Available online at www.sciencedirect.com
ScienceDirect
Carbohydrate Polymers 68 (2007) 495–501
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

Phase transition of starch granules observed by microscope under shearless and shear conditions

Pei Chen [a], Long Yu [a,b,*], Tim Kealy [c], Ling Chen [a], Lin Li [a]

[a] *Center for Polymers From Renewable Resources, School of Light Industry and Food, SCUT, Guangzhou, PR China*
[b] *CSIRO Manufacturing and Materials Technology, Melbourne, Victoria, Australia*
[c] *Rheology Solutions Pty Ltd., Bacchus Marsh, Victoria, Australia*

Received 31 August 2006; received in revised form 2 November 2006; accepted 7 November 2006
Available online 11 December 2006

Abstract

The phase transitions of starch granules of cornstarches with different amylose/amylopectin contents (waxy: 0/100; maize: 23/77; Gelose 50: 50/50; Gelose 80: 80/20) were systematically studied under shearless and shear conditions. A microscope with a hot-stage was used to observe phase transition under shearless conditions during heating and isothermally at different temperatures. Phase transition under shear conditions was investigated using a rheoscope – a controlled rate/controlled stress rheometer with a built-in high-resolution video camera. An increase in starch granule diameter, disappearance of birefringence and granule disappearing were used to describe the phase transition. The diameter growth rate and final accretion ratio sequence of starch granules during heating under shearless conditions was waxy > maize > G50 > G80, which correspond with amylose/amylopectin ratio. The growth rate of granular diameter under shear conditions was controlled by two factors: swelling and dissolving. Starch granules were destroyed under shear conditions, but some could still be identified under shearless conditions up to about 100 °C. Granules totally disappeared in a very short time after gelatinization under shear stress conditions. Increased amylose content led to a lower granule diameter growth rate and accretion ratio, and a higher temperature before birefringence disappeared. It was found that the higher the melting temperature of the amylose–lipid complex, the higher the gelatinization temperature, and the amylose detected by differential scanning calorimetry in the high-amylose starches is proposed as an explanation of the results observed by microscope.

Keywords: Gelatinization; Cornstarch; Microscope observation; Amylose; Amylopectin; Shear

1. Introduction

It is well known that an order–disorder phase transition occurs when starch granules are heated in the presence of water. When sufficient water is present, this transition, referred to as "gelatinization", results in near-solubilization of the starch (Donovan, 1979; French, 1984; Lelievre, 1974). The concomitant changes of measurable properties such as viscosity, heat uptake, crystallinity and size variation of starch granules, have been used to detect the extent of starch gelatinization. The technologies developed include microscopic observation (Ghiasi, Hoseney, & Varriano-Marston, 1982; Olkku & Rha, 1978; Tester & Morrison, 1992; Yeh & Li, 1996; Ziegler et al., 1993), differential scanning calorimetry (DSC) (Liu, Yu, Xie, & Chen, 2006; Stevens & Elton, 1971; Yu & Christie, 2001), and X-ray diffraction (XRD) (Cameron & Donald, 1993; Cooke & Gidley, 1992; Jenkins et al., 1994). Each technology has its advantages and disadvantages. Direct comparison may not have real meaning, since the detected physical parameters are different, but the various results obtained can be used to support each other, especially when studying gelatinization mechanisms. For example, Liu, Lelievre, and Ayoung–Chee (1991) studied starch gelatinization using DSC, X-ray and birefringence measurement. They found that a decrease in crystallinity occurred both before the birefringence of granules started to disappear and after all birefringence was lost. Yeh and Li (1996) studied the swelling of rice starch and reported a

* Corresponding author. Tel.: +61 3 9545 2797; fax: +61 3 9544 1128.
E-mail address: long.yu@csiro.au (L. Yu).

0144-8617/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2006.11.002

coincidental result between microscopic observations and DSC measurements. It should be noted that all of these technologies were used only to detect phase transition under shearless conditions. Variation of viscosity could be used to detect gelatinization under shear conditions. However, previous studies have mainly focused on the viscosity of gelatinized products (Freeman & Verr, 1972; Zobel, 1984).

