatment time 45 min). Figure based on birefringence (▲), DSC (□), X-ray (■), and amylose–iodine complex formation (△) measurements.

Fig. 4 shows the degree of gelatinisation as a function of the treatment temperature for a 10 w/w % and a 60 w/w % wheat starch–water mixture. This figure shows the results that were obtained with DSC and X-ray measurements. The temperature at which the gelatinisation process started, seemed to depend on the starch concentration. However, the number of measurements in this region is limited and for this reason it is hard to draw firm conclusions.

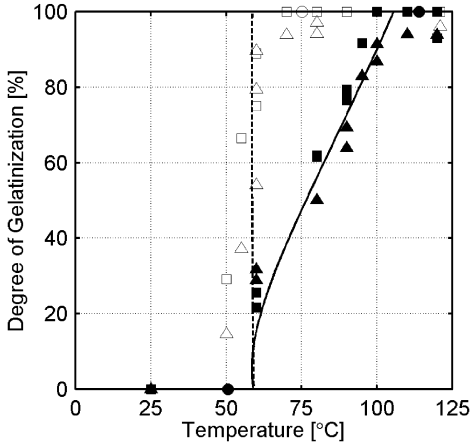

Fig. 4. Degree of gelatinisation of starch as function of the treatment temperature (treatment time 45 min) for 10 w/w % (□: DSC data, △: X-ray data) and 60 w/w % (■): DSC data, ▲: X-ray data) wheat starch–water mixtures. Dotted line: calculated values for a 10 w/w % wheat starch–water mixture, solid line: calculated values for a 60 w/w % wheat starch–water mixture. T_{oc1} and T_{cc1} of a 11.0 w/w % wheat starch–water mixture (○) and T_{oc2} and T_{cc2} of a 60.0 w/w % wheat starch–water mixture (●) obtained with DSC have also been added to this graph.

Table 2
Parameters of the Flory equation

T_m^0 (°C)	ΔH_u (kJ/mol)	χ_{12} (–)	Reference
291 ± 63[a]	29.2 ± 3.9[a]	0.53 ± 0.05[a]	This article
210	29.4	0.04	Lelievre (1974)
181	52.8	0	Donovan and Mapes (1980)
227 ± 13[a]	37.3	0	Burt and Russell (1983), Russell (1987)
263	22	0.57	Whittam et al. (1990), Moates et al. (1998)

[a] 95% confidence interval.

The temperature at which maximum degree of gelatinisation is reached, clearly depends on the starch concentration.

The temperature at which the gelatinisation starts and ends can also be obtained from DSC thermograms (see Fig. 1 and Table 1). The temperatures that we have found are comparable with the values reported in literature (see Table 1). Fig. 4 shows that T_{oc} and T_{cc} are also in line with the DSC and X-ray measurements.

Eq. (7) was fitted to the experimental data for which holds that the temperature is in between T_{oc} and T_{cc} and the results are shown in Fig. 4. The parameters T_m^0, ΔH_u and χ_{12} that resulted in the lowest sum of the squared residuals are given in Table 2. The calculated degree of gelatinisation vs. treatment temperature curves agree with the experimentally determined curves.

5. Discussion

According to Donovan (1979), the loss of crystallinity of the granule, the uptake of heat accompanied by the conformation change of starch, take up of water resulting in swelling of the granule and a decrease in the relaxation time of the water molecules occur simultaneously (or nearly so) during starch gelatinisation in excess water. Each method that can be used to study the gelatinisation of starch focuses on one or several of these specific aspects of the starch gelatinisation. During gelatinisation of a 10 w/w % wheat starch–water mixture, the microscopic changes in the crystallinity of the granule measured with the birefringence measurements, the endothermic gelatinisation transition measured with DSC, the long-range order determined with wide angle X-ray diffraction and the leaching of amylose chains with the amylose–iodine complexiometry occur more or less simultaneously. Because all these phenomena occur at the same time at these conditions, it does not matter which method is used for the determination of the degree of gelatinisation of starch in 10 w/w % wheat starch–water mixtures (see Fig. 2).

