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Relationship between Viscoelastic Properties and Starch Structure in Rice from Thailand

This study continues the studies of *Noosuk* et al. to investigate the properties of four Thai rice starches with different amylose content published in *Starch/Stärke* (**2003**, *55*, 337–344). The composition, gelatinisation behaviour and paste viscosity of these starches has been discussed previously. Here the amylopectin fine structure, amounts and size of the amylose are reported and discussed with reference to the viscoelastic behavior of the gels. The viscoelastic response was measured for 6 to 15% (w/w) pastes (60°C) and gels (25°C) by oscillation (pastes and gels) and creep (gels). Viscoelasticity was shown by all gels at concentrations >6% and the creep compliance could be represented by a four element Burger model. *G'* from oscillation, the instantaneous elastic modulus and Newtonian viscosity from creep, increased strongly with both starch and amylose concentration. Starch gels (30%, w/w) were monitored over 100 h, by Fourier Transform Infra Red spectroscopy (FTIR) and dynamic oscillatory rheology on storage at 25°C. Changes were seen at similar rates, using the Avrami model, for the two high-amylose (>20%) samples, but not for the other starches. Development of crystallinity, at both 25 and 5°C, was monitored by X-ray diffraction. Both the kinetics of retrogradation and extent of crystallinity suggest that amylopectin retrogradation is occurring in the high-amylose starch gels on storage but not in the lower amylose samples. It is suggested that amylopectin retrogradation is promoted by retrograded amylose. The fine structure of amylopectin may also be important, because samples that showed no retrogradation on storage had an amylopectin with a high proportion of amylopectin chains of DP 3–10 and a lower portion of chains of DP 11–22. In gels from waxy wheat it has been reported that retrogradation does occur, which may be related to the lower proportion of the shorter amylopectin chains in this material compared with low amylose rice.

Keywords: Rice starch; Granule structure; Gel viscoelasticity; Starch retrogradation

1 Introduction

Studies of starch gelatinisation and how it then changes on storage are complicated, as both gelatinisation and retrogradation do not proceed to thermodynamic equilibrium. Pasting and storage conditions, as well as the amount of water present, alter not only the amount of structures present but also their size and stability. Many methods are used to follow changes to the starch and all will address slightly different issues [1]. Relating the properties of the starch pastes to their constituents is also difficult due to the differing components, both chemically and structurally. In a previous paper by *Noosuk* et al. [2] the structures of some rice starches from Thailand were reported and discussed in terms of their pasting characteristics, swelling volumes and viscosities at 60°C immediately after pasting. This paper uses some of the same starches and investigates the viscoelastic properties of the rice pastes, particularly after cooling. Further details of the fine structures of the rice amylopectin have also been determined to assist in the interpretation of the data.

Rice starches because of their uniform granules (shape, size and size distribution) and the vast range of varietal diversity offer an excellent model system to study the effect of composition and physical characteristics on the properties of pasted starches. The eating property most associated with gelatinisation and storage is the marked change in the rheology of starch suspensions [3], therefore this study concentrates on monitoring the viscoelastic response of rice starch pastes at different concentrations and on storage.

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Traits within rices that correlate with the physicochemical properties and eating properties of rice can be difficult to define. Of a range of measures the apparent amylose level might explain the most variance, but still only 30% of the properties can be explained by amylose levels alone [4]. Amylose within the pasted starch will influence structure on several levels. This mostly linear molecule can reptate out of the swollen granule and can be present in the continuous phase. On storage amylose, at appropriate concentrations, will rapidly (hours) reorder and aggregate [5, 6]. These changes are linked with a marked change in the rheological properties of a starch paste [7]. The amylose interactions are very stable and temperatures $>$100°C are required to melt out their association [7]. Amylose left within the swollen granule might also play a role in the changing rheological properties on storage.

The amount and definition of "amylose" is also complex. Lipid can combine with the amylose and this amylose may not be measured. When amylose levels are quoted the quantity can include a portion of the amylopectin, as some chains within the branched polymer are long. Therefore starches that have high apparent amylose levels may contain more of the extra long amylopectin B chains [8]. Amylopectin fine structure is thought to affect the properties of rice or rice starch. For example, *Ong* and *Blanshard* [9] found that hard cooking rice tended to have more longer chain amylopectin than soft cooking rice. The texture of the rice was critically controlled by the proportion of the longest (DP 92–98) and shortest (DP $\leq$ 25) amylopectin chains. *Han* and *Hamaker* [10] reported that content of long chains of amylopectin of rice starch was negatively correlated with paste breakdown, while the proportion of short chains of amylopectin was positively correlated.

A feature of cooked rice grains or pastes is the amount of residual granule structure remaining and therefore the matrix is not homogenous. Models of the starch paste are of a discontinuous phase (the remaining swollen granules) and a continuous phase of amylose. Additional phase separation can occur during the retrogradation process. Therefore granule size, amylose-amylopectin ratio, macromolecular organisation of the granules, starch concentration and temperature-time regimes are all important for the viscoelasticity of starch gels [11, 12].

The objective of this study was therefore to investigate the viscoelastic properties of four rice starch gels, using dynamic and creep-compliance rheological tests, and obtain sufficient information about the composition and structure of the starch so that composition, structure and properties of these rice starches could be related.

2 Materials and Methods

2.1 Materials

2.1.1 Starch source

Starches were obtained from four sources. These were RD6 waxy rice (Rice Research Centre, Henchman, Thailand), aromatic Jasmine rice named Khao Dawk Mali 105 (Rice Research Centre, Patumthani, Thailand) and Supanburi1 (Rice Research Centre, Patumthani, Thailand) and a commercial rice starch sample (Cho Heng Rice Vermicelli Factory Co. Ltd., Nakhon Pathom, Thailand). Starch was extracted by wet milling using 0.3% sodium chloride and presoaked wet rice grains (soaked in 0.3% sodium chloride solution at 35°C for 5 h). Protein was removed from the starch using an alkaline method followed by centrifugation [13, 14]. Starches were dried in a hot air oven at 50°C for 20 h.

