Carbohydrate Polymers 90 (2012) 140–146

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Devitrification of the amorphous fractions of starch during gelatinisation

Sami Bulut[a,b,*], Christoph Schick[a,1]

[a] University of Rostock, Inst. of Physics, Wismarsche Str. 43-45, 18051 Rostock, Germany
[b] Trakya University, Faculty of Engineering and Architecture, Food Engineering Department, Edirne, Turkey

ARTICLE INFO

Article history:
Received 5 March 2012
Received in revised form 10 April 2012
Accepted 1 May 2012
Available online 8 May 2012

Keywords:
Waxy maize starch
Glycerol
Gelatinisation
Heat capacity
Rigid amorphous
TMDSC

ABSTRACT

Gelatinisation of waxy maize starch (WMS) in excess glycerol was investigated by a temperature modulated DSC in quasi-isothermal and non-isothermal mode. By using the measured specific heat capacities (c_p) at different stages of gelatinisation and the hypothetical enthalpy of melting for 100% crystalline amylopectin, a method was developed to reveal the amorphous fraction which remains glassy in the presence of excess glycerol what can be called “rigid amorphous”. It was suggested that the amorphous part of starch was plasticised at around 40 °C whereas, rigid amorphous fraction underwent a second stage glass transition which showed itself by an increase in c_p under the gelatinisation endotherm. It was also shown that holding WMS in excess glycerol at 100 °C (10 °C above the gelatinisation onset temperature) for 10 days was not enough to gelatinise the whole WMS implicating that the most stable crystals did not melt before the required threshold temperature was reached.

1. Introduction

Starch, one of the most important natural macromolecules due to its universal form of packing and storing carbohydrates in green plants, is a mixture of two molecular entities, a mainly linear fraction; amylose, and its branched counterpart; amylopectin (Randzio, Flis-Kabulska, & Grolier, 2002). Amylose is an essentially linear molecule of α-(1 → 4)-linked D-glucopyranosyl units with a few branches, whereas amylopectin has large molecular weight and highly branched structures consisting of α-(1 → 4)-linked D-glucopyranosyl units with 5–6% non-randomly distributed α-(1 → 6)-D-glucopyranosyl units. These two polymers are organised into a semi-crystalline structure (Chung, Liu, Lee, & Wei, 2011). When starch is composed primarily of amylopectin, it is referred to as “waxy” (Chakraborty et al., 2004). Waxy maize starch is a specialty material characterised by a high (>98%) amylopectin content (Sablani et al., 2007).

Understanding of structure, properties and processing of starch is important to improve the quality of starch based food products, pharmaceuticals and development of functional foods (Appelqvist & Debet, 1997). Starch is also an important raw material for biodegradable plastics due to its low cost, its availability as a renewable resources and its thermal processability using conventional plastics processing equipment such as injection moulding, film blowing, thermoforming and extrusion (Tan, Wee, Sopade, & Halley, 2004).

Starch gelatinisation can be generally defined as an irreversible order–disorder transition, which involves disruption of molecular organisation within the starch granules upon heating in the presence of plasticisers (Tan et al., 2004). Due to the complexity of the underlying mechanism of gelatinisation, many features of gelatinisation are not yet understood completely. However, increase in precision of analytical methods and instruments as well as the accumulation of information through various techniques employed up to date lead to better understanding of the gelatinisation. Detailed information on gelatinisation of starch could be found elsewhere (Ratnayake & Jackson, 2008).

Starch, as a natural semi-crystalline macromolecule carries some aspects of synthetic semi-crystalline polymers. Waigh, Gidley, Komanshek, and Donald (2000) suggested that there was an analogy between starch and a chiral side-chain polymeric liquid crystal and they proposed a gelatinisation theory based on this approach, which is still widely accepted and provides basis for emerging new gelatinisation theories (Tan et al., 2004).

The aim of the present contribution is to investigate the existence of an intermediate phase between crystal and amorphous phases which is well known and called “rigid amorphous” in the field of synthetic polymers (Suzuki, Grebowicz, & Wunderlich, 1985; Wunderlich, 2003). This intermediate phase is non-crystalline and includes amorphous portions of macromolecules

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* Corresponding author. Present address: Trakya University, Faculty of Engineering and Architecture, Food Engineering Department, Edirne, Turkey.
Tel.: +90 284 226 1217; fax: +90 284 226 12 25.
E-mail addresses: samibulut@trakya.edu.tr, sami_bulut@hotmail.com (S. Bulut), christoph.schick@uni-rostock.de (C. Schick).
[1] Tel.: +49 381 498 6880.