Similar results were obtained by Liu et al. (1991) and Cooke and Gidley (1992). Liu et al. (1991) observed small differences between birefringence and relative crystallinity (measured with X-ray) values of a 2 w/w % corn starch mixture as a function of the temperature that are comparable to the differences that are shown in Fig. 1. Cooke and Gidley (1992) have shown that the crystallinity loss (measured with X-ray) and the loss in endothermic peak area (measured with DSC) of starch in 5 w/v % aqueous wheat starch suspensions (they have also used maize, potato, waxy maize and tapioca) as a function of the treatment temperature are comparable.

At the beginning of the gelatinisation process, the same phenomenon occurs whether the amount of water is abundant (e.g. in case of a 10 w/w % wheat starch–water mixture) or limiting (e.g. in case of a 60 w/w % wheat starch–water mixture). Assuming that we have prepared a homogeneous starch–water mixture, every starch granule (independent of size) is surrounded by a thin layer of water and it increases in size due to the uptake of water by the amorphous growth rings (Waigh et al., 2000). Because the granule consists of alternating amorphous and semi-crystalline growth rings, increase of the amorphous growth rings due to the uptake of water results in disruption of the semi-crystalline growth rings and loss of crystallinity. The similarity between the beginning of the gelatinisation process at high and limiting water concentrations can be explained as follows. The semi-crystalline, concentric growth rings on the outside of the granule are the first growth rings that have access to the available water. This part of the granule will gelatinise and hold the available water. As a result, there is an excess of water at the outside of the granule at both high and limiting water concentrations during the onset of the gelatinisation process. For this reason, the same process can take place initially in both cases resulting in the same starting temperature for gelatinisation. Therefore, the first peaks in the DSC thermograms of both diluted and concentrated starch–water mixtures start at the same temperature (see Fig. 1). Because all water has been absorbed and bound by the gelatinised starch fraction in the concentric growth rings on the outside of the granule, there is no water available to induce swelling and disruption of the remainder of the starch granule at high starch–water ratios. For this reason, a heterogeneous mixture is formed that consists of amorphous, gelatinised starch and a part that has remained semi-crystalline. Complete loss of crystallinity can only be achieved by further increasing the temperature resulting in melting of the remaining semi-crystalline starch fraction (Waigh et al., 2000). An increased temperature is required because the amount of water in the remaining starch fraction is very low. This can also be observed in the additional peak in the DSC thermogram of 60 w/w % wheat starch in water in Fig. 1. In addition, Eq. (1) also predicts that the temperature required for complete loss of crystallinity increases with an increasing starch–water ratio.

At a wheat starch–water concentration of 60 w/w %, the differences between the curves obtained with birefringence, X-ray, DSC and amylose–iodine complex formation measurements become significant. Apparently, the phenomena that take place during the gelatinisation process at higher starch concentrations do not occur simultaneously anymore. The qualitative model based on a liquid-crystalline theory presented by Waigh et al. (2000) might provide an explanation here. They suggest that the gelatinisation of starch is a two-stage process. In the first stage, the amylopectin side chains that form double helices become separated from their duplex helical neighbours. In the second stage, a helix-random coil transition takes place resulting in the unwinding of the double helix. According to these authors the intermediate phase between the first stage and the second stage is not birefringent for A-type starches (e.g. wheat starch) and therefore birefringence measurements will already indicate that gelatinisation has taken place after the first stage. The unwinding of the double helices during the second stage is followed with WAXS and for this reason it is expected that the typical degree of gelatinisation determined with WAXS is lower than the degree of gelatinisation determined with birefringence at the same temperature. Both stages during the gelatinisation process give rise to an endothermic DSC peak. Only after the second stage has taken place, the sample is completely gelatinised and the DSC thermogram will not show an endothermic peak anymore. For this reason, the gelatinisation curve that is obtained with DSC is comparable to the curve obtained with WAXS. The theory of Waigh et al. (2000) does not provide a direct explanation for the observation that the amylose chains seem to be released after all ordening has disappeared. The behaviour of amylose during the gelatinisation of a concentrated starch–water mixture might be explained by considering the reduced mobility of the amylose chains in the granule (or part of the granule) caused by the low amount of water. Perhaps the disappearance of all ordening in the granule is needed to increase the mobility of the amylose chains within the granule to enable the release of amylose.