2.1.2 Paste preparation

Samples used in the estimation of swelling, dynamic viscoelastic properties and retrogradation were prepared in a Rapid Visco Analyser (RVA series 4, Newport Scientific, Warriewood, NSW, Australia) that enables accurate control of time-temperature and shear profiles. The stirring speed was 960 rpm for the first 10 s and 160 rpm for the remainder of the test. Temperature and time were set as follows; the sample was held at 50°C for 1 min, ramped up to 95°C at 12°C/min, held at this temperature for 2.5 min, cooled to 80°C, again at 12°C/min, and held at this temperature for a further 2 min.

2.1.3 Gel preparation

Starch pastes prepared in the RVA were then poured immediately into moulds. After cooling at room temperature for 1 h, the pastes were covered and then stored at 5°C for 12 h and transferred to the rheometer for creep or oscillation tests at 25°C.

2.2 Chemical composition

2.2.1 Proximate analysis

The moisture [15] and fat [16] content were determined according to AOAC methods. The amount of protein was assessed by the Dumas method [17] using a nitrogen analyser (NA 2000, Fisons Instruments, Rodano, Italy).

2.2.2 Amylose content

Amylose content was determined using a commercial assay kit from Megazyme (Wicklow, Ireland) according to the procedure described by *Gibson* et al. [18]. This method uses the specific binding properties of Concanavalin A to non-reducing end-groups of amylopectin and thus precipitates this fraction of the starch.

2.3 Structure and properties characterisation

2.3.1 Molecular weight of straight chain molecules

The molecular weight of debranched starch was determined. Defatted starch (100 mg) was dispersed with 1 mL ethanol and added to 10 mL of 1 M aqueous NaOH [19]. The suspension was left at room temperature for 24 h and then heated in a boiling water bath for 15 min. After cooling the solution was neutralised with 1 M aqueous HCl. To debranch the starch 2 mL of this solution was treated with isoamylase (20 μL) from *Pseudomonas amylodermosa* (Hayashibara Biochemical Laboratories Inc., Okayama, Japan) dispersed in 1 mL of acetate buffer (30 mM, pH 3.5) and incubated in a water bath at 40°C for 24 h. In order to stop the reaction, samples were heated for 5 min in a boiling water bath. After cooling and filtering through a 0.45 μm membrane samples were fractionated on a size exclusion chromatography-multi angle laser light scattering-refractive index (SEC-MALLS-RI) system as described by *Ramesh* [8]. After a guard column, five analytical columns (one TSK GEL G4000 PWXL, two Asahipak GS-320H, one TSK G2500 PWXL, and one TSK G-Oligo PWXL) were used to separate the sample. The eluent was a phosphate buffer at pH 8.6, flow rate 0.5 mL/min and a temperature of 30°C was used. Two in-line detectors were used; a MALLS (Wyatt Technology Inc., Santa Barbara, CA, USA) and a RI concentration detector (Optilab 903, Wyatt Technology Inc., USA). The refractive index increment value (d*n*/d*c*) for this solute-solvent system is 0.152 mL/g [20]. The absolute weight average molecular weight values, M_w, the number average molecular weight, M_n, and polydispersity (M_w/M_n) value of amylose were calculated from a Zimm plot using ASTRA™ software (Wyatt Technology Inc., USA).

2.3.2 Amylopectin branch chain analysis

The size of the amylopectin branch chains was determined by high performance anionic exchange chromatography (HPAEC) performed using a Dionex 100 system and equipped with pulsed amperometric detector (PAD) as described by *Blennow* et al. [21]. Starch samples (150 mg) were dispersed in 5 mM aqueous sodium acetate (20 mL) and heated in a boiling water bath for 6 min. The samples were then cooled to room temperature and isoamylase (15 μL) was added to debranch the amylopectin during incubation at 37°C for 4 h. After heating to denature the enzyme the samples were cooled to 4°C and centrifuged. The supernatant was collected and diluted five times before injection in the HPAEC system. The samples were separated using a flow rate of 1 mL/min, 100 mM NaOH gradient profile: 0–2 min, 100 mM NaOH and the following 0.6 M NaOAc gradient profile: 2–30 min, and 100 mM NaOH gradient profile: 30–35 min.

2.3.3 Swelling mass ratio

The swelling mass ratio (*Q*) was determined for 0.7% (w/w) starch pastes. The hot solution sampled from the RVA canister was immediately centrifuged at $1000 \times g$ for 15 min. The supernatant was removed by suction and the weight of the residue determined [22, 23]. The swelling mass ratio *Q* was calculated as the weight of the residue divided by the weight of dry starch in the sample prior to centrifugation.

2.3.4 Intrinsic viscosity

Samples (3%, w/v) were prepared in 5 M aqueous KOH and were heated at 95°C in a water bath for 10 min. The samples were stirred overnight at ambient temperature. The resulting clear solutions were filtered through glass wool. Filtrates were diluted to give five different concentrations (1–4 mg/mL). The intrinsic viscosity measurements were performed at 25±0.05°C using a Schott Gerate automated measuring unit with a 2 mL capacity Ostwald type viscometer that gave a flow time for distilled water of 77.90 s. The intrinsic viscosity was determined by measuring the flow times of the sample and using the Huggin plot of $(1/c(t/t_0 - 1)$ against c, where t_0 is the flow time for the solvent, t the flow time for the solution and c the solution concentration.

