

Effect of high-pressure treatment on normal rice and waxy rice starch-in-water suspensions

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Abstract

The effects of treatment pressure (≤ 700 MPa), temperature at treatment (10–60 °C), and treatment duration (0–30 min) on the gelatinization of normal and waxy rice starches were investigated. Pressure-treated starch suspensions were examined for pasting behaviour, initial apparent viscosity (η_{initial}), degree of swelling, birefringence changes, and leaching of starch and amylose. The η_{initial} measurements provided an objective and analytical means of determining the degree of pressure-induced gelatinization of starch. Both normal and waxy rice starches exhibited sigmoidal-shaped pressure-induced gelatinization curves. The degree of gelatinization was dependent on the type of starch, the pressure, the temperature, and the duration of treatment. Different combinations of these factors could result in the same degree of gelatinization. There was a linear correlation between the degree of swelling and η_{initial} . After treatments at ≥ 500 MPa, both starches lost all birefringence although they experienced different extents of change in η_{initial} and the degree of swelling.

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1. Introduction

Starch is a major food reserve substance in plants, and occurs in discrete granules. Starch consists of two biopolymers: an essentially linear polysaccharide called amylose and a highly branched polysaccharide called amylopectin (Parker & Ring, 2001). Amylose and crystalline amylopectin are organized into alternating radial layers to form the mechanical structure of starch granules (Parker & Ring, 2001). Applications of starch in food products often involve its gelatinization for functional and nutritional properties. Starch gelatinization is defined as the disruption of molecular orders within the starch granule, manifested in irreversible changes in properties such as granular swelling, native crystallite melting, loss of birefringence, and starch solubilization (Atwell, Hood, Lineback, Varriano-

marston, & Zobel, 1988). Although heating starch in the presence of water is a common method of inducing gelatinization, high-pressure treatment of starch can also induce its gelatinization (Katopo, Song, & Jane, 2002; Stute, Klingler, Boguslawski, Eshtiaghi, & Knorr, 1996).

High-pressure treatment, among other non-thermal technologies, is gaining interest in the food industry. For example, high-pressure treatment may provide a preservative technique that can satisfy the consumer demands for ‘fresh-like’ products while maintaining shelf life. High-pressure treatment causes disordering of biopolymers, including proteins and starch, as it modifies non-covalent intermolecular interactions (Balny, 2002). The pressure-induced disordering is similar to heat-induced disordering, but not identical (Balny, 2002). For starches, this disordering results in pressure-induced gelatinization. Understanding pressure-induced gelatinization of starch is, therefore, vital for applications of high-pressure treatment in starch-containing products in order to understand and achieve the desired product functionality.

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High-pressure-induced starch gelatinization has been investigated in a number of studies in recent years (Katopo et al., 2002; Stute et al., 1996). The pressure range in which the gelatinization occurs depends on the type of starch. For instance, gelatinization of wheat starch begins below 300 MPa and is achieved completely at 600 MPa (Douzals, Marechal, Coquille, & Gervais, 1996). In contrast, the treatment pressure needs to be at least 600 MPa for potato starch to start to gelatinize (Bauer & Knorr, 2005). The findings of Stute et al. (1996) suggest that, for all starches, the characteristics of pressure-induced gelatinization can be different from those of heat-induced gelatinization. The authors showed that some starches, including normal corn starch, did not swell under pressure as much as they did during thermal gelatinization. In addition, Douzals, Cornet, Gervais, and Coquille (1998) reported that pressure-induced starch gelatinization resulted in a lower release of amylose compared with that from heat-induced gelatinization.

In this study, the effects of different treatment pressures, temperatures at pressurization, and treatment durations on normal and waxy rice starch suspensions were investigated. Pressure-treated starch suspensions were analyzed for pasting profile, initial apparent viscosity, degree of swelling, birefringence changes, and leached starch and amylose to explore different aspects of high-pressure-induced gelatinization. Pasting profiles and initial apparent viscosity provided information on physical changes that indicate the degree of gelatinization. The information gathered from the rheological measurements was related to the other analyses results to characterize the high-pressure-induced gelatinization of each starch. The observed differences and similarities in the behaviour of normal and waxy rice starches are compared and discussed.

2. Materials and methods

2.1. Materials

Unmodified normal rice starch (12% moisture, 0.09% fat, 0.13% protein, 0.06% ash) and waxy rice starch (11% moisture, 0.07% fat, 0.06% protein, 0.08% ash) were supplied by Remy Industries (Leuven-Wijgmaal, Belgium) and were used as supplied. The starches were stored in air-tight containers. A Megazyme amylose/amylopectin assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) was used for the analysis of leached starch and amylose.

2.2. Preparation of starch suspensions

Starch was dispersed in purified water (reverse osmosis followed by filtration through a Milli-Q apparatus) by stirring at room temperature ($\sim 20^\circ\text{C}$) to produce starch suspensions with a final concentration of 10% (w/w). Sodium azide (0.02%, w/v) was added to all samples as a preservative. Beckman Polyallomer centrifuge tubes

(13 mm internal diameter \times 51 mm high, or 16 \times 76 mm, Beckman Instruments, Inc., Spinco Division, Palo Alto, CA, USA) were used to hold the samples for high-pressure treatment. Once the centrifuge tubes had been filled with sample, the tubes were heat sealed.

2.3. High-pressure treatment

Pressure treatments of samples were conducted using a laboratory-scale high-pressure unit (Food-Lab, model S-FL-850-9-W, Stansted Fluid Power Ltd., Stansted, Essex, UK). Various treatment conditions were used: pressures ranged between 100 and 700 MPa, treatment durations ranged from 0 to 30 min, and temperatures at pressurization were between 10 and 60°C . The samples were equilibrated to the pressure treatment temperature in a water bath for 20 min before treatment commenced. The 65 \times 220 mm cylindrical high-pressure chamber was filled with a pressure-transmitting fluid consisting of an emulsion of 10% vegetable oil in water with small amounts of Tween 80, Span 60, and potassium sorbate. Control samples were prepared and kept in a water bath at the set pressurization temperature for the duration of the relevant pressure treatment.

The pressurization rate was 4.4 MPa/s and the depressurization rate was 9.2 MPa/s. The average adiabatic heating during pressurization was $\sim 1.9^\circ\text{C}/100\text{ MPa}$. The cooling rate during depressurization was $\sim 2.2^\circ\text{C}/100\text{ MPa}$. Samples from three separate runs with identical set conditions were collected to produce enough volume for analyses. The samples were transferred into storage containers after depressurization. Any sediment was mixed carefully by hand with the rest of the sample to ensure sample homogeneity. Lids were placed on the sample containers and the samples were held at ambient temperature (20°C) overnight ($\sim 10\text{ h}$) before analysis.