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http://dx.doi.org/10.1016/j.carbpol.2012.05.006

whose mobility is hindered by the near crystalline structures. The different amorphous fractions are usually classified on the basis of their mobility. The unconstrained amorphous phase, that relaxes at the glass transition (T_g), is usually addressed as the “mobile amorphous fraction” (MAF), whereas the term “rigid amorphous fraction” (RAF) refers to the amorphous chain portions whose mobility is hindered by the near crystalline structures (Maria Laura, 2009; Righetti, Tombari, & Lorenzo, 2008; Schick, Wurm, & Mohammed, 2003; Suzuki et al., 1985; Wunderlich, 2003). The determination of RAF and MAF is commonly based on calorimetric measurements. Despite the calorimetry, particularly DSC, is a standard tool in food research, there are only a few quantitative heat capacity data on starch plasticiser systems available (Bizot et al., 1997; Pyda, 2002; Tan et al., 2004).

In the present study, we measured heat capacity and enthalpy change of starch–glycerol–water systems during gelatinisation by using a high sensitivity heat flux temperature modulated differential scanning calorimeter (TMDSC) in order to understand changes in molecular mobility of starch during gelatinisation and to investigate wheatear a rigid amorphous fraction exists. For this purpose, starch in excess glycerol was exposed to different temperatures within the range of gelatinisation temperatures in quasi-isothermal mode, while the change in c_p was monitored for 15 h to 10 days.

2. Materials and methods

2.1. Reagents

2.1.1. Starch

Waxy maize starch was from National Starch Co. (Manchester, UK). In order to minimise the moisture gain/loss during weighing of the starch, the moisture content of the starch was equilibrated with relative humidity of the closed cabinet where the weighing scale (Sartorius, Micro M500P) used for the experiments was stationed. After a week of equilibration time, the moisture content of the starch determined by theromgravimetry (Setaram Labsys TG/DSC) was ca. 10% (w/w).

2.1.2. Glycerol

Glycerol obtained from Sigma–Aldrich (Germany) was 99% pure however, due to its high hygroscopic nature it is assumed that it might have gained some humidity from the air during sample preparation.

2.2. DSC instrument

A Mettler-Toledo 822e DSC equipped with a Star sensor HSS7 and temperature modulated DSC software was used for the experiments. A Julabo (FT900) cooler was used to achieve the programmed modulation temperature. Dry air was purged through the DSC cell with a flow rate of 20 ml/min. Temperature and heat flow calibrations were done in the standard DSC mode without temperature modulation, using the onsets of the transition temperatures and the heat of fusions for indium and zinc.

2.3. Sample preparation

Starch (8.00 ± 0.01 mg) was weighed in standard Mettler aluminium pans with 40 μl volume. After addition of 19.5 ± 0.1 mg of glycerol, the pans were hermetically sealed and left at room temperature for 1 h before the start of the experiments to ensure standardised starting conditions for all measurements. As reference, 19.5 mg of glycerol sealed in a similar aluminium pan was used. Considering the moisture content of starch, it could be stated that the starch is plasticised with a mixture of water and glycerol at a concentration of 4% and 96%, respectively.

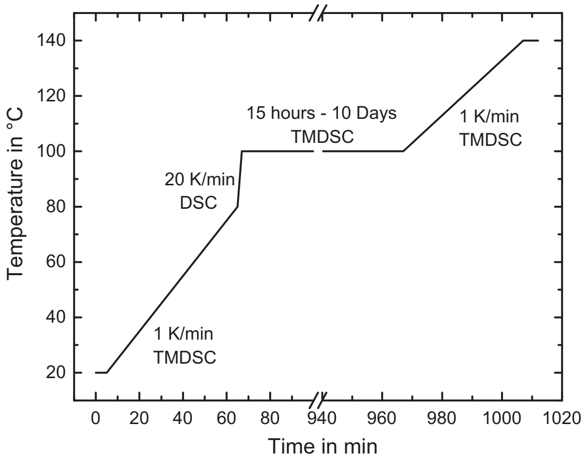

Fig. 1. Schematic presentation of temperature program for quasi-isothermal experiments. Temperatures shown are the mean temperatures of the modulated temperature program except the 20 K min^{-1} segment where modulation was not applied. For temperature scan, $A = 3$ K, $p = 2$ min, $q = 1$ K min^{-1}, for quasi-isothermal segment, $A = 3$ K, $p = 2$ min, $q = 0$ K min^{-1}.

2.4. Evaluation of DSC curves

After a set of experiments to determine the optimum modulation conditions for the chosen pan type, sample and glycerol weights, an underlying heating rate (q_o) of 1 K min^{-1} with 3 K amplitude (A_T) and 2 min period (p) was used for the temperature scanning experiments. Under these experimental conditions thermal lag does not falsify the measured heat capacity or the temperature measurement within 0.5 K. After recording an initial quasi-isotherm at 25 °C for 5 min, temperature scan was performed up to 140 °C. After the end of temperature scan, a second 5 min isotherm at 140 °C was recorded for baseline correction. In the case of any difference in between the initial and final isotherms, a linear correction was performed in such a way that the heat flow signals were reduced to zero for both initial and final isotherms.