The advantage of microscopy is the ability to directly observe simultaneous granular swelling and crystallinity disappearance. The technical development of digital image analysis makes quantitative microscopy more convenient and accurate. Currently, the combination of a microscope and a high-end rheometer, i.e., the rheoscope, is the only way to directly observe phase transition under shear conditions (Yu, Kealy, & Chen, 2006).

Cornstarch has particular attraction for scientific research since multi-endotherms have been detected at different water contents, and different amylose/amylopectin contents have been found in natural resources. In a previous paper (Liu et al., 2006), we reported on the gelatinization of cornstarches with different amylose/amylopectin contents, studied using a DSC with a stainless steel high-pressure pan. It was found that the number of endotherms, and the temperature and enthalpy of gelatinization, depend on the amylose/amylopectin content. However, the DSC thermograms did not explore the detailed progress of the phase transition, such as granular swelling and crystal structure destruction, and the gelatinization detected by DSC was limited under shearless conditions. In this work, cornstarches with different amylose/amylopectin contents (waxy: 0/100; corn: 23/77; Gelose 50: 50/50; Gelose 80: 80/20) were systematically studied under shearless and shear conditions using a microscope with a hot-stage, and a rheoscope. The observed results combined with DSC measurement were used to investigate the mechanism of starch gelatinization under shear and shearless conditions.

2. Experimental

2.1. Materials

Cornstarches with different amylose/amylopectin contents were used in the experimental work. Table 1 shows details of the starches studied. Gelose 50 and Gelose 80 had amylose contents of 50% and 80%, respectively. All the starches were commercially available. Most of starches (waxy-1, maize-1, G50, G80) were supplied by Penford (Australia), while waxy-2 and maize-2 were obtained from China. An infra-red heating balance (Model DHS-20) was used to measure moisture content through heating samples to 110 °C for 20 min.

Table 1
List of starches studied and their characterization

Starch	Amylose content (%)	Molecular weight (g/mol)[a]	Average size (μm)	Crystallinity (%)[b]
Waxy-1	0	20,787,000	12.1	38.8
Waxy-2	0	–	12.3	37.4
Maize-1	26	13,000,000	10.9	27.9
Maize-2	23	–	11.1	26.7
G50	50	5,115,000	9.6	17.4
G80	80	673,000	8.1	16.7

[a] Molecular weight measured by GPC [provided by Penford (Australia)].
[b] Measured by wide-angle X-ray diffraction.

2.2. Microscope with hot-stage

A polarization microscope (Axioskop 40 Pol/40 A Pol, ZEISS) equipped with a 35 mm SLA camera was used in the experimental work. A hot-stage (CI94, Linkam Scientific Instruments Ltd.) thermosystem was used in combination with the microscope system. In this work the magnification was 500 × (50 × 10).

Both normal and polarized light were used to investigate the phase transition of the starches. Suspensions with 0.5% starch were prepared to study their phase transition. A certain mass of a starch sample was weighed then put into a glass vial, followed by the addition of 25 μL of water using a microsyringe. The total moisture content in the suspension was taken as the original moisture content in the starch together with the added water. The starch suspension was sealed between two microscope glass slides using silicon adhesive. Each specimen was heated from room temperature to 105 °C at 2 °C/min. The camera interval timer was set as 30 s so that an image was captured at each 1 °C temperature increase. Each field was photographed under normal and polarized light.

The measurement of diameter variations of starch granules during heating or isothermally at a certain temperature was conducted using the Gun Image Manipulation Program. Images were continuously recorded during heating. The diameter of a granule was calculated using a sphere equal in area to the image. The accretion ration of starch swelling is calculated by $AR = (D_t - D_0)/D_0$, where D_0 and D_t represent the diameter of starch granule at initial and at a specific time, respectively.

2.3. Rheoscope

A Thermo Haake rheoscope was used in the experimental work to study gelatinization under shear conditions. Fig. 1 shows a schematic representation of the rheoscope, which comprises a controlled rate/controlled stress rheometer, with a 70 mm diameter 1° polished cone and plate measuring geometry. It also contains a built-in, high-resolution, black and white video camera with a microscope lens and light source.