2.4 Viscoelasticity

2.4.1 Oscillation

Starch pastes and gels (6–12%, w/w) were prepared in the RVA and transferred to a Bohlin CVO rheometer (Bohlin, Malvern, UK) equipped with cone and plate geometry (CP4/40). The measurements were performed in the linear viscoelastic range (2% strain) and over a frequency range of 0.1–10 Hz. The temperatures during measurement were controlled at 60 and 25°C for starch

pastes and gels, respectively. The dynamic rheological parameters (storage modulus *G*', loss modulus *G* and loss tangent tan δ) were recorded. The surfaces of pastes and gels were covered by paraffin oil during measurement to prevent moisture loss.

As part of the studies on the effects of ageing for the starch pastes, samples prepared at 30% (w/w) concentration in the RVA were immediately transferred to a Bohlin rheometer fitted with CP4/40 geometry. At intervals during a holding period of up to 100 h, at a constant temperature of 25°C, the rheology of the sample was measured at a frequency of 0.2 Hz.

2.4.2 Creep

Rice starch gels prepared in the RVA were transferred to a Bohlin CVO rheometer equipped with cone and plate geometry (PP20). The creep test was performed at 25°C by measuring the deformation while applying a constant stress (10 Pa) in the linear viscoelastic range for 200 s, the creep recovery was followed for a further 200 s. The gel surface was covered by paraffin oil during measurement to prevent moisture loss.

2.5 Measurement of starch order

2.5.1 Fourier transform infrared spectroscopy (FTIR)

Starch pastes (20–40%, w/w) prepared in the RVA were poured onto the diamond crystal of a temperature-controlled single attenuated total reflectance (ATR) sampling cell (Golden Gate, SpecAc, Orpington, UK) and then were cooled immediately to 25°C. An IFS48 spectrometer (Bruker, Coventry, UK) equipped with a room temperature deuterated triglycine sulphate (DTGS) detector was used. Spectra were recorded at 25°C at an angle of incidence 45°. The spectra (32 scans) were recorded at a resolution of 4 cm^{-1}. The spectrum of liquid water was subtracted [24–26]. In order to monitor the changes in the sample during storage, spectra were acquired at hourly intervals. The change in molecular order was assessed using the ratio of absorbance at 1047 cm^{-1} to that at 1022 cm^{-1} for the deconvoluted spectra [26, 27].

2.5.2 Wide-angle X-ray diffraction

Starch pastes of 30% (w/w) prepared in the RVA were cooled down immediately to 25°C, covered and stored at 5 and 25°C for 0, 1, 2, 3, 4 and 5 days. After storage a wide angle X-ray diffractogram was acquired using a Bruker AXS D5005 X diffractometer with an X-ray generator equipped with a copper target, operating at 40 kV and 50 mA and producing CuK_α radiation of approximately 1.54 Å wavelength. The diffractogram recorded was over the 2θ range of 4° to 38° with an interval of 0.1°. Relative crystallinity indices were determined from the area of the diffractions at 2Θ between 15° and 20.5°, calculated after normalisation by the standard deviation of the diffraction intensities over the entire 2θ range available and after the subtraction of the diffractogram acquired on the fresh paste.

3 Results and Discussion

3.1 Composition, structure and properties of rice starches

Tab. 1 displays the composition, structure and properties of the rice starches studied. The starches can be ranked in terms of their amylose content as: Supanburi 1 > commercial rice > Jasmine > RD6. The amylose M_w obtained (1.8–2.9 $\times 10^5$ Da) were in the range previously

Tab. 1. Composition, structure and properties of rice starches.

Starches derived from	Fat content[1] [%]	Protein content[1] [%]	Amylose[2]				Swelling mass ratio[2] [g swollen/g dry starch]	Intrinsic viscosity[1] [mL/g]
			Content [%]	M_w [$\times 10^5$]	M_n [$\times 10^5$]	M_w/M_n		
1 RD6 rice	0.66 ± 0.10	0.64 ± 0.00	2.08 ± 0.22	NA	NA	NA	34.64 ± 0.03	192 ± 0
2. Jasmine rice	0.67 ± 0.14	0.70 ± 0.01	15.14 ± 0.21	2.3 ± 0.5	1.6 ± 0.3	1.4 ± 0.5	21.89 ± 0.06	185 ± 6
1 Commercial rice	0.41 ± 0.07	0.56 ± 0.03	21.21 ± 0.19	2.8 ± 0.5	2.3 ± 0.4	1.2 ± 0.3	17.83 ± 0.02	171 ± 6
1 Supanburi 1 rice	0.67 ± 0.07	0.79 ± 0.05	22.43 ± 0.15	2.9 ± 0.2	2.4 ± 0.2	1.2 ± 0.4	14.69 ± 0.06	156 ± 7

[1] means of duplicate ± SD.
[2] means of triplicate ± SD.
NA not available.

reported for rice starch amylose using the same technique (enzyme debranching followed by SEC-MALLS) [8, 28]. However, the molecular weight of Jasmine amylose was notably lower than that of the high-amylose containing samples. The intrinsic viscosity [η] was in the range 156–192 mL/g and increased with increasing amounts of amylopectin, which is in line with other values reported for rice [29, 30]. The [η] values fall between that of potato starch (224 mL/g) and wheat starch (137 mL/g) [31, 32].

The lengths of the amylopectin chains are thought to have a major impact on the properties of rice starch and the lengths of the chains can vary [9, 10, 28, 29, 33–35]. Often three types of chain are defined: short- (DP 5–10), medium- (DP 11–22) and long-chain fraction (DP 23–40). Chains much longer than these are often considered to originate from the amylose fraction, but there is an increasing body of evidence that amylopectin can contain chains in excess of DP 50 and these can be found after debranching amylopectin. Chains of this length will bind iodine to produce absorbances the same as those normally associated with amylose [36]. We have previously shown that the amount of long B chains increases with the apparent amylose content of the starches [8].

