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Architectural changes of heated mungbean, rice and cassava starch granules: Effects of hydrocolloids and protein-containing envelope

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Abstract

Architectural changes of starch granules induced by heat were demonstrated using light microscopy and confocal laser scanning microscopy. Heat treatment (80 °C, 30 min) on mungbean starch, cassava starch and rice flour suspensions resulted in the rearrangement of amylose and granule-associated proteins within the deformed granules. The presence of alginate and carrageenan influenced the RVA pasting characteristics of starch/flour-hydrocolloid mixed suspensions by maintaining the granular structure of amylose-rich swollen granules or inducing the aggregation of the swollen ones. Generally, the addition of hydrocolloid increased peak viscosity, lowered breakdown and reduced setback of the flour-hydrocolloid mixed paste. This study demonstrated that the heat treatment in excess water generated the protein-containing granule envelope encasing the mungbean and cassava starch content within the deformed granules.

Keywords: Architecture; Cassava; Granule; Mungbean; Rice; Starch

1. Introduction

Rice (*Oryzae sativa*, L.), cassava (*Manihot esculenta* Crantz) and mungbean (*Vigna radiata* (L) Wilczec) are major starch crops in Asia. The native starches have been used extensively in traditional starchy foods such as noodles, snacks and desserts in the Orient. In addition, it is a common practice to use mixed flour in creating different textures and appearances. The textural properties of starch pastes and gels can range from firm and chewy mungbean starch vermicelli to soft and sticky cassava-based desserts. These starches/flours have different physicochemical properties; i.e., granular size distribution, swelling power, pasting, gelatinization and retrogradation characteristics (Hongsprabhas, 2006; Kasemsuwan, Bailey, & Jane, 1998; Xu & Seib, 1993).

Although the benefits of hydrocolloid addition to starch functionalities are apparent, the influences of hydrocolloids appear to vary due to the botanical origins of starch. The molecular interactions between starch and gum, extensively investigated during the past ten years, are critical to the overall functionalities of starch-hydrocolloid composites (Alloncle, Lefebvre, Llamas, & Doublier, 1989; Christianson, Hodge, Osborne, & Detroy, 1981; Funami et al., 2005a, 2005b, 2005c; Sae-kang & Suphantharika, 2006; Shi & BeMiller, 2002). However, little attention has been emphasized on the roles of starch granular structure and starch ghosts, which also impart the overall starch functionalities in food products.

The importance of starch ghosts has been underestimated in many of the physically analytical techniques, which treated gelatinized starch as a uniform amylopectin, amylose and non-starch constituents. The starch granule ghosts have been classified as starch granule surface, the granule envelope and the starch granule ghost (Atkin, Abeysekera,

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& Robards, 1998). The starch granule surface is the outermost layer of the granule; while the swollen surface surrounding most of the starch during gelatinization is called the granule envelope. The granule ghost is the remnants of the envelope after the structural collapse, where most of the starch has been liberated. The ghost remnants have been shown to be composed of amylopectin (Atkin et al., 1998). The large structure of starch ghosts, which possesses elastic properties (Atkin et al., 1998), could affect molecular progression after gelatinization, such as phase separation, re-crystallisation and retrogradation of amylose and amylopectin, which contribute to the overall functional properties of starch. Sae-kang and Suphantharika (2006) have demonstrated that the granular ghost structure of cassava starch still exists after gelatinization under shear.

The effects of botanical origins of starch and the architecture of starch granules, before and after heating, were emphasized in this study. The research strategies were to use the anionic hydrocolloids; namely alginate and carrageenan, to alter pasting characteristics and subsequent molecular interactions between hydrocolloids and starch constituents. We hypothesized that the existence of the swollen starch granules in the cooked starch paste, as well as the characteristics of the granule envelope, have played an important role in determining the overall pasting characteristics. The insights in the characteristics of the swollen starch granules, at microscopic scale, could help understanding the contributions of the excluded volume of swollen granules during and after starch/flour pasting.