Based on the section above, it is expected that all birefringence is completely lost before X-ray measurements indicate that the gelatinisation process starts, because the first stage of the gelatinisation process should be completed before the second stage of the gelatinisation process can start. However, granules in wheat starch do not gelatinise at the same temperature due to the bimodal granule size distribution (Eliasson & Karlsson, 1983). Small granules gelatinise over a broader temperature interval than large granules. These differences can be caused by differences in composition between small and large granules. Meredith (1981) found that the distribution of saturated and unsaturated lipid is different for small granules and this author also found that the total lipid concentration varies with granule size. Morrison (1995) mentioned that the amount of lipid might affect the gelatinisation temperature and therefore differences in lipid content between small and large granules might affect the gelatinisation temperature. Furthermore, the chain length distribution of amylose chains might differ between small and large granules. Amylose chain length results in a different gelatinisation temperature (Moates, Noel, Parker, & Ring, 1997). For these reasons, it seems that within different granules different stages of the gelatinisation process can take place. Therefore, X-ray measurements can indicate that the gelatinisation process has started, although birefringence measurements indicate that part of the granules have not been gelatinised yet.

DSC and X-ray measurements can be used to determine whether the final stage of the gelatinisation process (unwinding of the double helices) has been completed. For this reason, we have used the experimental data based on these measurements for comparison between the 10 w/w % and 60 w/w % wheat starch–water mixtures and for fitting of the adapted Flory equation. To our knowledge, this is the first time that the Flory equation has been adapted in such way that it can quantitatively describe the degree of starch gelatinisation as a function of temperature and the starch–water ratio, even at very high ratios. According to this equation, the gelatinisation process starts at the same temperature independent of the concentration. This behaviour was also expected based on the theory mentioned above and the thermograms of 11 w/w % and 60 w/w % wheat starch–water mixtures (see Table 1 and Fig. 1). Furthermore, Eq. (7) predicts a steep increase in the degree of gelatinisation with increasing temperature for a 10 w/w % wheat starch–water mixture and a more gradual increase of the degree of gelatinisation with increasing temperature in case of a 60 w/w % wheat starch–water mixture. These differences are also present in our experimental data.

Fitting Eq. (7) to the experimental data resulted in useful T_m^0, ΔH_u and χ_{12} values. We have determined the degree of gelatinisation of an 80 w/w % wheat starch solution as a function of the treatment temperature (results not shown). At the maximum temperature that has been applied (180 °C), the degree of gelatinisation was slightly higher than 50%. Extrapolation of the trend indicates that the temperature required to achieve complete gelatinisation of this starch–water mixture is much higher than 200 °C. For this reason, the value of T_m^0 should be much higher and therefore our value of T_m^0 seems reasonable. The Flory–Huggins interaction parameter χ_{12} that we have obtained is close to 0.5. A negative or small positive χ_{12} value is a characteristic of a stable mixture (Rudin, 1999) which is the case for a starch–water mixture. In addition, a χ_{12} value close to 0.5 indicates that the second virial constant is close to zero (Rudin, 1999). Banks, Greenwood, and Sloss (1969) have also found that the second virial coefficient is equal to zero for both amylose and amylopectin with use of light scattering experiments. It is expected that starch, a mixture of amylopectin and amylose, will therefore also have a second virial coefficient that is approximately equal to zero indicating that χ_{12} is close to 0.5.

The parameters that we have obtained with the adapted version of Flory's equation (Eq. (7)) are comparable to the

values of other researchers that were obtained after they have fitted the original Flory equation (Eq. (1)) to their experimental data (see Table 1). It should be noted that the majority of reported values did not contain a confidence interval. In addition, in other publications the Flory equation was not modified to account for the temperature dependence of the heat of fusion and entropy of fusion. The parameters obtained by these researchers should therefore be used with caution. The T_m^0 value that we have obtained is higher than the values reported by others (see Table 2), but the values reported by Burt and Russell (1983) and Whittam et al. (1990) fall within our confidence interval and therefore one cannot conclude that our value is different. The value of ΔH_u is lower than the value reported by Donovan and Mapes (1980), but close to the values reported by other researchers (see Table 2). All reported χ_{12} values are in this range 0–0.6 indicating that starch–water mixtures are stable. The Flory–Huggins interaction parameter of Whittam et al. (1990) is close to the value that we have found and falls within our confidence interval. The χ_{12} value obtained by Lelievre (1974) is closer to zero. Other authors have assumed that χ_{12} is zero (e.g. Burt & Russell, 1983; Donovan & Mapes, 1980), but do not verify this assumption.