Fig. 1 shows the chain length distribution profiles for the current samples. The high-amylose rice starches (Supanburi 1 and Commercial rice) had the largest proportion of medium and the lowest proportion of short chains. Conversely, the waxy and medium rice (RD and Jasmine) starches had the largest proportion of short- and the lowest proportion of medium-chains. It would therefore seem that when high levels of amylopectin are present these macromolecules seem to contain more short chains. This is in accord with the findings of *Reddy* et al. [19], *Takeda* et al. [33] and *Vandeputte* et al. [37] for rice starches and other workers for a wide range of starches of differing botanical origin [38–40]. Also the Jasmine rice seems to have a higher proportion of long chains compared to the other three rice samples. Therefore the characteristics of the amylopectin, as well as the amount of the branched polymer differ, for the samples.

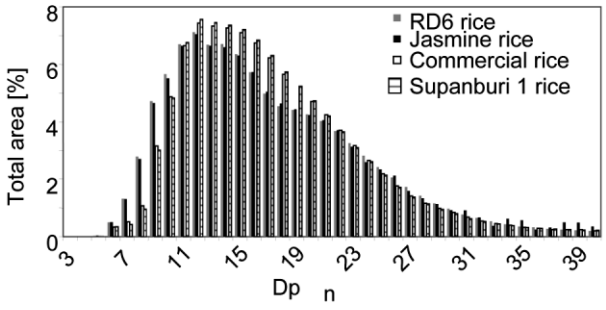

Fig. 1. Chain length distribution of starch amylopectin.

3.2 Viscoelastic properties of rice starch gels

The rheology of the starch pastes will depend on the amount and type of polymer in solution and the amounts retained in the swollen granule. In the previous paper [2] the viscosities for the Thai rice starches were measured in the shear rate range 50–1000 s^{-1} and on pasting using an RVA. An inverse correlation was found between the swelling volumes and the amylose content. The consistency index and viscosity showed a dependence on the concentration and the notional volume fraction parameter *cQ* (concentration % × swelling mass ratio). Similar results were found for the dynamic viscosity values for the starch pastes (6–12%) measured at 60°C immediately after pasting. Fig. 2 displays the complex viscosity measured at a frequency of 1 Hz as a function of *cQ*. Both the complex viscosity and the steady shear viscosity show a much weaker dependence on *cQ* for the waxy rice (RD6). For the higher amylose rice starches, one would expect greater amounts of exuded linear starch molecules at the intergranular space. It is this amylose that is important for the development of rigid three-dimensional network structures.

Of primary interest to this current work are the properties of the pastes when they have cooled. When first formed and measured at 60°C the oscillatory response of starch pastes showed higher values of *G*' than *G*" in all cases. Storage overnight at 5°C and measurements at 25°C resulted in increased viscosity. Both *G*' and *G*" increased, but this was particularly notable for the *G*' values. Fig. 3 shows *G*' measured at a frequency of 1 Hz, at starch concentrations of 6–15%. The order for the values of *G*' is: high-amylose > medium-amylose > waxy rice starch. An approximately linear relationship between *G*' and concentration seems to exist and this is particularly clear for the highest amylose starch (regression value R^2 =0.98) where the slope is most pronounced. This linear relation-

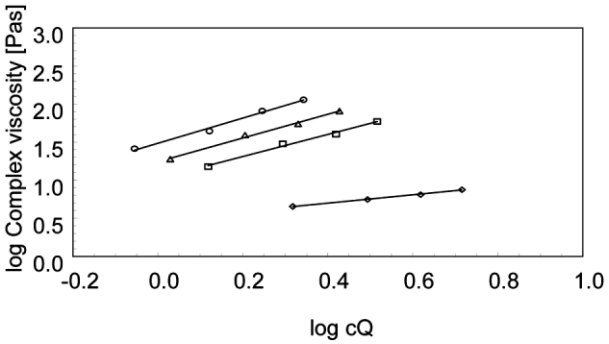

Fig. 2. The relationships between log complex viscosity (η*) and log *cQ* measured at 1 Hz and 60°C. Starch gels derived from RD6 (◇), Jasmine (□), commercial (△) and Supanburi 1 rice (○).

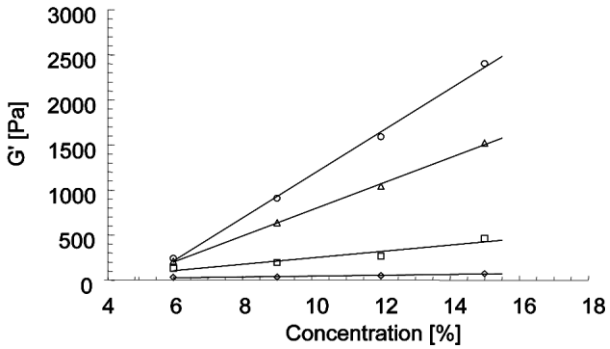

Fig. 3. The relationships between concentration and elastic modulus (G') of starch gels measured at 25°C. Starch gels derived from RD6 (◇), Jasmine (□), commercial (△) and Supanburi 1 rice (○).

ship between concentration and *G'* has been noted for other starches (e.g. over the range 6–30% for potato, wheat and maize starch gels [41] and for 4–8% corn starch pastes [42]). If the concentration dependences of *G'* are fitted to the power of the concentration ([*C*]), high R^2 values are still obtained, and the dependency varied between $[C]^{2.61}$ for Supanburi 1 and $[C]^{0.92}$ for RD6. The dependency of the elastic modulus on starch concentration is normally reported to be $[C]^{2.1–2.9}$ [19, 43]. However, these values are for starches containing substantial amounts of amylose and the dependency is less for waxy samples [44].