2.4. Rheological properties

The rheological properties of the samples were analyzed using a stress-controlled rheometer, the Physica UDS200 rheometer (Anton Paar GmbH, Graz, Austria) equipped with a starch cell and stirrer arrangement (C-ETD 160/ST). The starch cell was filled with 22 mL of sample and the contents were stirred at 100 rev/min for 1 min at 20°C before pasting. The pasting procedure entailed measuring the viscosity of the sample while increasing the temperature from 20 to 95°C at a constant rate of $2^\circ\text{C}/\text{min}$ with a constant rotational speed of 100 rev/min. The viscosity was measured at 30 s intervals. This experiment was carried out in duplicate for all samples.

2.5. Degree of swelling

A simple centrifugation technique, modified from that developed by Hemar and Horne (1998), was used to examine the degree of swelling of the starch granules. Glass capillary tubes (75 mm long) were filled with sample, leaving

about 10 mm of the tube void so that the sample was not overheated when sealing the end of the tube with a bunsen flame. After sealing, the tubes were placed into a Haemofuge centrifuge (Heraeus-Christ, Hanau, Germany), sealed ends to the outer rim, and centrifuged at 12,000 rev/min for 10 min at ambient temperature. Magnified images of the centrifuged tubes were obtained by scanning the tubes using a scanner (hp Scanjet 5590, Hewlett-Packard Development Company, USA). The degree of swelling was calculated using Eq. (1):

$$\text{Degree of swelling (\%)} = \frac{\text{Height of centrifuged sediment}}{\text{Height of sample}} \times 100 \quad (1)$$

Three tubes were analyzed for each sample.

2.6. Light microscopy

An aliquot of each sample was put on to a glass slide and a cover slip was placed on top of the sample for microscopic examination. A polarizing light microscope (Nikon Eclipse E600 Pol, Nikon Corporation, Tokyo, Japan) with a 50× objective was used to observe birefringence of the starch granules. The microscope was also used without the polarizing filter to observe the appearance of the sample.

2.7. Total starch and amylose assay

The assay procedure developed by Gibson, Solah, and McCleary (1997) was followed to measure the amounts of amylose and total starch leached from starch granules. The solution phase of the sample was first separated by centrifugation. A sub-sample of the aqueous phase (8 g) was transferred into a 10 mL centrifuge tube and centrifuged at ~4000 rev/min (2000g) for 10 min in a Mistral 2000 centrifuge (MSE (UK) Ltd., London, UK). The supernatant was weighed and freeze dried. The freeze-dried samples were dispersed by heating in dimethyl sulfoxide (DMSO). Lipids were removed by successive ethanol washing and the precipitated starch was recovered. The precipitated starch was then dissolved in an acetate/salt solution and a sub-sample was taken. Concanavalin A was added to precipitate amylopectin, which was then removed by centrifugation. A sub-sample of the supernatant was taken after the centrifugation. The total starch in a sub-sample of the acetate/salt solution with dissolved starch and the amylose in a sub-sample of the supernatant were enzymically hydrolyzed to glucose. Glucose oxidase/peroxidase reagent was then added to each sub-sample and the absorbances at 510 nm of these mixtures were measured. The relative concentration of amylose in the starch sample was estimated as the ratio of the absorbance of the supernatant to that of the total starch sample. The total starch in the sample (%) was calculated using the total starch content equation in McCleary, Gibson, and Mugford (1997). This assay was carried out in duplicate.

Analysis of variance (ANOVA, $p < 0.05$) using MINITAB Statistical Software was conducted to examine the significance of observed differences.

3. Results

3.1. Pasting behaviour

In this study, “pasting” was defined as the heating of the starch suspension from 20 to 95 °C at 2 °C/min while stirring at 100 rev/min. Changes in apparent viscosity were recorded during pasting while stirring the sample, to construct a pasting curve. Pasting curves for normal and waxy rice starch suspensions that had received no pressure or heat treatment are shown in Fig. 1. Several parameters, which provide information about gelatinization characteristics, can be extracted from a pasting curve; these are marked in Fig. 1. The initial viscosity, “ η_{initial} ”, is the apparent viscosity at 20 °C before pasting begins. The onset temperature of gelatinization, “ T_{onset} ”, is the temperature at which the apparent viscosity starts to increase. The peak viscosity, “ η_{peak} ”, is the maximum apparent viscosity attained during pasting and “ T_{peak} ” is the temperature at η_{peak} . The two types of rice starch had similar η_{initial} values (approximately 0.007 Pa.s) but showed different pasting patterns (Fig. 1). T_{onset} was 64.5 °C for normal rice starch and 60.1 °C for waxy rice starch. Untreated waxy rice starch suspensions showed a rapid increase in viscosity over a narrow temperature range, so that T_{peak} was 72 °C. The viscosity increase for normal rice starch after T_{onset} was more gradual and over a wider temperature range, so that T_{peak} was 92 °C. The η_{peak} value was 2.1 Pa.s for normal rice starch and 3.5 Pa.s for waxy rice starch.

Selected pasting curves for normal and waxy rice starches after pressure treatment are shown in Fig. 2. The pressure treatments were carried out for 30 min at 40 °C

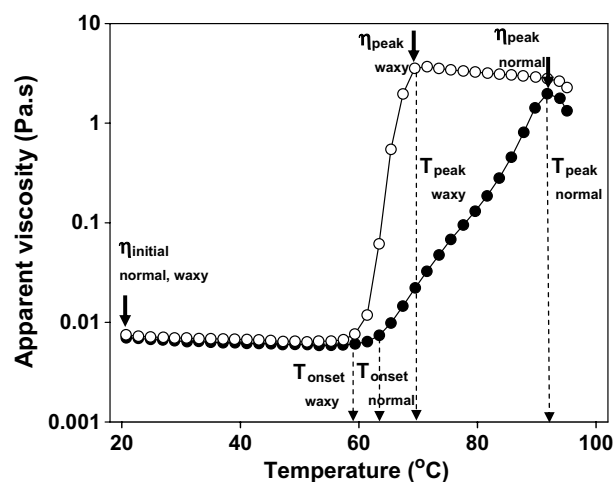


Fig. 1. Pasting curve. Apparent viscosity of starch suspensions (10% w/w) as a function of temperature for untreated normal rice starch (●) and untreated waxy rice starch (○).