We have included the parameters reported by Whittam et al. (1990) in our comparison, although they have used A-type crystalline starch instead of native wheat starch. However, since we have assumed that the non-crystalline part of native starch is an inert part of the system, we only consider that part of the starch, which is crystalline in native starch, and that is therefore comparable with A-type crystalline starch.

The parameters that have been obtained with the fitting procedure appear to be reasonable. In addition, the model values are in good agreement with the experimental values. Thus, in our view Eq. (7) can be used as a practical and reliable way to quantitatively estimate the degree of gelatinisation of a starch–water mixture as a function of the starch concentration and treatment temperature.

6. Conclusions

Measurement of the degree of gelatinisation of starch in a 10 w/w % wheat starch–water mixture as a function of the treatment temperature based on birefringence, DSC, X-ray or amylose–iodine complex formation measurements give similar curves, because the physical–chemical processes involved occur simultaneously. If a 60 w/w % wheat starch–water mixture is used, however, the degree of gelatinisation vs. temperature curves are affected by the methods used to calculate the degree of gelatinisation. The highest values are obtained with birefringence measurements. Calculations based on DSC and X-ray measurements resulted in slightly lower values. Finally, amylose–iodine complex formation resulted in the lowest degree of gelatinisation. These differences were explained by considering the phenomena that take place during the gelatinisation at limiting water conditions.

Gelatinisation of 10 w/w % and 60 w/w % wheat starch–water mixtures started at approximately the same temperature. However, complete gelatinisation was reached at different temperatures. These results are in accordance with DSC measurements that were carried out independently.

An adapted version of the Flory equation provides a quantitative description of the degree of starch gelatinisation as a function of the starch–water ratio and the temperature. Fitting this equation to our experimental data results in T_m^0, ΔH_u and χ_{12} values that are reasonable and comparable to the values reported in literature. The adapted Flory equation can be used as a means to estimate the temperature that is needed to completely gelatinise starch in a starch–water mixture over the whole concentration range.

The procedure followed for the handling of the samples can be used for diluted and concentrated starch–water mixtures and makes sure that recrystallization is prevented. At low starch concentration it does not matter which method is selected to determine the degree of gelatinisation. However, at higher concentrations the degree of gelatinisation that is calculated depends on the analysis method used. DSC and X-ray measurements can be used to determine whether the final stage of the gelatinisation process has been completed. Birefringence and amylose–iodine complex formation measurements do not require specialized equipment and for this reason they are suitable alternatives. However, use of birefringence and amylose–iodine complex formation measurements will lead to different results.

Acknowledgements

The authors wish to thank Herman de Beukelaer for his assistance with the DSC measurements, Guus Frissen for his assistance with the X-ray measurements, and Aldo Kranenbarg for his assistance with the compression molder (all of them work at Agrotechnology and Food Sciences Group in Wageningen). In addition, the authors thank the Food Physics department at Wageningen University for using their microscope. Finally, the authors want to thank Rolf Renger for the valuable discussions concerning the manuscript.

References

Atkins, P. W. (1997). *Physical chemistry*. Oxford: Oxford University Press.

Banks, W., Greenwood, C. T., & Sloss, J. (1969). Light-scattering studies on aqueous solutions of amylose and amylopectin. *Carbohydrate Research, 11*(3), 399–406.

Bauer, B. A., & Knorr, D. (2005). The impact of pressure, temperature and treatment time on starches: pressure-induced starch gelatinisation as pressure time temperature indicator for high hydrostatic pressure processing. *Journal of Food Engineering, 68*, 329–334.

Birch, G. G., & Priestley, R. J. (1973). Degree of gelatinisation of cooked rice. *Starch, 25*, 98–100.

Burt, D. J., & Russell, P. L. (1983). Gelatinization of low water content wheat starch–water mixtures. A combined study by differential scanning calorimetry and light microscopy. *Starch, 35*(10), 354–360.