Another method to observe the viscoelastic behaviour of the stored gels is to look at creep compliance and recovery. Fig. 4 displays creep compliance – creep recovery curves, at 25°C for rice starch gels at 6 to 15% (w/w) concentration after overnight storage at 5°C. All the starches show viscoelastic behaviour except for the 6% RD6 rice starch gel, for which the compliance curve increases linearly as a function of time and no retardation phenomenon is recorded. For the other gels, after sufficient time has passed, the compliance increased linearly with time at a steady flow rate. When the stress was suddenly removed (t = 200 s), only the elastic and retarded elastic parts of gel were recovered. The response is often fitted to a model consisting of a Maxwell element in series with a single Voigt element (a four-element Burger model) as described by Equation (1):

$$J(t) = J_0 + J_1\left[1 - \exp\left(-\frac{t}{\tau_1}\right)\right] + \frac{t}{\eta_N} \quad (1)$$

Where $J(t)$ is the measured compliance, J_0 and J_1 are the compliances of the Maxwell and Voigt springs respectively, η_N is the viscosity of the Maxwell dashpot and τ_1 is the retardation time associated with the Voigt element. The parameters obtained are displayed in Tab. 2. The instantaneous elastic modulus (G_0=1/J_0) reflects the minimally disturbed sample with most structural bonds intact, while the Newtonian viscosity (η_N) represents the state of material in flow with structural bonds broken [42].

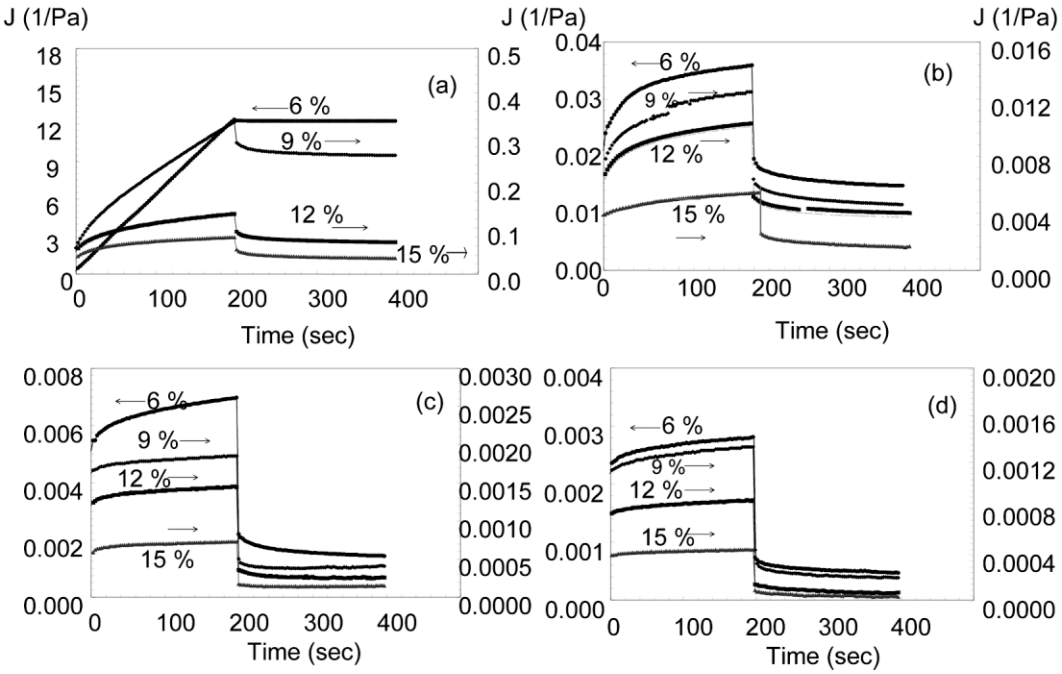

Fig. 4. Creep compliance and creep recovery of rice starch gels derived from RD6 (a), Jasmine (b), Commercial (c) and Supanburi 1 rice (d) at various concentrations.

Tab. 2. The viscoelastic parameters of the rice starches gels at 25°C analysed by creep and dynamic viscoelastic measurements at 1 Hz.

Starch derived from	Concentration [%, w/w]	Creep					Dynamic viscoelastic		
		G_0 [Pa]	G_v [Pa]	η_v [Pa·s]	η_N [P·s]	τ_v [s]	G' [Pa]	G'' [Pa]	tan δ
RD6 rice	6	–	–	–	1.64×10^1	–	30	9	0.305
	9	18	18	3.93×10^2	$8.66 \times x10^2$	22	34	13	0.388
	12	21	25	6.71×10^2	4.58×10^3	27	48	19	0.402
	15	31	35	1.02×10^3	1.16×10^4	29	71	27	0.403
Jasmine rice	6	48	93	2.22×10^3	5.00×10^4	24	132	15	0.111
	9	132	439	1.11×10^4	1.43×10^5	25	195	28	0.145
	12	172	396	1.01×10^4	1.33×10^5	26	266	41	0.161
	15	318	1 023	2.92×10^4	2.56×10^5	29	461	76	0.165
Commercial rice	6	196	1 001	2.00×10^4	2.32×10^5	20	208	20	0.096
	9	617	8 201	1.21×10^5	1.67×10^6	15	648	45	0.069
	12	833	7 458	1.06×10^5	1.78×10^6	14	1039	60	0.058
	15	1718	9 073	1.07×10^5	3.31×10^6	12	1520	93	0.061
Supanburi 1 rice	6	429	4 123	7.37×10^4	7.14×10^5	18	219	22	0.100
	9	909	9 358	1.61×10^5	1.49×10^6	17	908	54	0.059
	12	1330	18 657	3.16×10^5	2.17×10^6	16	1591	92	0.058
	15	2584	29 407	3.50×10^5	6.74×10^6	12	2403	134	0.056

G_0: Instantaneous elastic modulus; G_1: Retarded elastic modulus; η_v: Voigt viscosity; η_N: Newtonian viscosity; τ_1: Retardation time; G': Elastic modulus; G'': Viscous modulus.