Water at 0.2% w/w of each sample was used as a solvent. The plate and cone were heated to each set temperature, and the cone was allowed to remain in contact with the lower plate during this time to minimize equilibration time when the sample was added. Standard steady shear rheometric experimental techniques were followed to generate a viscosity–time profile, and the camera collected a corresponding image for each of the viscosity data. The

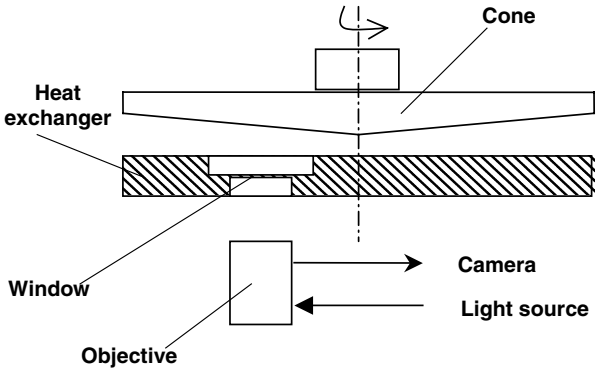

Fig. 1. Schematic representation of the rheoscope. The rotation of the cone produced steady shear and the heat exchanger controlled the measurement temperature.

suspensions were heated from room temperature to 95 °C at 2 °C/min. If the outer edge of the plate and cone were exposed, the water evaporated significantly when the temperature was above 90 °C (the highest temperature used in the rheoscope experimental work was 95 °C). To mitigate this issue, a small amount of low-viscosity (2 mPa·s) silicone oil was pipetted around the exposed edge. The rheoscope images showed no evidence of oil droplets in the samples, and there was no evidence of evaporation during the experiments.

2.4. Differential scanning calorimetry

A Perkin-Elmer DSC Diamond-I with an internal coolant (Intercooler 1P) and nitrogen purge gas was used in the experimental work to confirm the gelatinization behaviors. The melting temperature and enthalpies of indium were used for temperature and heat capacity calibration. High-pressure stainless steel pans (PE No. B0182901 with a gold-plated copper seal (PE No. 042-191758) were used to study the thermal behaviors up to 300 °C with high moisture content. The slow heating rate of 2 °C/min was used to match the observations under the microscope with the hot-stage, and to minimize any temperature lag due to the large mass of the samples.

2.5. Scanning electron microscopy (SEM)

SEM (Philips X230) was used to investigate the appearance and surface details of the starches, and their internal morphologies. The surfaces of the samples were coated with