From the measured modulated heat flow rates the total specific heat capacity, $c_{p\ total}$, and the reversing specific heat capacity, $c_{p\ reversing}$, were determined according the standard procedures (Wunderlich, Jin, & Boller, 1994). In the case of our study with starch, $c_{p\ total}$ represents the sum of baseline and excess heat capacities, while $c_{p\ reversing}$ is a measure of baseline heat capacity alone. For simplification $c_{p\ reversing}$ is abbreviated by c_p.

The specific heat capacity (c_p) of glycerol was determined separately. Any variation in the weight of the glycerol (±0.1 mg) in sample and reference side was taken into account when calculating the total c_p of starch with 10% (w/w) moisture in excess glycerol. Unless otherwise stated, all the enthalpy and c_p values reported in this study are for WMS (containing ca. 10% moisture) in excess glycerol.

2.5. Quasi-isothermal measurements for determination of rigid amorphous fraction of starch

Fig. 1 shows the temperature program and the parameters of modulation used for quasi-isothermal experiments. Initial 1 K min^{-1} heating up was designed to understand the starch–water–glycerol interactions below gelatinisation temperatures. Non modulated segment of temperature program (20 K min^{-1}) was designed to bring the sample to quasi-isothermal temperature without exposing the samples to higher temperatures

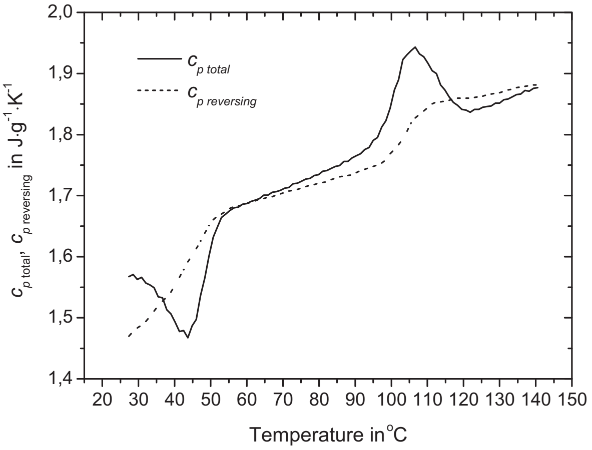

Fig. 2. Total specific heat capacity, $c_{p\ total}$, and reversing specific heat capacity, $c_{p\ reversing}$, of WMS in excess glycerol as revealed by a TMDSC scan (A = 3 K, p = 2 min, q = 1 K min^{-1}, solid line: $c_{p\ total}$, dashed line: $c_{p\ reversing}$).

due to peak modulation temperature. The crystal fraction that was not melted during the 15 h to 10 days quasi-isothermal heat treatment was detected by a modulated temperature scan immediately after the quasi-isothermal heat treatment. From the total area under the peak in the $c_{p\ total}$ curve, the enthalpy of fusion for the remaining ungelatinised WMS was calculated by integration. This enthalpy was compared with the enthalpy required to melt all the crystallites in the starch. In the literature the enthalpy required to melt the crystallites in starch studied by different methods was reported to be between 32 and 36 J g^{-1}. Noel and Ring (1992) investigated the melting behaviour of native and highly crystalline spherulites of A and B-type starches that was prepared under controlled conditions and reported a value of 32 J g^{-1} for the melting enthalpy for A-type crystalline spherulites. Cooke and Gidley (1992) reported that if they extrapolated values of enthalpy obtained from waxy starches to hypothetical starches containing 100% molecular order (double helices by ^{13}C-CP/Mas-NMR), they obtained enthalpy of 33 J g^{-1}. In this study we used the value of 33 J g^{-1} as a reference for calculation of crystalline fraction of WMS.

3. Results

The $c_{p\ total}$, and $c_{p\ reversing}$ of WMS in excess glycerol determined by a modulated temperature scan between 20 °C and 140 °C are shown in Fig. 2. In the presence of glycerol, increased gelatinisation temperature as presented by the endotherm appearing around 106 °C (peak gelatinisation temperature, T_p) is a known phenomenon. Addition of glycerol to starch–water systems is known to elevate starch gelatinisation temperature (Blanshard, 1987; Perry & Donald, 2000; Tan et al., 2004; van Soest, Bezemer, de Wit, & Vliegenthart, 1996).

Fig. 2 shows an exotherm appearing at low temperature (around 45 °C). Similar exothermic peaks for starches in the presence of glycerol were observed by other researchers (Perry & Donald, 2002; van Soest et al., 1996). Perry and Donald (2002) reported that the exotherms were shifting upward on temperature scale and the enthalpy of exothermic peaks was increasing with increasing concentration of glycerol. The authors named these peaks as "self-assembly exotherms". Assuming that the structure of starch was analogous to that of a side chain liquid crystalline polymer, it was proposed that lamellar assembly occurs within the starch granule when the degree of plasticisation-induced by an appropriate combination of solvent and heat-exceeds a certain minimum level to allow smectic-like ordering within the amorphous lamellar regions of the granule.