Tab. 2 also compares the creep and oscillatory parameters for the starch gels. G_0 and η_N were found to increase with increasing amylose content and concentration of starches. G_0 was in the same order of magnitude as G' at 1 Hz for all rice starches' gels, as also reported by other authors [45–47].

The τ values followed the same trend as tan δ, in that they decreased with increasing amylose content of the starches. Increasing concentrations of starches for the low-amylose gels correlated with increased values of τ and tan δ, but for samples containing $>$20% amylose the values fell as the concentration increased. Tan δ values for non-waxy rice starch gels are quoted as < 0.2 at starch concentrations 15% on first cooling to 25°C [44, 47]. The lower values obtained here correspond to the longer ageing and solidification of the gels, but tan $\delta > 0.2$ are indicative of the low-amylose gels [44]. At the higher starch levels these no-/ low-amylose gels appear to be less "solid" (tan δ increases) when the gels contain more total starch. The concentrations of starch used are far in excess of the c^* values and these starches have high swelling volumes and therefore the paste will mostly consist of close-packed swollen granules with little or no leached amylose between. The amylose contributes not only to the firmness within the swollen granule, but also to interactions between close-packed granules. This results in a network formation and will make an important contribution to the overall modulus. Without the linking of the granules one with another, and perhaps as the concentration becomes greater and the granules contain less water, the elastic nature of the sample is reduced.

For equal concentrations of starch, it can be considered that the amylose is much more effective in producing a high storage modulus than the swollen granule. The leached amylose will self-associate and form strong structures in which the granules are embedded. At high concentration much of the amylose will not leach out of the granule and therefore reinforce the granule as the amylopectin self-associates.

3.3 Kinetics of retrogradation of rice starches gels

Having established the properties of the four Thai starches over a range of concentrations, the changes occurring within the pastes were followed as they were stored for up to 100 h at 5 and 25°C. All the starches studied for this part of the work had a total starch concentration in the range 20–40% and the amylose levels would have been $<$0.7, 4.5, 6.3, 6.7% for the RD6, Jasmin, commercial and Supanburi 1 rice starch, respectively. Amylose could be expected to gel at concentrations in excess of 1.2% [48]. The major impact of the amylose gelation should have

already occurred by the time the samples had cooled from pasting [7], but additional and continuing changes to the amylose should not be completely ruled out.

The development of order within 30% starch gels on storage at 25°C was measured by FTIR and inferred from the storage modulus. The results are shown in Fig. 5. The storage modulus shows no development in structure on storage for the low- and medium-amylose rice whereas the two high-amylose samples show substantial retrogradation, the extent being greatest for the Supanburi 1 sample. There are some differences in the ratio for the FTIR data that corresponds to the amount of order in the samples at time zero. The high-amylopectin samples had the highest initial values, but these did not increase with time. This contrasted with the Supanburi 1 and commercial sample where the amount of FTIR order increased with storage.

The effect of concentration on retrogradation rate of different concentrations of rice starch gels was studied (Fig. 6). It was found that an increase in short-range molecular order with time at 25°C to an apparent plateau

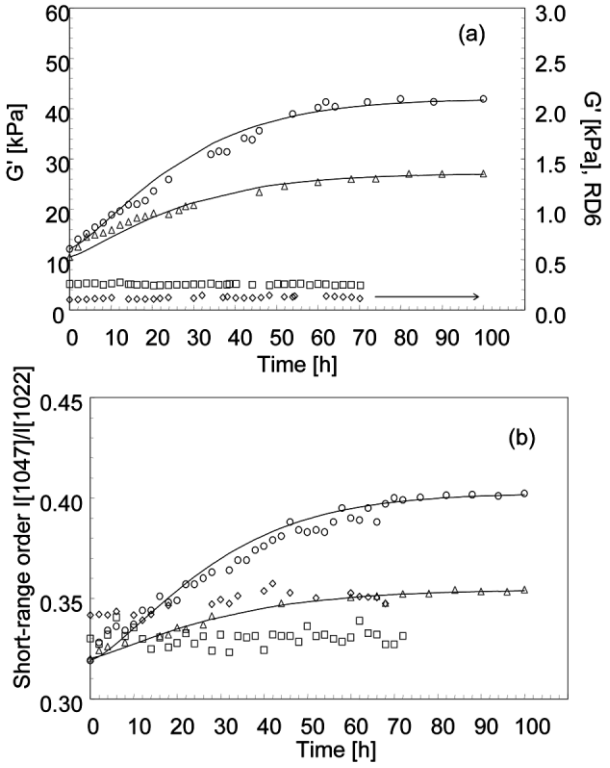

Fig. 5. Structure development during the retrogradation at 25°C of rice starches gels (30%, w/w) measured by rheometer (a) and FTIR ratio of peaks at 1047/1022 (b). Starch gels derived from RD6 (◇), Jasmine (□), commercial (△) and Supanburi 1 rice (○). The lines depict the fit using the Avrami equation.