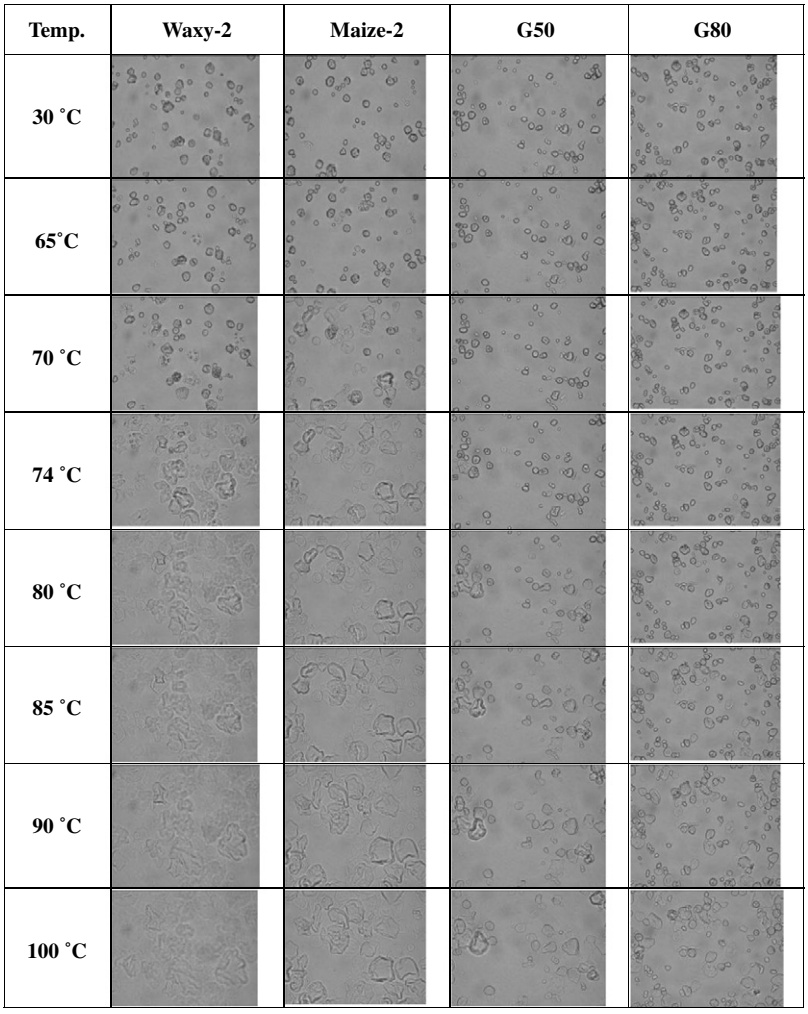

Fig. 2. Microscope images of different starches at different temperatures under normal light.

carbon. A lower voltage of 2 K was used in this part of the experimental work to avoid damaging the surfaces.

3. Results and discussion

The phase transition of starches continuously heated under shearless conditions was studied using a microscope with a hot-stage. Variations in granular morphologies and birefringence during heating were recorded automatically every 30 s. Figs. 2 and 3 show some microscope images taken at different temperatures, collected under normal and polarized light, respectively, for different starches heated at 2 °C/min. The images collected at 30 °C represent the initial morphologies of the different starches. It can be seen that all the granules appear as spherule particles and semi-crystalline black crosses under polarized light. The birefringence sequence of the granules was waxy > maize > G50 > G80. The granular size sequence was waxy > maize > G50 > G80. The phase transition observed under microscope was nonlinear and the images in Figs. 2 and 3 mainly represent the variation points. It can be seen that the diameter of granules increased with increasing temperature for all starches, and birefringence of all the starches disappeared with increasing temperature. These behaviors are expected and have been widely reported. It is important to note that the temperatures at which birefringence disappeared and the accretion ratios are significantly different for the different starches, with lower temperatures of birefringence disappearance and higher accretion ratios being recorded for the amylopectin richer starches. Table 2 lists the observed initial and final temperatures of starch granular swelling and birefringence disappearance for different starches, and their final accretion ratio. An important phenomenon is that the final morphologies of the different starches were significantly different. The granules of the waxy and maize starches were destroyed at about 75 and 85 °C, respectively. The granules of G50 and G80 remained as spherule up to 100 °C, which was the maximum experimental temperature.

Fig. 4 shows the diameter variation measured during heating of the different starch granules. The growth rates and final accretion ratios for the different starches were significantly different. The granular growth rate was nonlinear and all materials exhibited a sharp shoulder, with the amylopectin richer starches having the narrower shoulders. For