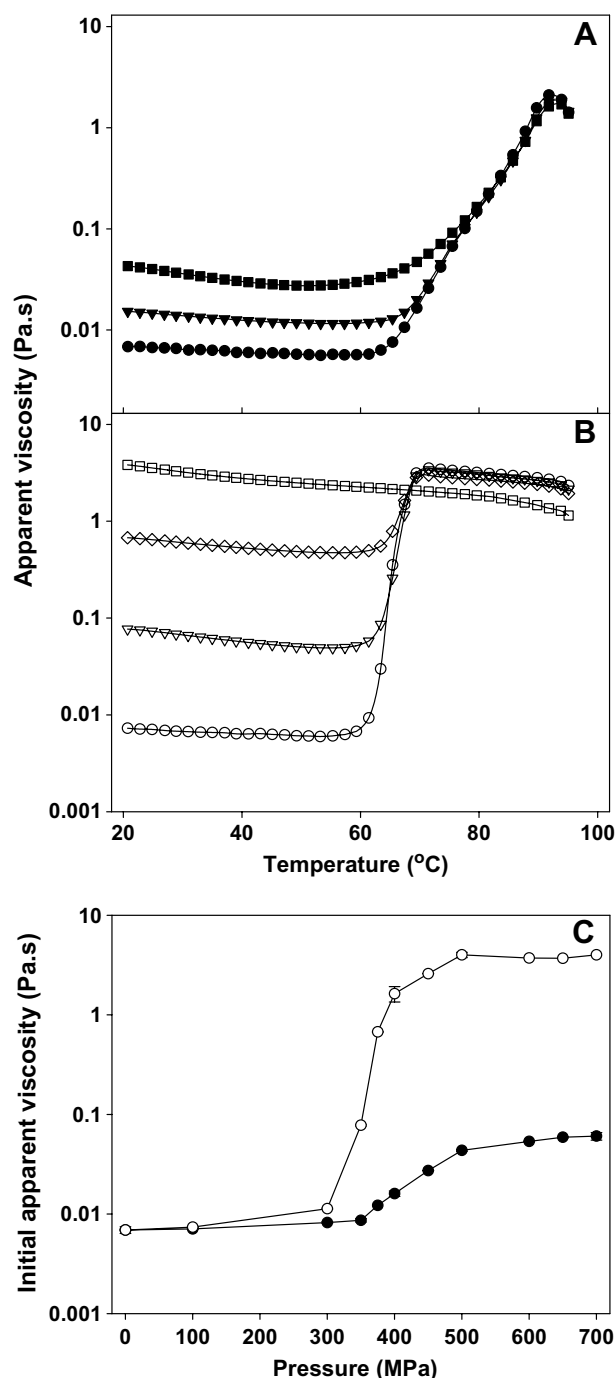


Fig. 2. (A) Pasting curves for normal rice starch after no pressure treatment (control) (●), pressure treatment at 400 MPa (▼), and pressure treatment at 500 MPa (■). (B) Pasting curves for waxy rice starch after no pressure treatment (control) (○), pressure treatment at 350 MPa (▽), pressure treatment at 375 MPa (◇), and pressure treatment at 500 MPa (□). (C) Initial apparent viscosity as a function of treatment pressure for normal rice starch (●) and waxy rice starch (○). The temperature at treatment was 40 °C and the treatment duration was 30 min.

at different pressures. The adiabatic heating was ~ 1.9 °C/100 MPa, which increased the temperature of the pressurizing unit during treatment. For example, the temperature increased to ~ 49.5 °C and cooled back to 40 °C over ~ 4 min during the 500 MPa treatment. Starch gelatiniza-

tion involves granule swelling and the release of starch material and results in an increase in viscosity (BeMiller & Whistler, 1996). The increased η_{initial} after pressure treatment indicates the degree of gelatinization of starch as a consequence of the pressure treatments. The viscosity of the starch suspensions subsequently increased with temperature during pasting when the starch had not been completely gelatinized by the pressure treatment.

The η_{initial} value of normal rice starch suspensions was 0.007 Pa.s when untreated and increased to 0.043 Pa.s after treatment at 500 MPa. However, even after treatment at ≤ 500 MPa, the initial viscosity of normal rice starch did not increase to the η_{peak} that could be attained on pasting. The η_{peak} value for the pressure-treated normal rice starch was approximately 2.1 Pa.s, was not notably different between suspensions that received different pressure treatments, and was very close to the value achieved in the untreated sample (Fig. 2A).

Waxy rice starch showed more noticeable changes in η_{initial} after pressure treatments, compared with normal rice starch (Fig. 2B). Pressure treatment at 350 MPa was enough to increase the η_{initial} value of the waxy rice starch suspension approximately tenfold, from 0.007 (untreated) to 0.078 Pa.s, followed by a further approximately tenfold increase after the 375 MPa treatment. On pasting, the viscosities of these samples increased to η_{peak} values that were similar to the values achieved for untreated waxy rice starch. After the 500 MPa pressure-treatment, the waxy rice starch suspension showed a η_{initial} value that was slightly higher than the η_{peak} value of the untreated suspension and the viscosity did not increase further during pasting. Instead, the viscosity of the suspension decreased as the temperature increased. This decrease may have been a consequence of the stirring, which may have broken down the swollen granules and remnants, therefore decreasing the viscosity of the suspension (BeMiller & Whistler, 1996).

3.2. Initial viscosity (η_{initial})

Fig. 2C shows the change in η_{initial} after different pressure treatments at 40 °C. For both normal rice starch and waxy rice starch, the plot of η_{initial} against pressure exhibited a sigmoidal-shaped curve. The η_{initial} value did not change markedly until a critical level of pressure was applied, which was approximately 350 MPa for normal rice starch and 300 MPa for waxy rice starch. Above these critical pressures, there was a phase where η_{initial} increased sharply as the treatment pressure was increased. This phase was between 350 and 500 MPa for normal rice starch and between 300 and 500 MPa for waxy rice starch. Above 500 MPa, both starch types showed no further increase in η_{initial} .

The effect of the duration of pressure treatment at 40 °C on η_{initial} of the starch suspensions was also examined (Fig. 3). At 300 MPa, a small but significant increase in η_{initial} of normal rice starch was observed with increased duration of pressure treatment (Fig. 3A). When the treat-

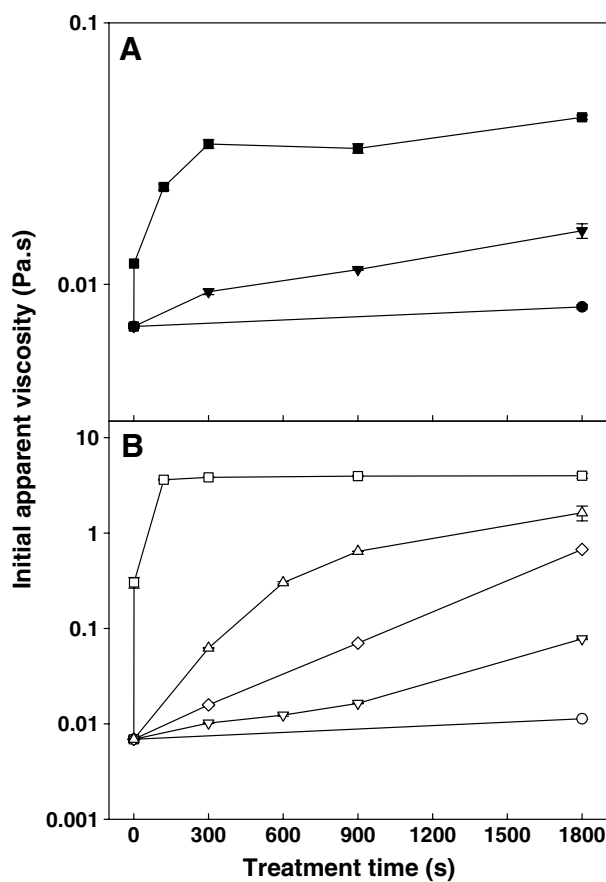


Fig. 3. Initial apparent viscosity as a function of treatment duration. (A) Normal rice starch after pressure treatment at 300 MPa (●), 400 MPa (▼), and 500 MPa (■). (B) Waxy rice starch after pressure treatment at 300 MPa (○), 350 MPa (▽), 375 MPa (◇), 400 MPa (△), and 500 MPa (□). The temperature at treatment was 40 °C.

ment pressure was increased to 400 MPa, η_{initial} increased gradually with treatment time. At 500 MPa, there was a sharp increase in η_{initial} over the first 300 s of pressure treatment but prolonged treatment did not result in a significant further increase in η_{initial} .