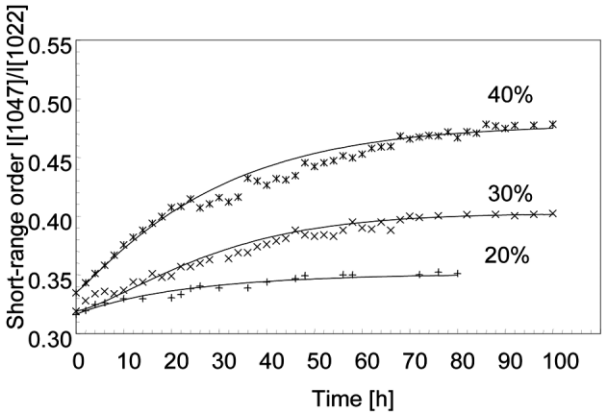

Fig. 6. The recovery of short-range molecular order during the retrogradation (as measured by FTIR) at 25°C of Supanburi 1 rice starch gels at various concentrations.

value was concentration-dependent for Supanburi 1 rice starch (20–40%, w/w). Results suggest that increasing starch concentration leads to a greater proportion of the available material participating in ordered structures, giving rise to more three-dimensional networks in starch gels during retrogradation.

The other method that can be used to see order in starch gels is X-ray diffraction. This revealed that rice starch retrograded, under the conditions used, to a B type polymorph, with peaks at angles of 6 and 17. It would generally be accepted that the crystallisation is due to clusters of short amylopectin chains. It is thought that longer amylopectin chain length favours the B polymorph [49, 50]. Crystallisation can be seen most clearly for the Supanburi starch gel stored at 5°C (Fig. 7), but was also obvious for the commercial rice at 25°C storage.

It is possible to get quantitative data from the diffractograms, but this is difficult for materials like gels where the concentration of the polymer is low. Even if these samples

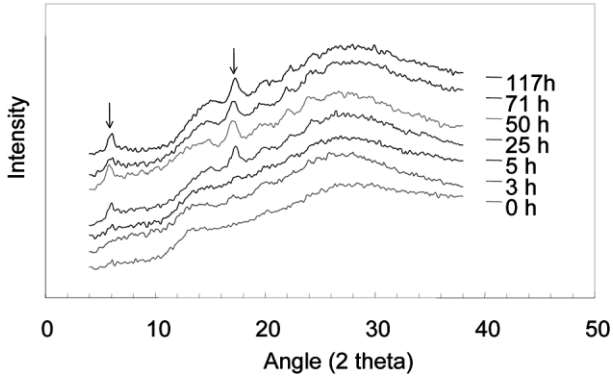

Fig. 7. Crystallinity development in rice starch gels during storage at 25°C and 5°C for 120 h. Arrows denote growing peaks.

had undergone full retrogradation only about 10% of the sample would be crystalline. Measures for the amount of crystallinity have been calculated for the samples on storage and again very low levels of crystallinity were seen for the Jasmine and RD6 samples, while substantial amounts were seen on storage at both 5 and 25°C for the commercial and Supanburi 1 samples. The best estimate of% crystallisation suggests that the commercial high-amylose starch had a higher final crystallinity than the Supanburi 1 starch for storage at 25°C. This is not the same order suggested by the FTIR and rheology data. It is possible that the calculations are complicated by some polymorphism being present in the sample. *Sasaki* et al. [51] used dynamic rheology to follow the development of structure in gels formed from wheat starches of different amylose contents stored at 5°C. The final moduli obtained for the higher amylose starches (amylose content range 18.5 to 28.6%) were similar to that observed in our rice work. The faster kinetics they found could be explained by the lower temperature. For waxy wheat starches (amylose contents 1.4% and 1.7%) they observed significant structure development at 5°C, which we did not see in our X-ray study for the RD6 waxy rice and the Jasmine rice starch. To directly compare the starches it would be necessary to use the same techniques at the same temperatures, but there is a suggestion that gels from the lower amylose rice starches are less susceptible to retrogradation than wheat starches. A possible explanation for these differences in retrogradation is the higher proportion of the very short amylopectin chains (DP < 9) for the lower amylose rice starches (Fig. 1), Compared to high amylose wheats the waxy samples had a higher proportion of the shorter chains, however the numbers of very short chains in the wheat amylopectin were less than in the rice samples. *Shi* and *Seib* [52] found from studies on waxy starches that the short chains reduced retrogradation, but *Würsch* et al. [53] showed that if the A chain length is reduced below DP 10 than retrogradation will not occur.

The rates for retrogradation for our data can be modelled from the Avrami equation:

$$Y(t) = Y_\infty - (Y_\infty - Y_0)\exp(-kt^n) \quad (2)$$

Where Y_∞ is the value of the measured parameter (FTIR, rheology or crystallinity by X-ray) at the longest time and Y_0 is the initial value. The exponent n was taken as 1, because it has been demonstrated that the basic mechanism of retrogradation of starch is instantaneous nucleation followed by a rod-like growth of crystals, but values up to two have also been determined for amylopectin retrogradation [54].

The values obtained for the retrogradation rate (k) are given in Tab. 3. The retrogradation rates tended to decrease with increasing starch concentration over the range investigated. However, if the concentrations were further increased the rates could be expected to be greater [55]. No difference in retrogradation rate was found between the two varieties of high-amylose rice (Tab. 3).

Another useful way of looking at the data for all the methods that show reordering of the starches on storage is to normalise the values to those obtained for the longest storage period. These data are shown in Fig. 8. The Avrami parameters (Tab. 3) that predict the kinetics determined by the FTIR and rheological methods are very similar and this is born out by Fig. 8. However, the kinetic for the development of X-ray crystallinity was much slower compared to the development seen for the other two measurement techniques. If storage was at 5°C the rate of X-ray order was much faster then when the sample was stored at 25°C (Fig. 7).