Calabrese, V. T., & Khan, A. (1999). Amylose–iodine complex formation without KI: evidence for absence of iodide ions within the complex. *Journal of Polymer Science: Part A: Polymer Chemistry, 37*, 2711–2717.

Chaiwanichsiri, S., Ohnishi, S., Suzuki, T., Takai, R., & Miyawaki, O. (2001). Measurement of electrical conductivity, differential scanning calorimetry and viscosity of starch and flour suspensions during gelatinisation process. *Journal of the Science of Food and Agriculture, 81*(15), 1586–1591.

Cooke, D., & Gidley, M. J. (1992). Loss of crystalline and molecular order during starch gelatinisation: origin of the enthalpic transition. *Carbohydrate Research, 227*, 103–112.

Donovan, J. W. (1979). Phase transitions of the starch–water system. *Biopolymers, 18*(2), 263–275.

Donovan, J. W., & Mapes, C. J. (1980). Mutiple phase transitions of starches and Nägeli amylodextrins. *Starch, 32*(6), 190–193.

Donovan, J. W., Lorenz, K., & Kulp, K. (1983). Differential scanning calorimetry of heat–moisture treatment of starches. *Cereal Chemistry, 60*, 381–387.

Douzals, J. P., Marechal, P. A., Coquille, J. C., & Gervais, P. (1996). Microscopic study of starch gelatinization under high hydrostatic pressure. *Journal of Agricultural and Food Chemistry, 44*(6), 1403–1408.

Eliasson, A. C. (1980). Effect of water content on the gelatinization of wheat starch. *Starch, 32*(8), 270–272.

Eliasson, A. C., & Karlsson, R. (1983). Gelatinization properties of different size classes of wheat starch granules measured with differential scanning calorimeter. *Starch, 35*, 130–133.

Flory, P. J. (1953). *Principles of polymer chemistry*. Ithaca: Cornell University Press.

Gidley, M. J., & Bociek, S. M. (1985). Molecular organization in starches: a carbon ^{13}CP/MAS NMR study. *Journal of the American Chemical Society, 107*(24), 7040–7044.

Hoffman, J. D. (1958). Thermodynamic driving force in nucleation and growth processes. *Journal of Chemical Physics, 29*, 1192–1193.

Jenkins, P. J., & Donald, A. M. (1998). Gelation of starch: a combined SAXS/WAXS/DSC and SANS study. *Carbohydrate Research, 308*(1–2), 133–147.

Jenkins, P. J., Cameron, R. E., Donald, A. M., Bras, W., Derbyshire, G. E., Mant, G. R., & Ryan, A. J. (1994). In situ simultaneous small and wide angle X-ray scattering: a new technique to study starch gelatinization. *Journal of Polymer Science, Part B: Polymer Physics, 32*(8), 1579–1583.

Karapantsios, T. D., Sakonidou, E. P., & Raphaelides, S. N. (2000). Electrical conductance study of fluid motion and heat transport during starch gelatinization. *Journal of Food Science, 65*(1), 144–150.

Karim, A. A., Norziah, M. H., & Seow, C. C. (2000). Methods for the study of starch recrystallization. *Food Chemistry, 71*(1), 9–36.

Lelievre, J. (1974). Starch gelatinization. *Journal of Applied Polymer Science, 18*(1), 293–296.

Lepori, L., & Gianni, P. (2000). Partial molar volumes of ionic and nonionic organic solutes in water: a simple additivity scheme based on the intrinsic volume approach. *Journal of Solution Chemistry, 29*(5), 405–447.

Liu, H., & Lelievre, J. (1993). A model of starch gelatinization linking differential scanning calorimetry and birefringence measurements. *Carbohydrate Polymers, 20*(1), 1–5.

Liu, H., Lelievre, J., & Ayoung-Chee, W. (1991). A study of starch gelatinization using differential scanning calorimetry, X-ray, and birefringence measurements. *Carbohydrate Research, 210*, 79–87.

Mandelkern, L. (1989). In *Comprehensive polymer science*. In C. Booth & C. Price (Eds.). *Polymer properties* (Vol. 2). Oxford: Pergamon Press.