Waxy rice starch showed a similar behaviour to that observed for normal rice starch (Fig. 3B). The treatment pressure of 300 MPa led to a slight increase in η_{initial} with increased duration of pressure treatment. At 350 MPa, η_{initial} increased steadily with treatment time, whereas, at 375 MPa, the increase in η_{initial} was linear ($R^2 = 0.98$) with treatment duration. When the treatment pressure was increased to 400 MPa, the increase in η_{initial} was no longer proportional to the treatment duration. At 400 MPa, η_{initial} increased considerably after 300 s of pressure treatment and the increase in η_{initial} was more gradual with longer treatment. At 500 MPa, η_{initial} increased abruptly after the first 120 s of pressure treatment and did not change further as the treatment duration was extended.

The effect of temperature at pressure treatment on η_{initial} of the starch suspensions is shown in Fig. 4. The set temperatures at pressurization were 10, 20, 40, and 60 °C. As a result of the adiabatic heating (~ 1.9 °C/100 MPa), the

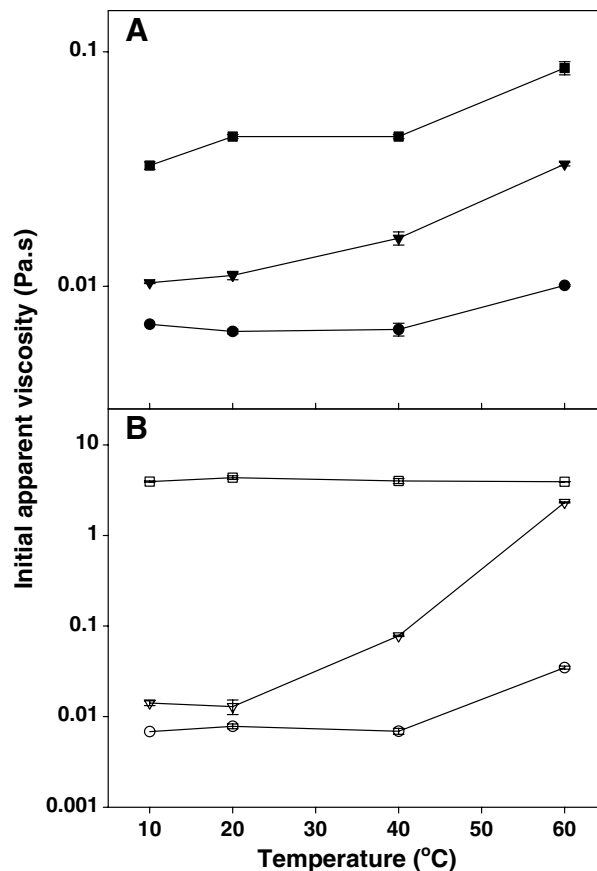


Fig. 4. Initial apparent viscosity as a function of temperature at treatment. (A) Normal rice starch after no pressure treatment (control) (●), pressure treatment at 400 MPa (▼), and pressure treatment at 500 MPa (■). (B) Waxy rice starch after no pressure treatment (control) (○), pressure treatment at 350 MPa (▽), and pressure treatment at 500 MPa (□). The treatment duration was 30 min.

temperature increased by up to 9.5 °C at 500 MPa and then decreased back to the set temperature within 5 min of the set holding time of 30 min. As T_{onset} was 64.5 °C for normal rice starch and 60.1 °C for waxy rice starch when the set temperature at treatment was 10, 20, or 40 °C, the temperature of the unit stayed below T_{onset} for both starch types even though adiabatic heating occurred. However, when the set temperature at treatment was 60 °C, the adiabatic heating increased the temperature of the unit above T_{onset} for both starch types, especially at 500 MPa when the temperature increased to ~ 69.5 °C.

The η_{initial} value of untreated normal rice starch was essentially constant when it was held at temperatures between 10 and 40 °C, which were well below T_{onset} (64.5 °C) (Fig. 4A). There was a small but significant increase in η_{initial} of the untreated control sample at 60 °C as it was held near T_{onset} . At 400 MPa, η_{initial} increased gradually as the temperature was increased from 10 to 60 °C. At 500 MPa, normal rice starch increased in η_{initial} when the temperature was increased from 10 to 60 °C. For untreated waxy rice starch, η_{initial} increased only when the temperature was increased to 60 °C, which was T_{onset} (Fig. 4B). At 350 MPa, η_{initial} increased with an increase

in temperature at treatment from 20 to 60 °C. However, η_{initial} of waxy rice starch was not affected by the temperature at treatment when the treatment pressure was increased to 500 MPa, with a high η_{initial} observed at all temperatures. At 500 MPa, waxy rice starch was completely gelatinized even at the lowest temperature at pressurization (10 °C).

3.3. Degree of swelling

Degree of swelling was defined here as the volume fraction of the centrifuged sediment relative to the volume of total sample, calculated using Eq. (1). During thermal gelatinization, water molecules form hydrogen bonds with the exposed hydroxyl groups of amylose and amylopectin in starch, causing swelling of the starch granules (Ratnayake, Hoover, & Warkentin, 2002). Similarly, when pressure is applied to starch-in-water suspension, water molecules enter into starch granules and form hydrogen bonds with starch polymers. At the individual granule level, this means an increase in granule size (swelling). However, when considering the whole system at the suspension level, such linkages between starch polymers and water reduce the bulk suspension volume (Douzals et al., 1996). Since phenomena that result in volume reduction is favoured under pressure, hydration of starch granules (swelling) can be induced by pressure instead of heating. The degree of swelling after different pressure treatments is shown in Fig. 5A.

In normal rice starch, the degree of swelling did not change until the treatment pressure was greater than 300 MPa and then increased rapidly as the treatment pressure increased up to 500 MPa. The maximum degree of swelling was approximately 50%. Waxy rice starch showed a minor increase in the degree of swelling at treatment pressures below 300 MPa. The degree of swelling then increased very sharply between 300 and 400 MPa and reached 100% at 400 MPa.