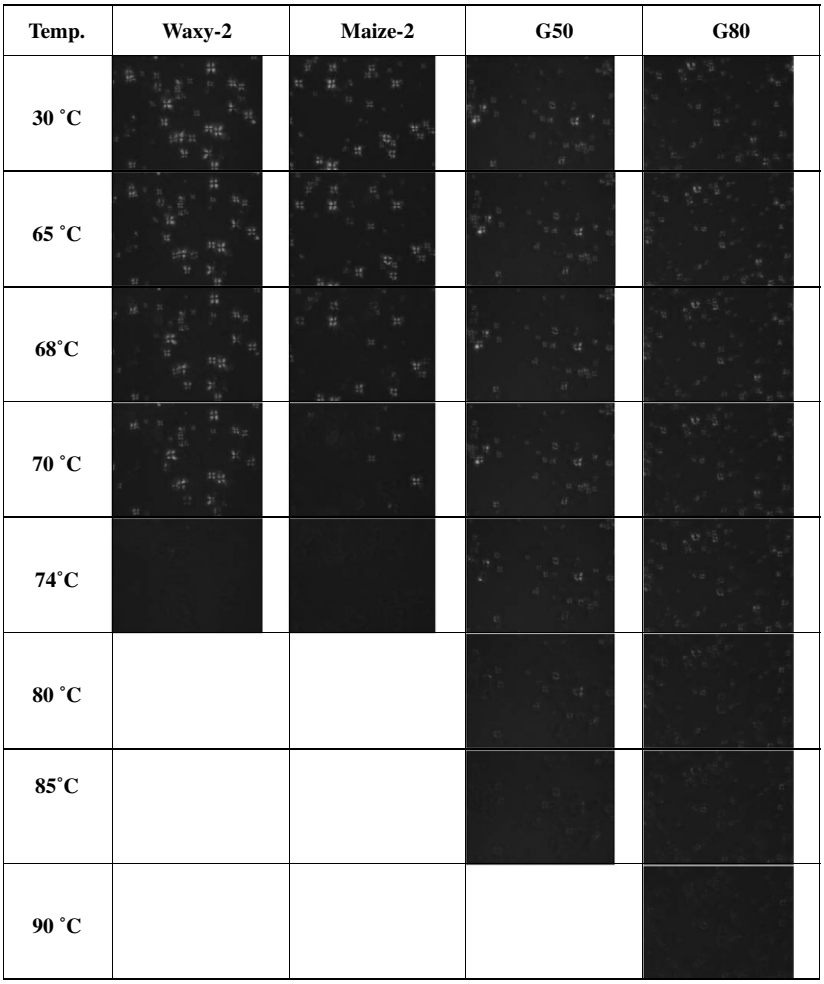

Fig. 3. Microscope images of different starches at different temperatures under polarized light.

Table 2
Observed variation in different starch granules under shearless conditions

Starch	Initial temperature (°C)	Destruction temperature (°C)	Accretion ratio (%)	Birefringence variation detected	
				Initial temperature (°C)	End temperature (°C)
Waxy-1	64	75	190	64	73
Waxy-2	63	75	197	60	72
Maize-1	66	80	151	64	70
Maize-2	66	82	173	65	74
G50	71	>100	77	73	85
G80	74	>100	43	75	90

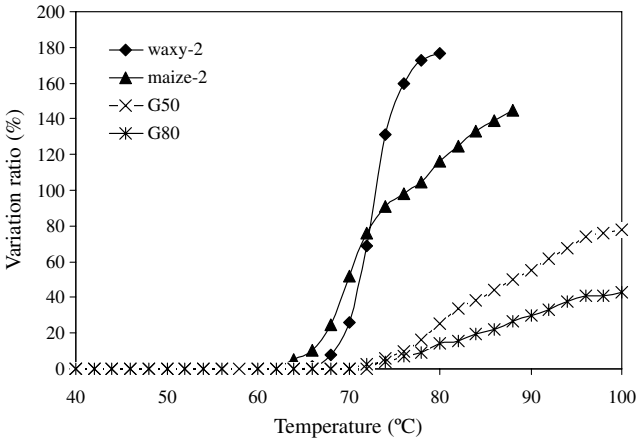

Fig. 4. Diameter variation measured by microscope during heating of different starches.

example, the diameter of the waxy-1 starch increased only slightly when the temperature was below 70 °C. The growth rate increased sharply in the temperature range of 70–75 °C, then the increase rate slowed significantly. The growth rate of G80 was much smoother and continued to the maximum experimental temperature of 100 °C. These phenomena correspond with other measurement techniques such as DSC (Liu et al., 2006; Yu & Christie, 2001). The sequence of diameter increase rate and final accretion ratio of the starch granules during heating was waxy > maize > G50 > G80 in the temperature range used in this experimental work. The disappearance of birefringence of the starches showed similar patterns, but the initial and final temperatures were different. Generally, the temperature variation range of birefringence was much narrower than that of diameter. The results also indicated that during heating, the loss of crystalline structure occurred at lower temperatures than the loss of molecular order for all starch samples. This was expected, since starch granules continuously swell even after the crystalline structure of the starch granules has been destroyed. Liu et al. (1991) has reported that a decrease in crystallinity occurred both before the birefringence of granules started to disappear and after all birefringence was lost.