Mark, J. E. (1999). *Polymer data handbook*. Oxford: Oxford University Press.

Meredith, P. (1981). Large and small starch granules in wheat – are they really different?. *Starch 33*(2), 40–44.

Moates, G. K., Noel, T. R., Parker, R., & Ring, S. G. (1997). The effect of chain length and solvent interactions on the dissolution of the B-type crystalline polymorph of amylose in water. *Carbohydrate Research, 298*(4), 327–333.

Moates, G. K., Parker, R., & Ring, S. G. (1998). Preferential solvent interactions and the dissolution of the B-type crystalline polymorph of starch in aqueous solutions. *Carbohydrate Research, 313*(3–4), 225–234.

Morrison, W. R. (1995). Starch lipids and how they relate to starch granule structure and functionality. *Cereal Foods World, 40*(6), 437–438 (440–441, 443–446).

Ottenhof, M.-A., Hill, S. E., & Farhat, I. A. (2005). Comparative study of the recrystallization of intermediate water content waxy maize, wheat, and potato starches. *Journal of Agricultural and Food Chemistry, 53*(3), 631–638.

Parker, R., & Ring, S. G. (2001). Aspects of the physical chemistry of starch. *Journal of Cereal Science, 34*, 1–17.

Qu, D., & Wang, S. S. (1994). Kinetics of the formations of gelatinized and melted starch at extrusion cooking conditions. *Starch, 46*(6), 225–229.

Randzio, S. L., Flis-Kabulska, I., & Grolier, J.-P. E. (2002). Reexamination of phase transformations in the starch–water system. *Macromolecules, 35*(23), 8852–8859.

Rolee, A., & Le Meste, M. (1997). Thermomechanical behavior of concentrated starch–water preparations. *Cereal Chemistry, 74*(5), 581–588.

Roussel, L., Vieille, A., Billet, I., & Cheftel, J. C. (1991). Sequential heat gelatinization and enzymic hydrolysis of corn starch in an extrusion reactor. Optimization for maximum dextrose equivalent. *Lebensmittel-Wissenschaft und -Technologie, 24*(5), 449–458.

Rudin, A. (1999). *The elements of polymer science and engineering*. New York: Academic Press.

Russell, P. L. (1987). Gelatinization of starches of different amylose/amylopectin content. A study by differential scanning calorimetry. *Journal of Cereal Science, 6*(2), 133–145.

Shahidi, F., Farrell, P. G., & Edward, J. T. (1976). Partial molar volumes of organic compounds in water. III. Carbohydrates. *Journal of Solution Chemistry, 5*(12), 807–816.

Svensson, E., & Eliasson, A.-C. (1995). Crystalline changes in native wheat and potato starches at intermediate water levels during gelatinization. *Carbohydrate Polymers, 26*(3), 171–176.

Tang, H., & Hills, B. (2001). Starch granules. A multinuclear magnetic resonance study. *Special Publication – Royal Society of Chemistry, 262(Magnetic Resonance in Food Science)*, 157–164.

Tester, R. F., & Sommerville, M. D. (2001). Swelling and enzymatic hydrolysis of starch in low water systems. *Journal of Cereal Science, 33*(2), 193–203.

Van Krevelen, D. W. (1976). *Properties of polymers*. Amsterdam: Elsevier.

Van Soest, J. J. G., Tournois, H., de Wit, D., & Vliegenthart, J. F. G. (1995). Short-range structure in (partially) crystalline potato starch determined with attenuated total reflectance Fourier-transform IR spectroscopy. *Carbohydrate Research, 279*, 201–214.

Waigh, T. A., Gidley, M. J., Komanshek, B. U., & Donald, A. M. (2000). The phase transformations in starch during gelatinisation: a liquid crystalline approach. *Carbohydrate Research, 328*(2), 165–176.

Wang, W.-C., & Sastry, S. K. (1997). Starch gelatinization in ohmic heating. *Journal of Food Engineering, 34*(3), 225–242.

Whittam, M. A., Noel, T. R., & Ring, S. G. (1990). Melting behaviour of A- and B-type crystalline starch. *International Journal of Biological Macromolecules, 12*(6), 359–362.