Although waxy rice starch showed 100% swelling and normal rice starch could reach only 50% swelling, the degree of swelling curves of both starches had similar sigmoidal shapes and were also similar to the η_{initial} curves in Fig. 2C. In fact, there was a linear relationship between the degree of swelling and η_{initial} of starch suspensions after all pressure treatments and a single regression line represented the results from both normal rice starch and waxy rice starch (Fig. 5B). It was clear that the swelling of starch granules was correlated with the increase in η_{initial} .

3.4. Light microscopy

The radial orientation of crystallites in native starch granules causes the characteristic birefringence (Maltese cross-pattern) under a polarized light microscope (Yuryev, Wasserman, Andreev, & Tolstoguzov, 2002). As starch undergoes a phase transition from the ordered state to a disordered state during gelatinization, it loses crystallinity which leads to loss of this birefringence (Ratnayake

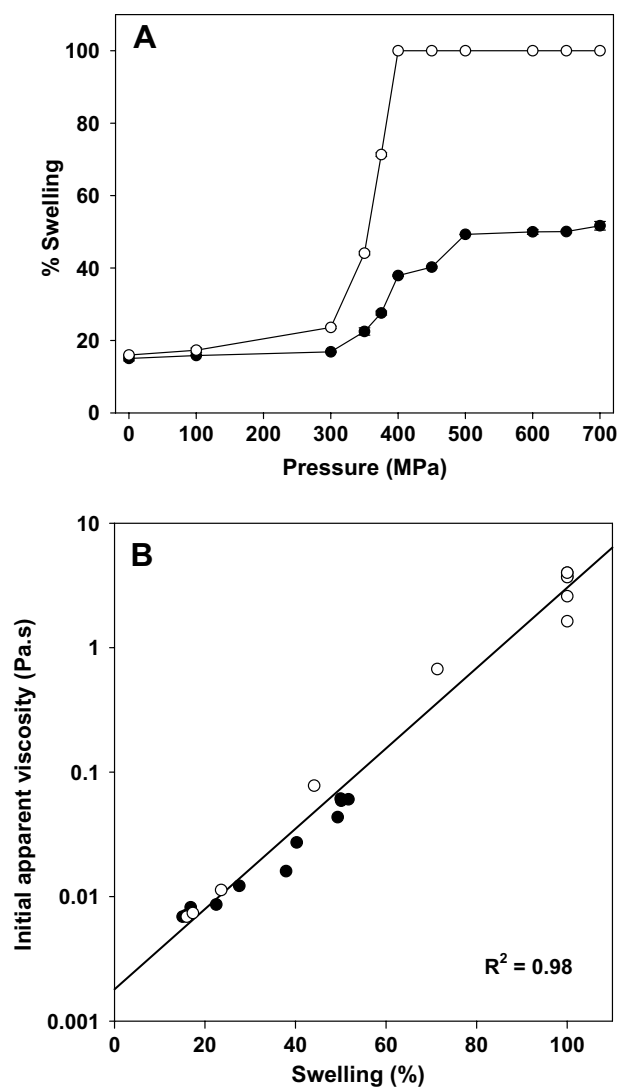


Fig. 5. (A) Degree of swelling (%) as a function of treatment pressure. (B) Plot of swelling (%) versus initial apparent viscosity for normal rice starch (●) and waxy rice starch (○). The temperature at treatment was 40 °C and the treatment duration was 30 min.

et al., 2002). Fig. 6 shows the change in birefringence of starch granules at different stages of starch gelatinization. Untreated starch granules of both starch types had characteristic birefringence patterns (Fig. 6A1 and B1). The starch samples shown in Fig. 6A2 and B2 were those that corresponded to the midpoints (approximately) of the rapid increasing phase on the η_{initial} and degree of swelling curves for normal and waxy rice starch, respectively (Fig. 2C and Fig. 5A). The treatment pressures at the midpoints were 400 MPa for normal rice starch and 350 MPa for waxy rice starch. A number of normal rice starch granules lost birefringence after treatment at 400 MPa (Fig. 6A2). Similarly, some loss of birefringence was observed in waxy rice starch after treatment at 350 MPa (Fig. 6B2). After treatment at 500 MPa, at which point the η_{initial} and degree of swelling curves for both starches had plateaued (Fig. 2C and Fig. 5A), no birefringence