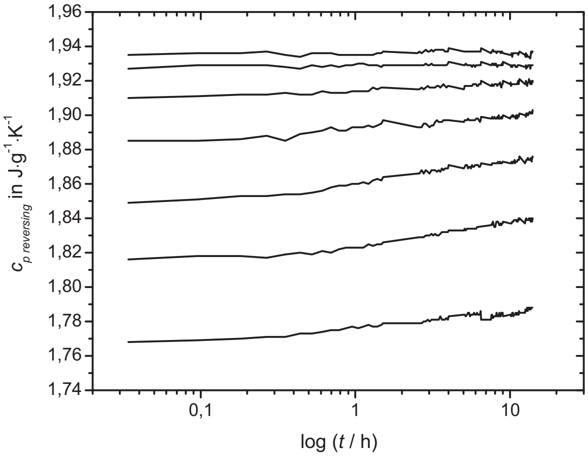

Fig. 3. Reversing specific heat capacity ($c_{p\ reversing}$) of WMS in excess glycerol measured by TMDSC in quasi-isothermal mode (A = 3 K, p = 2 min) for 15 h. Mean temperatures for the curves from bottom to top are 85, 90, 95, 100, 105, 110 and 115 °C, respectively.

As seen in Fig. 2 the c_p is increasing during the exothermic event at about 50 °C where, we believe that the glass transition of MAF is taking place. Second stage of glass transition is observed under the gelatinisation endotherm at about 105 °C where c_p is increasing stepwise, indicating that the solid starch (crystals and RAF) is mobilised as the crystallites melt during gelatinisation. At temperatures above 120 °C we assume that heat capacity is the sum of the heat capacities of liquid starch and all the contributions originating from any interactions between starch, glycerol and water.

3.1. Quasi-isothermal experiments.

In order to study the progress of gelatinisation and to understand the temperature dependent melting of different fractions of starch granule, quasi-isothermal experiments were conducted for 15 h at different temperatures. The c_p changes during 15 h of measurement at different temperatures are shown in Fig. 3. Fig. 4 shows the $c_{p\ total}$ and $c_{p\ reversing}$ data for the remaining ungelatinised fraction of WMS after holding for 15 h at different temperatures which

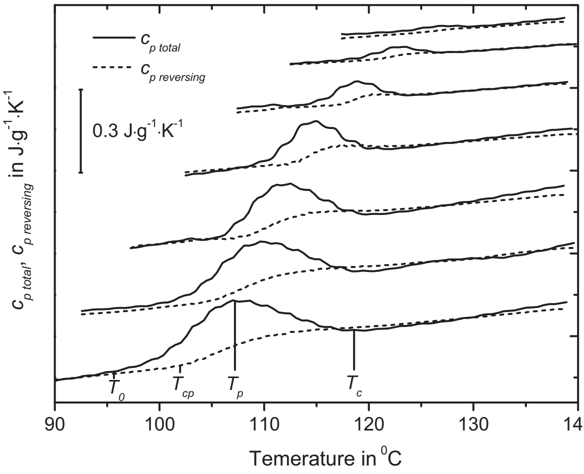

Fig. 4. Total specific heat capacity, $c_{p\ total}$, and reversing specific heat capacity, $c_{p\ reversing}$, of WMS in excess glycerol during temperature scans after holding at different temperatures for 15 h in quasi-isothermal TMDSC mode (A = 3 K, p = 2 min, q = 1 K min^{-1}, solid lines: $c_{p\ total}$, dashed lines: $c_{p\ reversing}$). Curves were transposed vertically, for the sake of clarity. Holding temperatures for 15 h in quasi-isothermal TMDSC mode from bottom to top are 85, 90, 95, 100, 105, 110 and 115 °C, respectively.