The data presented here could be explained in terms of molecular ordering and different length scales being detected by the methods. The amount of retrogradation and the rate of retrogradation are also important to distinguish. Previous work has indicated that FTIR gives an indication of order at very small length scales. In studies with waxy maize changes in the FTIR absorbance patterns are seen before changes in NMR or DSC can be detected [54]. It would appear that there is a change in FTIR order in the first five hours of storage and this may well reflect ordering of parts of the amylose chains as well

Tab. 3. Retrogradation rates of rice starches gels (30%, w/w) calculated by the Avrami equation.

Data analysed from	Retrogradation rate [$\times 10^{-5}$ s^{-1}]			
	Commercial rice starch (30%)	Supanburi 1 rice starch		
		20%	30%	40%
Short-range molecular order from FTIR[1]	0.86	1.17	0.88	0.83
Elastic modulus (G')	0.97	–	0.93	–

[1] Ratio of absorbance at wave number 1047 to 1022.

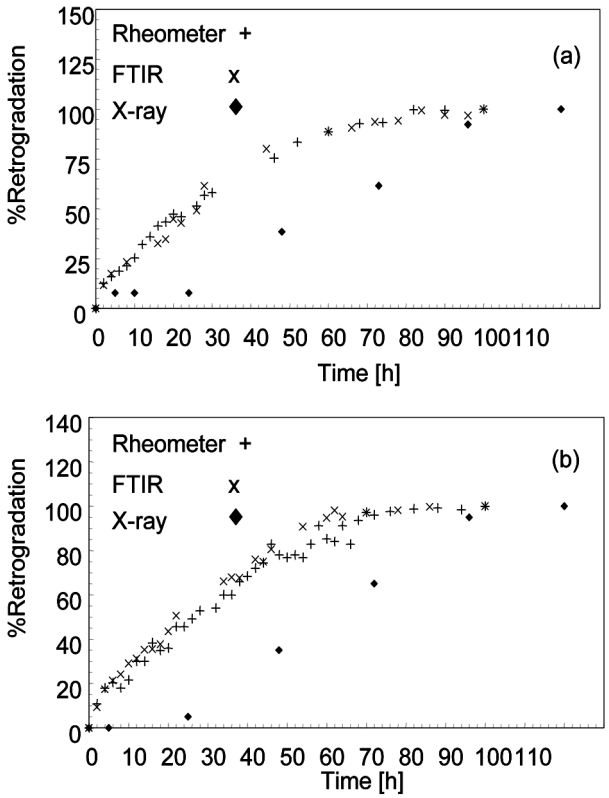

Fig. 8. Comparison of the relative extent of the retrogradation of starches derived from commercial (a) and Supanburi 1 rice (b) as measured by the different physical techniques at 25°C.

as the amylopectin. At the start of storage there is more order for the high-amylopectin samples, but they are quickly superseded by the high-amylose containing samples. In this study the change in the rheology occurs at the same rates as the changes in the FTIR.

The change in the X-ray pattern is due to the amylopectin crystallites and occurs after the changes seen for the viscosity and FTIR. This could be expected if the mechanism is considered to be a helical formation, followed by association of the helices to form structures of longer order. Despite the higher quantities of amylopectin in the waxy samples, no amylopectin retrogradation was seen. From the rheology results and FTIR one would infer that the greatest amount of order occurs in the Supanburi 1 and commercial rice samples, while the low-amylose and waxy rice show no change in structure on storage. When calculated from the X-ray diffractograms the commercial rice sample did seem to produce more crystals than the comparable Supanburi 1 sample. However, much more work would be required to show that under the conditions used the two varieties of rice produced differing amounts and / or types of amylopectin crystals.

Changes during longer term storage is normally ascribed to amylopectin retrogradation. Here the total amount of order and the rate at which this occurs is greatest for samples containing high levels of amylose. The extent to which recrystallisation occurs, as evidenced by the X-ray pattern observed at 5°C, cannot be explained by changes to only the amylose. There are two possibilities as to why there is greater amylopectin retrogradation in the high-amylose starches:

Firstly the low-amylose starches (RD6 and Jasmine rice) have a greater proportion of short chains in their amylopectin and as previously discussed these may reduce the effective recrystallisation of the amylopectin.

Secondly, amylose may play a direct role. It has been suggested [56] that amylose that retrogrades rapidly will form an ordered matrix on a molecular level, which can act as seed nuclei for amylopectin molecules, accelerating the aggregation and crystallisation of amylopectin. Another factor is the possible co-crystallisation of the amylopectin and amylose molecules and the location of these macromolecules [7, 57, 58]. The role of lipids interacting with the amylose or directly or indirectly with the amylopectin may also need to be considered [59, 60].

4 Conclusions

The work in the study shows the strong dependence of the viscoelastic properties of starch gels on the amylose content. These can only be partly explained by an amylose network between granules and reinforcement of swollen granules. The rapid change that occurs on storage is considered to be due to the amylose gelation. This is used to explain the cooling and holding pattern seen in viscograms where “setback” is not high for low-amylose samples. Granule rigidity after pasting will increase with amylose content, not only because of interactions between amylose chains within the swollen granule, but also because of interactions between long B chains of amylopectin. These long chains are present at higher levels in the high-amylose starches. Interactions involving long regions of unsubstituted glucan chains will occur at 60°C and increase in extent with time as the temperature decreases. This gives greater granule rigidity and causes interactions between close-packed granules resulting in network formation and results in a further important contribution to the overall modulus. Changes occurring over long time periods almost certainly involved amylopectin reordering but this is promoted by the presence of amylose.

No single measure would explain the differences seen for these starches, but the concept that short-term changes to starch pastes are due to amylose while the change

during longer term storage is solely due to amylopectin needs to be reconsidered if an understanding of the changes in the eating quality of starch on storage is be understood and predicted.

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