2. Materials and methods

2.1. Materials

Food grade mungbean starch (MB, Pine Brand, SithiNan, Thailand), rice flour (RF) and cassava starch (CS) (Jade Leaf Brand, Bangkok Interfood, Thailand) were obtained from a local supermarket. MB had 12.01% moisture content and 0.17% protein while RF contained 10.28% moisture and 7.59% protein. The CS contained 12.17% moisture but there was no protein detected by the Kjeldahl method (AOAC, 2000). Commercial grade sodium alginate (Algogel 6021) and sugar standardized carrageenan (Satiagel ME10) were obtained from Degussa Texturant Systems (Thailand). Potassium iodide (APS Finechem, Australia) and Rhodamine B (Invitrogen, USA) were used to stain amylose and protein, respectively.

2.2. Microstructure of starch granules before and after heating

One milliliter of MB, CS or RF suspensions were prepared in distilled water (unless specified) at a concentration of 0.67% (w/v), shaken vigorously and allowed to stand for 10 min at room temperature to absorb water. The suspension was heated in a water-bath at 80 °C for 30 min in a quiescent condition. The sample was cooled down at room temperature (27 °C) for 1 min and centrifuged at 14,000 rpm for 5 min (Spectrafuge 16M, USA) as described by Hongsprabhas (2006). The supernatant liquid was discarded. The sediment obtained after centrifugation was suspended in a 0.5 mL of distilled water and stained with 10% Lugol's iodine solution (Autio & Salmenkallio-Marttila, 2001). The microstructure was examined before and after heating at 80 °C for 30 min under the Leica DME Light Microscope (USA).

A solution of Rhodamine B (0.01% in 95% ethanol) was added to the unheated and heated starch suspensions prepared as described above. After incubation for 5 min, each sample was loaded into a slide well and observed for a location of fluorescent-labelled protein using the Confocal Laser Scanning Microscopy or CLSM (Axio Imager MI, Carl Zeiss PTe Ltd, Germany). An HeNe laser with an excitation wavelength of 543 nm was used. CLSM digital image were acquired using the LSM 5 PASCAL program.

2.3. Viscosity of commercial hydrocolloid gums

The commercial alginate (0.1–1.0% w/v) and carrageenan (0.1–0.3% w/v) were prepared in distilled water, boiled until clear solutions were obtained, cooled down at room temperature for 1 h and equilibrated at 25 °C for 2 h. The viscosity was measured at a shear rate of 12.2–122 s^{-1} using a Brookfield Rheometer (DV III+, USA) equipped with a small sample cell adaptor (UL-adapter, spindle no. 0). The cell was maintained at 25 °C throughout measurement. Reading was taken in triplicates.

2.4. Pasting characteristics

Rapid Visco Analyzer (RVA, Newport Scientific, Warriwood, Australia) was used to characterize pasting properties of mixed flour suspensions containing hydrocolloid gums at specified concentration. The flours were dispersed in the hydrocolloid suspension and incubated at 8 °C overnight to ensure that both starch and hydrocolloid were hydrated prior to heating. Twenty-five milliliters of mixed flour-hydrocolloid suspension containing 12% (w/v) of flour and different concentration of hydrocolloid was heated from 50 to 95 °C at the rate of 12 °C/min, held at 95 °C for 2.50 min, cooled to 50 °C at the rate of 12 °C/min and finally kept at 50 °C at 160 rpm. Amylogram describing pasting characteristics included pasting temperature (temperature where viscosity first increased), peak time (minutes at which the peak viscosity occurred), peak viscosity (the maximum viscosity developed soon after the heating cycle ended), holding strength (viscosity after holding at 95 °C for 2.5 min), breakdown (the viscosity difference between peak viscosity and holding strength), final viscosity (viscosity after cooling at 50 °C for 2 min) and setback viscosity (the viscosity difference between final viscosity and peak viscosity).

2.5. Statistical analysis

The experiments were carried out in two separated trials. Each trial was run in duplicates. The data were analyzed by either Analysis of Variance (ANOVA) with significance at $p < 0.05$, or Non-linear Regression. Significant differences among mean values were determined by Tukey Test. All statistical analyses were performed using the GraphPad Prism Software Version 4.00(Trial) (2003).