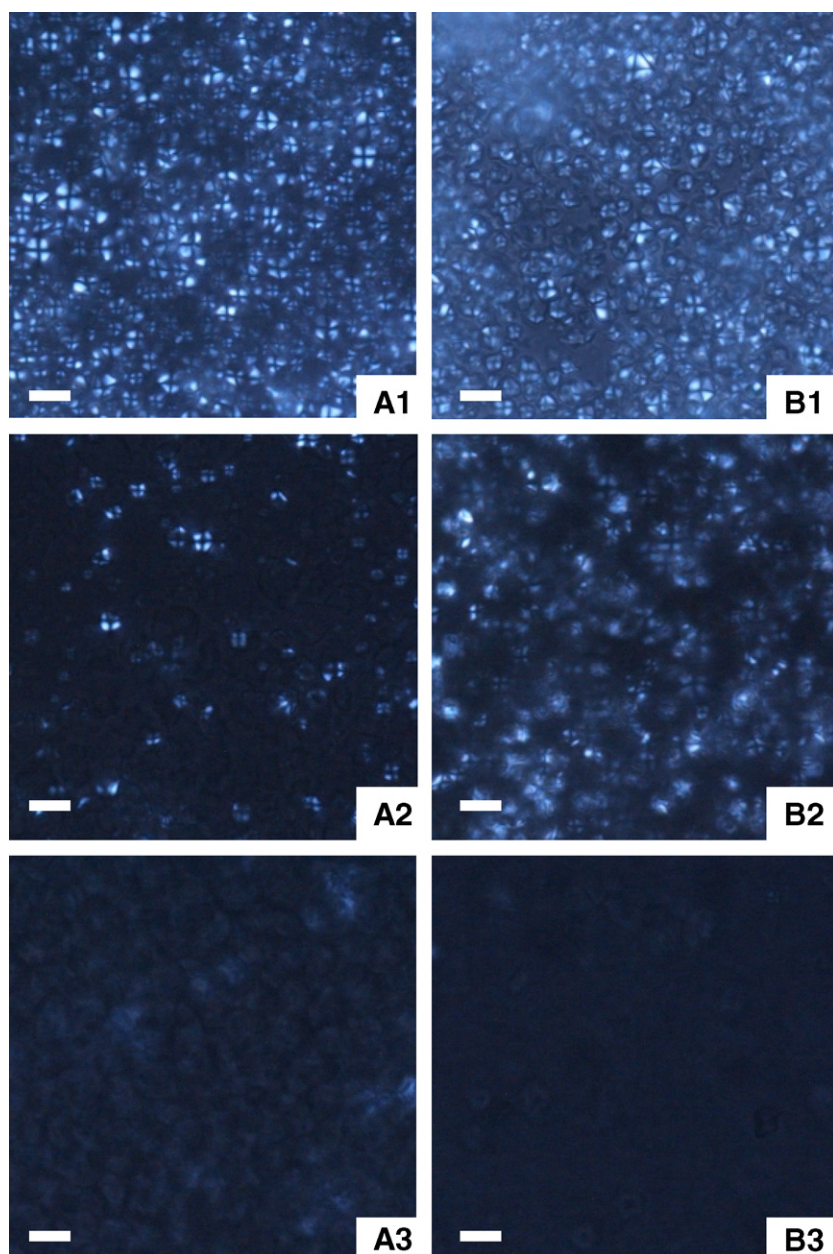


Fig. 6. Polarized light micrographs. (A) Normal rice starch suspension after [A1] no pressure treatment, [A2] pressure treatment at 400 MPa, and [A3] pressure treatment at 500 MPa. (B) Waxy rice starch suspension after [B1] no pressure treatment, [B2] pressure treatment at 350 MPa, and [B3] pressure treatment at 500 MPa. The bar is 20 μm . The temperature at treatment was 40 $^{\circ}\text{C}$ and the treatment duration was 30 min.

was observed in either of the starches (Fig. 6A3 and B3) despite the different extents of change in η_{initial} and the degree of swelling between the two starches (Fig. 2C and Fig. 5A).

The micrographs in Fig. 7 were taken without the polarizing filter to observe the granular structure of the starches. Untreated samples of both normal rice starch and waxy rice starch showed intact granular structures (Fig. 7A1 and B1). After the treatment at 500 MPa, normal rice starch appeared to be swollen but still retained the granular structure (Fig. 7A2). In contrast, after the same treatment, waxy rice starch lost most of its granular structure and only a few swollen granules and

fragments were observed after the same treatment (Fig. 7B2). This suggests that, for normal rice starch, swelling of starch granules during the pressure treatment was sufficient to distort the crystalline region of starch, as indicated by the loss of birefringence, but not enough to disrupt the granular structure. The observation also confirms the incomplete swelling (to only 50%) of normal rice starch shown in Fig. 5A, which in turn resulted in the smaller increase in η_{initial} compared with waxy rice starch (Fig. 2C). For waxy rice starch, the almost complete disruption of the granule structure led to 100% swelling (Fig. 5A) and a much higher η_{initial} than measured for normal rice starch (Fig. 2C).

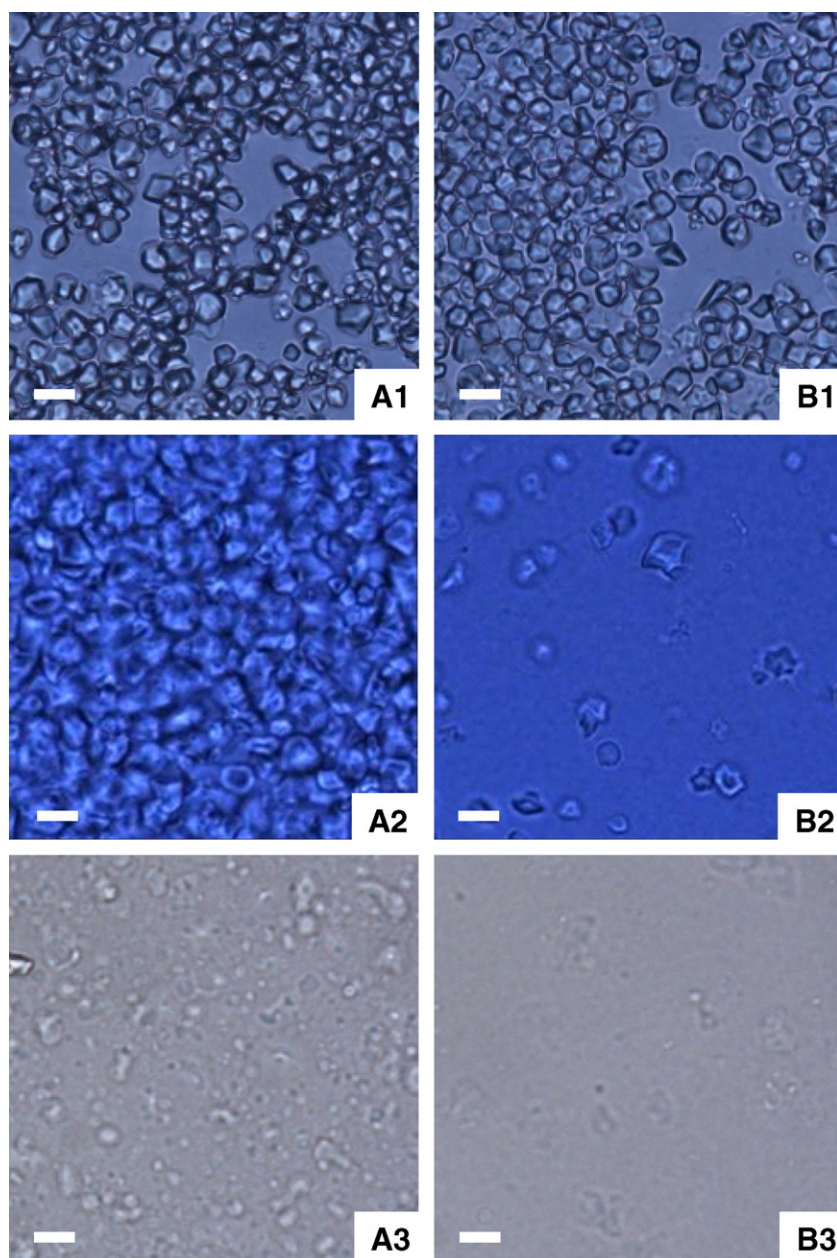


Fig. 7. Light micrographs without polarizing filter. (A) Normal rice starch suspension; (B) Waxy rice starch suspension. After no pressure treatment [A1 and B1], after pressure treatment at 500 MPa [A2 and B2], and after pressure treatment at 500 MPa and subsequent pasting [A3 and B3]. The bar is 20 μ m. The temperature at treatment was 40 $^{\circ}$ C and the treatment duration was 30 min.

The granular structure of normal rice starch, which was still observed after the pressure treatment, was destroyed after the subsequent pasting (Fig. 7A3). This breakdown of granules accounts for the apparent viscosity increase of the pressure-treated sample during pasting (Fig. 2A). The fragments of waxy rice starch observed in Fig. 7B2 was also further broken down after pasting (Fig. 7B3).

3.5. Leaching of starch and amylose

Table 1 summarizes the leached starch and amylose analyses. “Leached starch” was defined as the amount of

starch in the supernatant relative to the total starch in the suspension. “Amylose in leached starch” was defined as the percentage of amylose in the leached starch. “Leached amylose” was defined as the amount of amylose in the supernatant relative to the total amylose in the starch suspension. The amounts of leached starch and leached amylose after pressure treatment were very low in both starch types but some trends in the results were found.