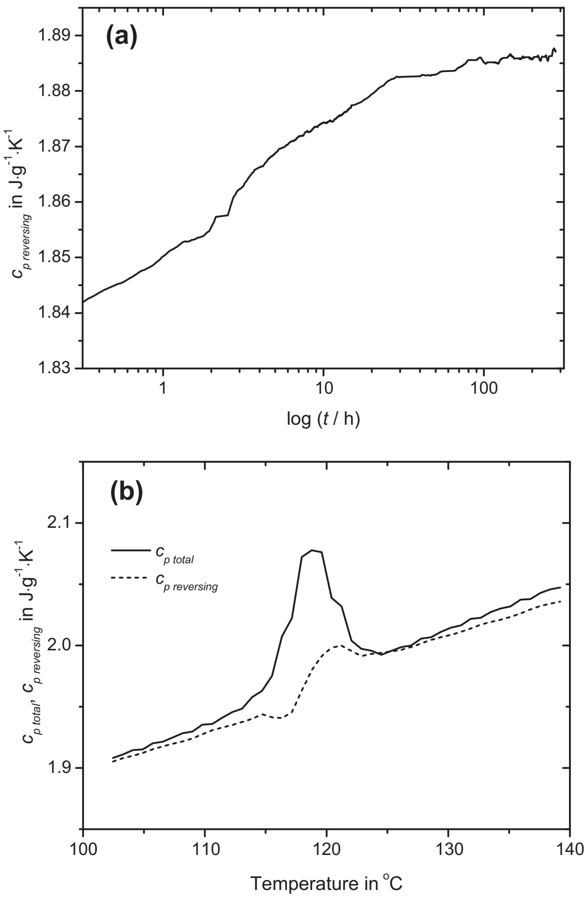

Fig. 5. Specific reversing heat capacity ($c_{p\ reversing}$) of WMS in excess glycerol measured by TMDSC in quasi-isothermal mode ($A = 3$ K, $p = 2$ min) for 10 days at 100 °C (a), total specific heat capacity ($c_{p\ total}$) and reversing specific heat capacity ($c_{p\ reversing}$) measured by a TMDSC scan ($A = 3$ K, $p = 2$ min, $q = 1$ K min^{-1}) from 100 °C to 140 °C after quasi-isothermal heat treatment (b).

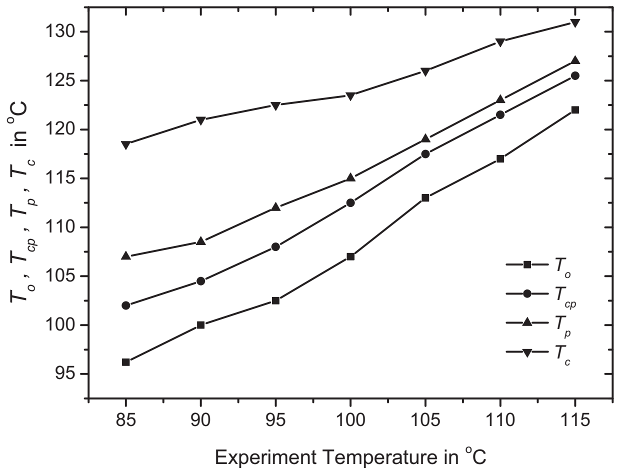

Fig. 6. Onset (T_o), peak (T_p) and conclusion (T_c) temperatures from $c_{p\ total}$ for the remaining ungelatinised starch after 15 h of quasi-isothermal experiments at different temperatures. The temperature where the $c_{p\ reversing}$ starts to increase stepwise is shown as T_{cp}. TMDSC scan at $A = 3$ K, $p = 2$ min, $q = 1$ K min^{-1}. The temperatures T_o, T_p, T_c and T_{cp} are illustrated in Fig. 4 at 85 °C curve.

reveals that even after 15 h heat treatment at 115 °C, some residual crystals left. Considering that peak gelatinisation temperature (T_p) was 106 °C when there was no annealing (Fig. 2), it can be concluded that the most stable crystals are not melting if required energy is not reached through increased temperature. This can also be seen from Fig. 5, where WMS in excess glycerol was hold at 100 °C in quasi-isothermal mode for 10 days. Some residual crystals did not melt during this time, which showed itself as a melting endotherm after a subsequent temperature scan (Fig. 5b).

Comparing gelatinisation onset temperature (T_o) and gelatinisation conclusion temperature (T_c) for the remaining crystals after 10 days at 100 °C (Fig. 5b) with the 15 h treatment at 100 °C (Fig. 6) revealed that the prolonged holding time at 100 °C increased T_o from 107 °C to 113 °C whereas, T_c remained unchanged at about 123 °C.

3.2. Calculation of rigid amorphous fraction of starch

After a complete gelatinisation, it is known that the crystal structure is lost and the amorphous part is undergone glass transition resulting in an overall amorphous structure. When an amorphous and linear polymer is above its glass transition temperature it is considered to be liquid (Sperling, 2006). In this respect, the amorphous part of starch may not be considered as liquid in real sense, but liquid-like. In terms of the c_p change during gelatinisation, both glass transition of the amorphous part and the melting of crystallites respond in the same way (i.e., increased c_p). If the c_p change during gelatinisation is taken as a reference to calculate the liquid and crystalline part of starch during partial gelatinisation, the error involved would be small since both glass transition and melting contributes to c_p in positive way. Based on these assumptions the liquid and crystalline parts of the starch after each quasi-isothermal treatment was calculated by using the measured c_p values and enthalpy required for melting of the remaining WMS crystallites (33 J g^{-1}) as reported in the literature (Cooke & Gidley, 1992). Fig. 7 shows the measured values of c_p before and after quasi-isothermal heat treatment. Extrapolation of c_p values before and after gelatinisation to 90 °C, which is the onset temperature for gelatinisation of WMS in excess glycerol, gave the following parameters as shown in the figure: heat capacity before the beginning of quasi-isothermal heat treatment; c_{p1}, heat capacity after the complete gelatinisation obtained by temperature scan after the quasi-isothermal heat treatment; c_{p2}, and the heat capacity after the quasi-isothermal heat treatment; c_p. Based on these parameters the liquid fraction of