Fig. 5 shows selected images taken by the rheoscope during heating (2 °C/min) under shear stress for different starches. It can be seen that the particles of the waxy and maize starches remained intact up to about 75 °C, and that above this temperature, they were completely destroyed and disappeared in a narrow temperature range. The granules of the amylose-rich starch G80 remained spherule at the highest experiment temperature of about 100 °C. It should be noted that the granular growth rates and final accretion ratios under shear stress were lower than those under shearless conditions for the amylopectin-rich starches (see Tables 2 and 3) but the final accretion ratio of G80 is higher. An explanation for this is that as the starch granules increased in size, the outside layer of the starch particles dissolved in water due to shear stress. One important effect observed by comparing successive images during the experiments, was that all of the starch systems tested displayed a distinct process of dissolution. At first the particle size increased due to the ingress of moisture through the amorphous region. This swelling was accompanied by a reduction in the disparity or definition of the granular epidermis, which one would intuitively suspect to be due to stretching caused by expansion and softening as a result of the elevated temperature. These phenomena are followed by partial or complete dissolution during particle swelling. Some cloudiness was observed around the starch particles in the suspension before total dissolution, which was attributed to the dissolved parts of the starch particles. The starch G80 has not fully swelled at the highest temperature and the granules have not started to dissolve in this experimental work under both shearless and shear conditions. The shear stress enhanced the swelling resulting in the higher of final accretion ratio.

Fig. 6 shows the gelatinization endotherms of various starches measured by DSC. It can be seen that the waxy and maize starches exhibited a large gelatinization endotherm at about 70 °C. This endotherm has been well accepted as representing the gelatinization of amylopectin, and is labeled "G" in Fig. 6 (Donovan, 1979; Evans & Haisman, 1982; Liu et al., 2006; Russel, 1987; Zobel, 1984). Apart from the G endotherm, a second endotherm was detected for maize starch at about 90 °C, and this has been recognized as the phase transition within an amylose–lipid complex, and is labeled "M2" in Fig. 6 (Biliaderis, Page, Slade, & Sirett, 1985; Jovanovich & Añón, 1999; Liu et al., 2006; Raphaelides & Karkalas, 1988). The thermal transitions of the high-amylose content starches (G50 and G80) were not as sharp as those of the normal and