For normal rice starch, the leached starch in the sample increased as the treatment pressure increased up to 400 MPa. However, the amount of leached starch did not change significantly when treatment pressure increased

Table 1

Average amount of starch leached from granules, amount of leached amylose and percentage of amylose in the leached starch from granules after pressure treatments ($n = 2$)

	Leached starch (% w/w)	Leached amylose (% w/w)	Amylose in leached starch (% w/w)
<i>Normal rice</i>			
No treatment	1.70	1.10	10.50
350 MPa	1.98	1.71	14.30
400 MPa	2.07	2.22	16.57
500 MPa	2.16	2.22	16.09
600 MPa	2.48	2.39	15.41
700 MPa	2.38	2.07	14.60
Pooled SD ^a	0.07	0.33	2.19
<i>Waxy rice</i>			
No treatment	1.53	2.70	4.90
100 MPa	1.62	2.80	4.87
300 MPa	2.43	5.72	6.63
350 MPa	2.81	6.20	6.17
Pooled SD ^a	0.17	1.04	0.69

The temperature at treatment was 40 °C and the treatment duration was 30 min.

^a Pooled standard deviation of values in the same column.

above 400 MPa. For waxy rice starch, the leached starch increased from 1.53% (w/w) when untreated to 2.81% (w/w) after the treatment at 350 MPa.

In untreated normal rice starch suspensions, approximately 1.1% (w/w) of amylose leached from starch granules into the aqueous phase and this figure increased slightly to 1.7% (w/w) after treatment at 350 MPa. The amount of leached amylose did not change significantly from 400 to 700 MPa and was around 2% (w/w). The percentage of amylose in leached starch was slightly higher after pressure treatment than in the untreated sample but the results for the samples that received pressure treatments (350–700 MPa) were not significantly different.

In waxy rice starch suspensions, the leached amylose increased steadily with treatment pressure, from 2.7% (w/w) when untreated to 6.2% (w/w) after treatment at 350 MPa, which was higher than for normal rice starch after treatment at all pressures, i.e., a maximum of ~2% (w/w). Above 350 MPa, the waxy rice starch suspensions were too viscous to separate the aqueous phase by centrifugation. However, given that the majority of the waxy rice starch granules had been disintegrated after the 500 MPa treatment (Fig. 7B2), it can be assumed that most starch material in the granules, including amylose, would have leached into the aqueous phase eventually as the treatment pressure increased. On a dry basis, waxy rice starch contains approximately 3% amylose and normal rice starch contains a higher amount, ~16% amylose.

4. Discussion

The effects of increasing temperature are essentially energy and volume effects due to thermal expansivity (Balny, Masson, & Heremans, 2002). In contrast, the

effects of pressure are mainly volume effects through compressibility of the system (Balny et al., 2002). According to Douzals et al. (1996), at pressures over 300 MPa, the reduction in volume is greater for a wheat starch suspension than for pure water at the same pressure. This indicates that the water molecules linked with starch occupy a smaller volume than the molecules in pure water. Consequently, uptake of water by starch granules occurs under pressure in order to reduce the suspension volume; hence gelatinization of starch is induced. The in-situ FTIR study by Rubens, Snauwaert, Heremans, and Stute (1999) showed that the amorphous regions of the starch granule are hydrated first, similar to heat-induced gelatinization. This hydration induces swelling of the granules, leading to distortion of the crystalline regions which then become more accessible for water.

The present study explored aspects of the pressure-induced gelatinization of normal and waxy rice starches. By using η_{initial} as an indicator for the degree of gelatinization, we showed that pressure treatments gelatinized normal rice starch and waxy rice starch to different extents depending on the treatment pressure, the duration, and the temperature at treatment. Although loss of birefringence is often used as an indicator of starch gelatinization, whether induced by heat or pressure, its limitation in determining the degree of pressure-induced gelatinization objectively and quantitatively has been acknowledged in the literature (Kawai, Fukami, & Yamamoto, 2007; Stute et al., 1996). For example, Stute et al. (1996) reported that many corn starch granules showed a partial loss or “fading out” of birefringence so that distinguishing between gelatinized and non-gelatinized granules was difficult.

In contrast, the η_{initial} method used in this study provides an objective and analytical means of determining the degree of pressure-induced gelatinization of starch. The viscosity measurement encompasses the swelling and the leaching of starch material that would occur during starch gelatinization. This study established that the increase in η_{initial} of starch suspensions after pressure treatment is directly correlated with the degree of swelling (Fig. 5B). Likewise, in thermal gelatinization of starch, Bagley, Christianson, and Beckwith (1983) showed that the viscosity of starch suspensions correlated directly with the volume fractions of the swollen granules when the leaching of soluble material was insignificant.

The relationship between η_{initial} and treatment pressure followed a sigmoidal-shaped curve in both starches (Fig. 2C). Such sigmoidal curve shapes, which also represented the relationship between the degree of swelling and the treatment pressure (Fig. 5A), seem to be typical of pressure-induced starch gelatinization. Bauer and Knorr (2005) used the ratio of starch granules having lost birefringence as an indicator for the degree of gelatinization and reported similar sigmoidal curves for pressure-induced gelatinization of wheat and tapioca starches. The swelling index curve for wheat starch presented by Douzals et al. (1998) also exhibited a sigmoidal shape.

The sigmoidal-shaped gelatinization curve means that pressure-induced gelatinization occurred over a pressure range and that the treatment pressure had to be above a critical level for gelatinization to occur effectively. Individual granules of starch in the population of normal rice starch or waxy rice starch have different degrees of association between starch polymers in the amorphous regions (Glicksman, 1969), which suggests that they will pose different resistances to water uptake. Therefore, it can be assumed that the critical pressure is the pressure at which granules with, overall, the weakest associations between starch polymers start breaking and that granules with stronger associations will subsequently swell over a pressure range. Likewise, the effect of treatment duration on the degree of gelatinization (η_{initial}) observed in Fig. 3 can be explained. Granules with weaker associations between starch polymers will gelatinize early and those with stronger associations will gelatinize later during a pressure treatment (Glicksman, 1969).

However, treatment duration and η_{initial} displayed different types of relationship depending on the treatment pressure (Fig. 3). Although different treatment pressure and duration combinations can result in the same η_{initial} value of a starch suspension, the channelling of water into the starch granules during pressurization may not necessarily occur in the same way under different treatment pressures. It is also possible that the observed effects of treatment pressure, temperature at pressurization, and treatment duration on starch gelatinization are kinetic effects (Figs. 3 and 4).