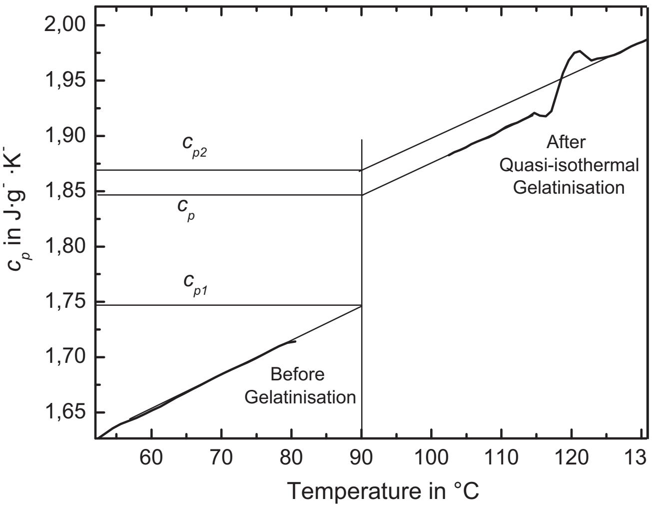

Fig. 7. Reversing specific heat capacities ($c_{p\ reversing}$) before and after a quasi-isothermal experiment at 90 °C showing the c_p values used for the calculation of the liquid fraction of WMS.

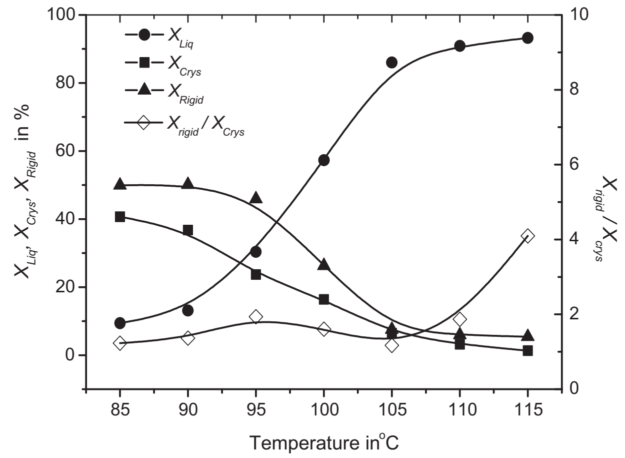

Fig. 8. Liquid, crystal and rigid amorphous fraction of WMS in excess glycerol after holding at different temperatures for 15 h in q-isothermal mode.

WMS after each quasi-isothermal treatment was calculated according to following formula:

$$X_{liq} = \frac{c_p - c_{p1}}{c_{p2} - c_{p1}}$$

where X_{liq} is the liquid fraction. Here we assume that additional contributions to the increase in heat capacity due to interactions between starch and plasticiser (Bocharnikova et al., 2003; Matveev et al., 1998) depend on the solid to liquid transition of the starch only. The additional contribution from the interaction is the same for the RAF to liquid and the crystal to liquid transitions. If this is not the case the value of X_{liq} would slightly change but it would not affect the general conclusions from this paper.

The remaining crystal fraction after quasi-isothermal heat treatment (X_c), was calculated by dividing the enthalpy of melting (in $\mathrm{J\,g^{-1}}$) obtained by the temperature scan after quasi-isothermal heat treatments by $33\,\mathrm{J\,g^{-1}}$, which is the enthalpy required for melting of 100% crystalline amylopectin (Cooke & Gidley, 1992).

If starch was composed of amorphous and crystalline fractions, the addition of X_{liq} and X_c as calculated above should have summed up to unity. However, this was not the case indicating that an intermediate structure existed. We suggested the term “rigid amorphous”, which is well known in the field of synthetic polymers (Schick et al., 2003; Suzuki et al., 1985; Wunderlich, 2003) to describe this fraction of starch. Fig. 8 shows the different fractions of starch after quasi-isothermal treatments at different temperatures.

4. Discussion

Because of the reaction of distilled water with aluminium pans during the long course of measurements (1–15 h) with DSC (unpublished results), glycerol was used as a plasticiser instead of water. Using different ratios of starch:glycerol:water, Perry and Donald (2002) investigated the effect of glycerol on gelatinisation and proposed that, whatever the solute (glycerol) concentration, or whether water is present at all, the basic parameters of gelatinisation remained unchanged, although the endotherm is shifted upward systematically due to relative inability of non-aqueous solutes to ingress and plasticise the starch granule.