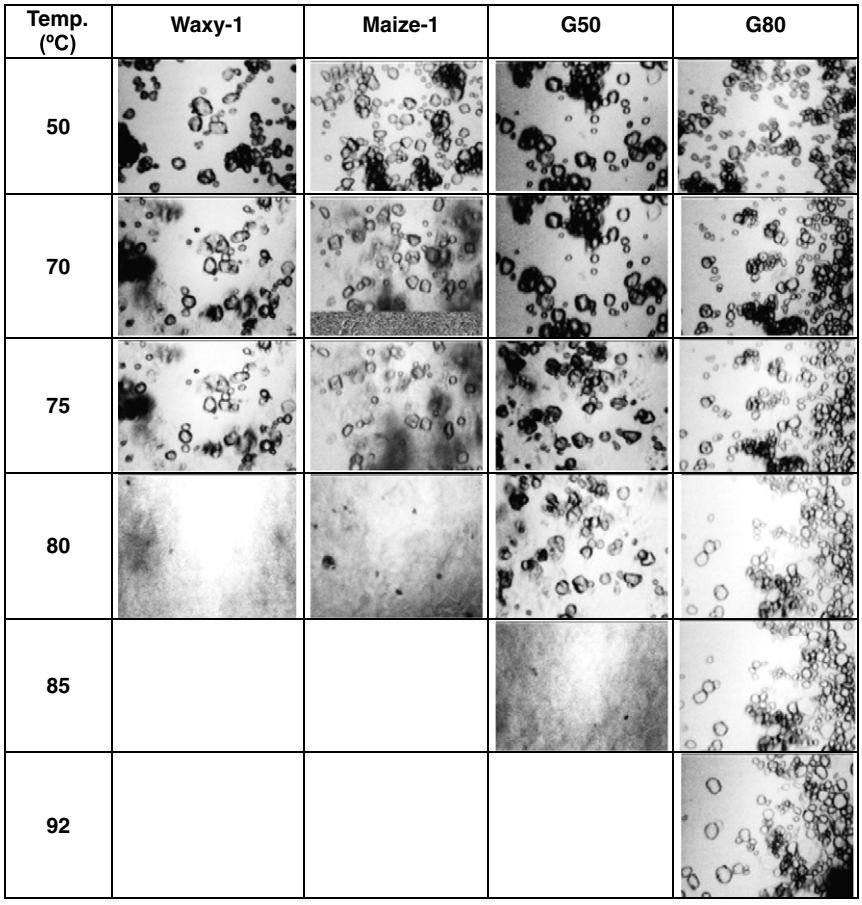

Fig. 5. Rheoscope images of different starches during heating (2 °C/min) under shear stress conditions.

Table 3
Observed size variation in different starch granules under shear conditions

Starch	Initial temperature (°C)	Destruction temperature (°C)	Accretion ratio (%)
Waxy-1	60	80	110.2
Maize-1	65	80	89.7
G50	70	85	73.8
G80	75	>95	96.7

amylopectin-rich starches; instead very broad endotherms were obtained in the temperature range of 65–115 °C. This broad peak is a composite of gelatinization G and a phase transition M2. Apart from this broad endotherm, a small endotherm was also detected at about 155 °C for G80 (labeled "M3"), which is considered as the melting temperature of amylase (Liu et al., 2006). The DSC results can be used to explain the phenomena observed under the microscope, even though the measurement conditions were different. The lower gelatinization temperature of the amylopectin-rich starches detected by DSC corresponded with a lower temperature of birefringence disappearance and a faster growth rate of starch granules. The broader endotherm of amylose-rich starches detected by DSC corresponded with the broader temperature range of birefringence disappearance observed under the microscope (see Fig. 3). The amylase for G80 at 155 °C can be used to explain why it remained undissolved.

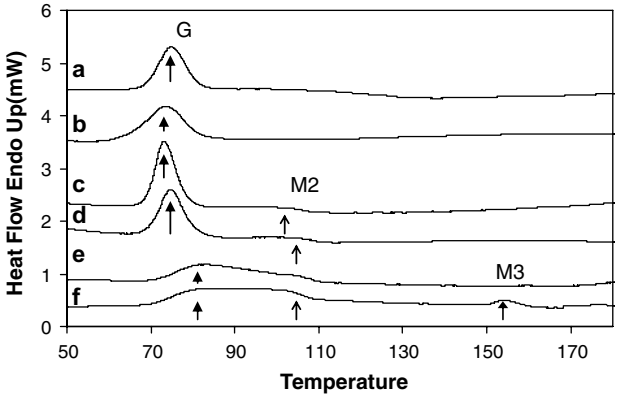

Fig. 6. DSC gelatinization endotherms of different starches with excess water (70%): (a) waxy-1; (b) waxy-2; (c) maize-1; (d) maize-2; (e) G50; and (f) G80.

4. Conclusions

The phase transition of granules of cornstarch with different amylose/amylopectin contents (waxy: 0/100; maize: 23/77; Gelose 50: 50/50; Gelose 80: 80/20) were systematically studied under shearless and shear conditions. The phase transition of starch granules under shear stress conditions was observed for the first time. The growth in starch granule diameter and the disappearance of

birefringence and particle were used to describe the phase transition.

The diameter growth rate and final accretion ratio sequence of starch granules during heating under shearless conditions was waxy > maize > G50 > G80 in the temperature range used in this experimental work. The disappearance of birefringence showed a similar variation pattern, but the initial and final temperatures were different. Generally, the temperature variation range of birefringence disappearance was much narrower than that of diameter growth rate. The results also indicated that during heating, loss of crystalline structure occurred at lower temperatures than loss of molecular order for all starch samples.

The growth rate of granular diameter under shear conditions was controlled by two factors: swelling and dissolving. Starch granules were destroyed under shear conditions, but some could still be identified under shearless conditions up to about 100 °C. The granules totally disappeared in a very short time after gelatinization under shear stress conditions.