The effect of temperature at treatment on the gelatinization of normal and waxy rice starches (Fig. 4) can be related to the pressure–temperature (P – T) diagram of wheat starch suspensions, shown by Douzals, Perrier-Cornet, Coquille, and Gervais (2001). The P – T diagram of wheat starch suspension was divided into three zones. “Zone A” corresponded to high temperatures (about 40–76 °C) and low pressures (<300 MPa) where the degree of gelatinization could be increased by increasing either the pressure or the temperature. “Zone B” corresponded to higher pressures (>300 MPa) and temperatures of 0–40 °C where there was almost no influence of temperature on gelatinization. “Zone C” corresponded to subzero temperatures, where an increase in pressure resulted in an increase in gelatinization temperature. Because of the differences in starch type and the method for determining gelatinization, the P – T diagrams of the normal and waxy rice starches used in this study would have a slightly different shape from that in Douzals et al. (2001). Nevertheless, assuming similar trends, examples for Zone A in our study include normal rice starch suspensions that received pressure treatment at 400 MPa at temperatures of 20–60 °C and waxy rice starch suspensions that received pressure treatment at 350 MPa at 20–60 °C (Fig. 4). The degree of gelatinization (η_{initial}) of these samples increased at a constant pressure as the treatment temperature was increased. At 500 MPa, the gelatinization of the waxy rice starch

suspension was unaffected by the temperature at treatment, which can be related to Zone B type behaviour (Fig. 4B).

Although the two different starches (normal rice starch and waxy rice starch) both lost all birefringence after pressure treatment at 500 MPa (Fig. 6), their pressure-induced gelatinization characteristics were different. This was demonstrated by examining the pasting curves (Fig. 2A and B) and the degree of swelling (Fig. 5A). Normal rice starch maintained the granular entity more effectively than waxy rice starch (Fig. 7). It can be assumed that the granular entities seen in the normal rice starch (Fig. 7A2) were swollen starch granules rather than starch “ghosts”, as the leaching of starch was minimal (Table 1) and the viscosity of the sample increased considerably during subsequent pasting (Fig. 2A). In contrast, the waxy rice starch sample can be considered to contain starch ghosts only (Fig. 7B2) as no further gelatinization-related changes occurred upon further heating. Blaszcak, Fornal, Valverde, and Garrido (2005) showed a similar trend when waxy corn starch and high-amylose corn starch suspensions were compared. Pressure treatment at 650 MPa for 6 min resulted in a complete breakdown of the granules in waxy corn starch, whereas the high-amylose corn starch retained a granular structure (Blaszcak et al., 2005).

Buckow, Heinz, and Knorr (2007) suggested that disintegration of the crystalline region was not completed by pressure because the side-by-side dissociation and helix unwinding of amylopectin units might be suppressed as van der Waals’ forces and hydrogen bonds are stabilized, which should favor the helix structure. Although this proposal may explain the pressure behaviour of normal type starches such as the maize starch used by Buckow et al. (2007) or the normal rice starch in this study, it does not explain how the crystalline structure of waxy type starches can be disintegrated completely by pressure.

Starch is composed primarily of a mixture of two polymers, amylopectin and amylose (Parker & Ring, 2001). Normal rice starch contains 16% (w/w of total starch) amylose and waxy rice starch contains considerably less amylose, 3% (w/w of total starch). Collapse of the crystalline structure of waxy rice starch, which contains only a small amount of amylose (3%), indicates that the amylopectin crystalline structure can be destroyed by pressure. Although crystallinity of starch granules is formed by the ordering of amylopectin chains (BeMiller & Whistler, 1996), the extent of interaction of the starch chains within the amorphous and crystalline regions can be affected by a number of other factors such as the amylose/amylopectin ratio and the characteristics of amylose and amylopectin in terms of molecular weight distribution, degree and length of branching, and conformation (Hoover, 2001). It seems possible that amylose, which occurs among the amylopectin molecules in starch, contributes to the different susceptibilities of normal and waxy rice starches to pressure in terms of preserving the granular entity.

It can be speculated that, when starch contains more amylose, such as normal rice starch, amylose and displaced

amylopectin in starch may develop a more pressure-stable arrangement. In other words, amylose may form thermodynamically favorable complexes with displaced amylopectin molecules instead of leaching into the aqueous phase. Similarly, in thermal gelatinisation, Debet and Gidley (2007) proposed that slower swelling starches (non-waxy or tuber starches) give sufficient time and polymer concentration for glucan cross-linking to take place. Also, the pressure treatments in this study were carried out at 40 °C, if the amylose in the normal rice starch was solubilized during pressure treatment, it may have formed a gel structure within the starch granules, incorporating displaced amylopectin units, similar to the amylose gel network that can be formed on cooling after thermal gelatinization (Morris, 1990).

Debet and Gidley (2006) stated that lipid, protein and amylose are all necessary to restrict swelling in wheat and maize starches (non-waxy). In particular, Kuakpetoon and Wang (2007) suggested that more amylose was present in the form of an amylose–lipid complex in the outer 10% layer of the starch granules than in the core. This could restrict swelling of granules and leaching of amylose. For amylose-containing corn starches, more long chain amylopectin molecules were found in this region which could also contribute to restricted swelling and leaching. Normal rice starch used in this study not only contained more amylose than waxy rice starch but also slightly more fat and protein. These small compositional differences between the two starch types may, in part, contribute to the different pressure-induced gelatinization characteristics. However, further studies are required to determine the significance of such compositional differences.

It remains unknown if further changes can occur in normal rice starch when the treatment pressure is increased beyond the levels used in this study. Bowler, Williams, and Angold (1980) described the swelling behaviour of cereal starches during heating as a two-stage phenomenon. In the first stage, starch granules swell radially. The rate of amylose leaching is relatively slow during the first stage of swelling (Hermansson & Svegmärk, 1996). Amylose–lipid complexes are believed to restrict swelling in the first stage as they do not dissociate unless heated above 94–98 °C (Hermansson & Svegmärk, 1996; Zeleznak & Hosey, 1987). Once the temperature is sufficiently high, the amylose–lipid complexes dissolve and amylose leaches out, and the granules swell tangentially, deform, and lose their original shape in the second stage (Hermansson and Svegmärk, 1996). If the swelling behaviour of starch under pressure follows the same stages as that described for thermal gelatinization, it can be speculated that the treatment pressures used in this study (up to 700 MPa) did not solubilize the amylose complexes that may have existed.

5. Conclusions

Both normal rice starch and waxy rice starch followed sigmoidal-shaped pressure-induced gelatinization curves.

The degree of starch gelatinization (η_{initial}) was dependent on the treatment pressure, the temperature at pressure treatment, and the treatment duration, and different combinations of these factors could result in the same degree of gelatinization. There was a linear relationship between the degree of swelling and η_{initial} , indicating contribution of swelling to η_{initial} increases. Disappearance of the characteristic birefringence in the starch granules was observed with an increase in treatment pressure for both starches. After treatments at ≥ 500 MPa, both starches lost all birefringence despite the different extents of change in η_{initial} and the degree of swelling. In terms of industrial application, the η_{initial} results show product viscosity changes due to pressure treatment and the pasting profiles show the further changes in viscosity that might occur in the subsequent heating processes.

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