The exothermic peak appearing around 45 °C (Fig. 2) is thought to be due to smectic-like organisation of double helices in the presence of a plasticiser or heat. Plasticisation, either due to the presence of plasticising solvent or the input of thermal energy, increases the mobility and entropy of the amylopectin chains within the amorphous lamellar regions of the semicrystalline growth ring. Mobility within these regions allows enthalpically driven assembly of the lamellar structure to be initiated, with amylopectin double helices moving into register, assuming a smectic-like order (Perry & Donald, 2000). Smectic-like organisation of double helices upon annealing in the presence of water was depicted by Tester and Debon (2000). The approach is plausible however, one question remains unanswered. Qualitative investigation of Perry and Donald (2000) data revealed that the enthalpy of the exothermic peak could be as much as 5 times higher than the enthalpy of gelatinisation peak when 100% glycerol is used as a plasticiser. In our case the observed exothermic peak is in similar magnitude of gelatinisation endotherm. This could be due to the fact that the glycerol concentration in the system (DSC pan) was about 96% due to moisture coming from starch and the DSC pans were left for 1 h at room temperature before DSC scans were performed during which, some plasticisation and smectic ordering could have taken place. However, we think that smectic like ordering should take place with lesser enthalpy change compared to melting of crystals during gelatinisation. It is therefore, more likely that the exothermic peak is occurring not only due to smectic-like ordering but there could also be some interaction forces between glycerol and starch polymer as suggested by van Soest et al. (1996).

It would be expected that an ordered structure (smectic) would exhibit a lower heat capacity. However, this is not the case during the exothermic event and the c_p is increasing at a higher rate than the rest of the gelatinisation process. The change in c_p during the exothermic event shown in Fig. 2 has not been recorded in previous studies mentioned above, due to limitations of conventional DSC used without temperature modulation. The increase in c_p during the exothermic event implies that despite of the smectic ordering, the mobility of the overall system is increasing. It is likely that the increase of c_p during the exothermic event is due to the glass transition of the amorphous phase as the ingress of glycerol is facilitated by heat. It has been suggested that lamellar assembly could take place in the presence of a low molecular weight plasticiser without heating, provided that long enough time is given for smectic ordering. However, heating in the presence of a plasticising solvent brings about far more rapid lamellar assembly due to the highly cooperative action of thermal and solvent plasticisation. In case of water acting as a plasticiser, it is proposed that hydration, plasticisation and resulting lamellar assembly and crystallisation occur effectively instantaneously at room temperature, due to the highly effective plasticising ability of water (Perry & Donald, 2000).

Fig. 2 shows that the onset of gelatinisation temperature is about 90 °C whereas, stepwise change in c_p starts at about 98 °C giving a glass transition temperature (T_g) of about 106 °C. This is in contradiction to the theory suggested by some studies (Biliaderis, Page, & Maurice, 1986; Slade & Levine, 1984, 1988) where it is assumed that the glass transition, which is responsible for the heat capacity change, is located at the leading edge of the gelatinisation endotherm. According to this approach, only after the glass transition is complete, the crystalline domains can undergo an irreversible melting process which results in gelatinisation. That is, melting cannot commence unless the characteristic glass transition temperature (T_g) of the glassy region is exceeded. Our results suggest that this is not the case and shows that the processes taking place between onset gelatinisation temperature, T_o (90 °C) and 98 °C does not result in a c_p increase. This was also observed from the experiments designed to reveal rigid amorphous fraction of starch where samples were exposed to quasi-isothermal heat treatment for 15 h at different temperatures and then temperature was increased to gelatinise the remaining crystallites. As seen in Fig. 4 the c_p for the remaining ungelatinised fraction of starch did not increase right after the T_o of ungelatinised starch. It can be seen from Fig. 6 that the temperature for onset of stepwise c_p increase (T_{cp}) was 3–6 °C higher than T_o. By employing a TMDSC, Tan et al. (2004) studied starch–water–glycerol systems and noted that a glass transition (increased c_p) was not a prerequisite for gelatinisation as the c_p change was taking place at higher temperatures than T_o.

Considering that starch is highly heterogonous, partition of water or plasticiser by different fractions of starch would be different. Amorphous growth ring is believed to be the first to absorb water (plasticiser) and swell whereas the intercrystalline amorphous phase (rigid amorphous) is restricted by the crystallites. It is therefore, likely that the glass transition of the amorphous phase is taking place in more than a single step. In excess water we believe that the first glass transition is taking place as soon the water is introduced at room temperatures. Considering the fact that starch can absorb significant amount of water, it would be the amorphous growth ring first to go through a glass transition as the water is absorbed by this part of the starch. That is probably the reason that there is no glass transition right after the onset of the gelatinisation in excess water. In our case (starch–water–glycerol system), we believe that the first glass transition is taking place during the exothermic event between 28 and 55 °C where, smectic-like ordering and other interactions such as breakage of hydrogen bonding between starch–starch molecules and formation of hydrogen bonding between glycerol and starch (Tan et al., 2004) is also taking place. The increase in c_p under the gelatinisation endotherm could be considered as the second step of glass transition where heat capacity increases due to melting of crystals, interactions between starch and plasticiser and mobilisation (glass transition) of the rigid amorphous part as the crystallites melt. Comparison of the magnitudes of c_p increase at first and second stage glass transitions indicates that the glass transition of MAF contributes to c_p increase at a higher rate than that of glass transitions of RAF, which is in line with the published results on synthetic polymers (Suzuki et al., 1985; Wunderlich, 2003).