It was found that the higher the gelatinization temperature, the higher the melting temperature of the amylose–lipid complex, and the amylose detected by DSC in the high-amylose starch is proposed as an explanation for the results observed by microscope.

Acknowledgement

The authors from SCUT, China, would like to acknowledge the research funds NFSC (50540420129), DHE (104148) and GNSF (No. 05200617). The equipment and time contributions from Rheology Solutions Pty Ltd. are also gratefully acknowledged.

References

Biliaderis, C. G., Page, C. M., Slade, L., & Sirett, R. R. (1985). Thermal behavior of amylose–lipid complexes. *Carbohydrate Polymers, 5*, 367–389.

Cameron, R. E., & Donald, A. M. (1993). A samll-angle X-ray scattering study of the absorption of water into the starch granule. *Carbohydrate Research, 244*, 225–236.

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

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

Evans, I. D., & Haisman, D. R. (1982). The effect of solutes on the gelatinization temperature range of potato starch. *Starch/Stärke, 34*, 224–231.

Freeman, J. E., & Verr, W. J. (1972). *Cereal Science Today, 17*(2), 46.

French, D. (1984). Organization of starch granules. In R. L. Whister, J. N. BeMiller, & E. F. Paschall (Eds.), *Starch Chemistry and Technology* (2nd ed., pp. 183–247). Orlando, FL: Academic Press Inc..

Ghiasi, K., Hoseney, R. C., & Varriano-Marston, E. (1982). Gelatinization of wheat starch: 3 – Comparison by differential scanning calorimetry and light microscopy. *Cereal Chemistry, 59*, 258–262.

Jenkins, P. J., Cameron, R. E., et al. (1994). In situ simultaneous small and wide angle X-ray scattering: a new technique to study starch gelatinization. *Journal of Polymers Science: Part B – Polymer Physics, 32*, 1579–1583.

Jovanovich, G., & Añón, M. C. (1999). Amylose–lipid complex dissociation: a study of the kinetic parameters. *Biopolymers, 49*, 81–89.

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

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

Liu, H., Yu, L., Xie, F. W., & Chen, L. (2006). Gelatinization of cornstarch with different amylase/amylopectin content. *Carbohydrate Polymers, 65*, 357–363.

Olkku, J., & Rha, C. (1978). Gelatinization of starch and wheat flour starch – a review. *Food Chemistry, 3*, 293.

Raphaelides, S., & Karkalas, J. (1988). Thermal dissociation of amylose–fatty acid complexes. *Carbohydrate Research, 172*, 65–82.

Russel, L. P. (1987). Gelatinisation of starches of different amylose/amylopectin content: a study by differential scanning calorimetry. *Journal of Cereal Science, 6*, 133–145.

Stevens, D. J., & Elton, G. A. H. (1971). Thermal properties of the starch water system. *Starch/Stärke, 23*, 8–11.

Tester, R. F., & Morrison, W. R. (1992). Swelling and gelatinization of cereal starches: III – Some properties of waxy and normal, nonwaxy barley starches. *Cereal Chemistry, 69*, 654–658.

Yeh, A., & Li, J.-Y. (1996). A continuous measurement of swelling of rice starch during heating. *Journal of Cereal Science, 23*, 277–283.

Yu, L., & Christie, G. (2001). Measurement of starch thermal transitions using differential scanning calorimetry. *Carbohydrate Polymers, 46*(2), 179–184.

Yu, L., Kealy, T., & Chen, P. (2006). Study of starch gelatinization in a flow field using simultaneous rheometric data collection and microscopic observation. *International Polymer Processing, 3*, 283–289.

Ziegler, G. R., Thompson, D. B., et al. (1993). Dynamic measurement of. starch granule swelling. *Cereal Chemistry, 70*, 247–251.

Zobel, H. F. (1984). Gelatinization of starch and mechanical properties of starch pastes. In R. L. Whistler, J. N. BeMiller, & B. F. Paschall (Eds.), *Starch: Chemistry and Technology (chapter IX.)*. New York: Academic Press.