4.1. Rigid amorphous fraction of starch

The liquid and crystalline fraction calculated through c_p and heat of fusion for amylopectin crystallites revealed that the endotherm recorded after quasi-isothermal heat treatment was not only due to the non-melted crystallites during the previous heat treatment but an amorphous fraction also existed. This amorphous fraction was probably closely associated with intercrystalline lamella and hence was not mobilised before the crystallites melted. By using the terminology of synthetic polymers we call this amorphous phase as rigid amorphous. Even though the term "rigid amorphous" was not used to describe the molecular structure of starch before, studies on starch structure describes similar definitions of rigid amorphous phase as in semi-crystalline synthetic polymers. On investigation of melting transitions of starch–monoglyceride systems, Biliaderis et al. (1986) described the morphology of starch granules and stated that the semicrystalline character of amylopectin arises from the presence of less organised regions of dense branching and compact highly ordered areas (crystallites) of DP 15–20 chains. However, the overall organisation of the molecule is such that it does not permit a sharp demarcation between the crystalline and amorphous regions since other longer chains (DP 50–60) run continuously from one phase to another. Thus, intercrystalline amorphous parts are under strain and therefore it is likely that they do not have any of thermal properties of an idealised amorphous phase (i.e., significant ΔC_p at T_g). Studies performed by SEM, TEM and AFM revealed that the crystalline and amorphous lamellae of the amylopectin are organised into larger more or less spherical structures termed "blocklets" ranging between 20 and 500 nm in diameter. No sharp demarcation between crystalline and amorphous phase of starch was observed (Gallant, Bouchet, & Baldwin, 1997). It has been suggested (Biliaderis et al., 1986) that the thermal behaviour of starch granules should be evaluated on the basis of its morphological and molecular features which could be considered as a three phase system: a fully ordered crystalline phase, a bulk amorphous phase and a nonordered intercrystalline phase. It is also generally believed that some or all the starch molecular chains run continuously from one phase to another creating so-called interphase. The interphase between the crystalline or semicrystalline and the amorphous phase as well as the interorganisation of these two phases is very important for understanding the behaviour of the starch–water (plasticiser) system (Randzio et al., 2002).

It is also worth noting that the heat treatment above T_o in the existence of excess glycerol efficiently created more stable structures similar to the effect of annealing. In the absence of a previous heat treatment, onset (T_o), peak (T_p) and conclusion (T_c) temperatures of gelatinisation endotherm were 90 °C, 106 °C and 122 °C, respectively (Fig. 2). As seen in Fig. 6, 15 h quasi-isothermal heat treatment at 115 °C shifted the onset (T_o), peak (T_p) and conclusion (T_c) temperatures of remaining ungelatinised fraction to 122 °C, 127 °C and 131 °C, respectively. This implies that a fraction of starch having a T_o of as high as 28 °C higher than the original sample could be obtained with a heat treatment of starch in excess glycerol which, could be valuable information for the production of resistant starch.

It should be noted that holding WMS in excess glycerol at 100 °C (10 °C above the onset gelatinisation temperature) for 10 days was not enough to gelatinise the whole WMS implicating that the most stable crystals did not melt before the required threshold temperature was reached.

5. Conclusions

The existence of an intermediate phase between amorphous and crystalline structure called "rigid amorphous phase" was shown by quasi-isothermal scans using a TMDSC.

It was shown that there was no glass transition at the leading edge of the gelatinisation endotherm in excess glycerol. We suggested that the glass transition was taking place in more than a single step. First stage of glass transition was taking place between about 25 and 55 °C in excess glycerol where the amorphous part of starch was mobilised. In case of water is used as plasticiser, this first stage could take place as soon as excess water is introduced in starch at room temperatures. The second stage of glass transition was more to do with the rigid amorphous fraction of starch and was taking place in parallel with gelatinisation as indicated by a stepwise c_p change under the gelatinisation endotherm where the rigid amorphous fraction was mobilised as crystallites melted.

It was also shown that the heat treatment above T_o in the existence of excess glycerol created more stable structures similar to the effect of annealing. Holding WMS in excess glycerol at 100 °C for 10 days was not enough to gelatinise the whole WMS implicating that the most stable crystals did not melt before the required threshold temperature was reached.

Acknowledgements

We would like to thank for the stimulating conversations with Prof. J.R. Mitchell and Drs. I.A. Farhat, B. McNaughton and D. Meissner. We would also like to thank to Nottingham University Food Sciences Division for providing us the WMS samples.

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