3. Results and discussion

The shape of MB starch granules was round to oval with the size ranging from 7 to 28 μm (Fig. 1a), which was slightly larger than that of CS (Fig. 1c). The latter possessed round shape with truncated characteristics. Both starches were much larger in size than the polygonal-shaped RF granule (Fig. 1e). When heating a diluted starch suspension, at 80 °C for 30 min under quiescent condition, it resulted in the deformation (swollen, ruptured and collapsed) of granules in the suspensions as shown in Fig. 1 (b, d, f). It appears that the amylose was retained in the deformed (swollen and collapsed) granular structure of MB and CS. However, the presence of amylose in the starch ghost of RF was not apparent. Our previous investigation suggested that the swollen amylose-rich MB granule could act as a spring determining the elastic properties of MB starch gel with the total solid content between 10%–

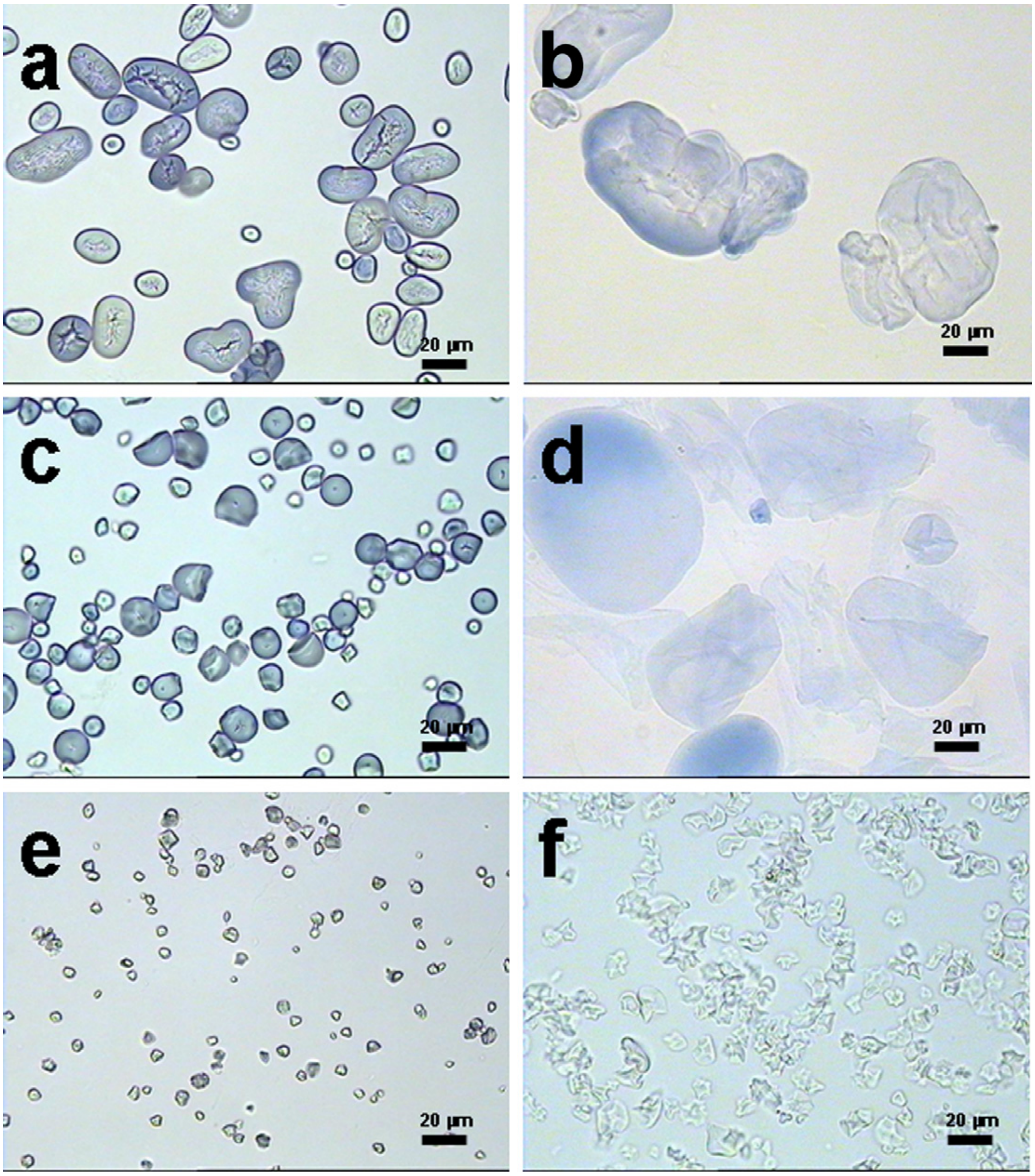

Fig. 1. Light micrographs of unheated (a, c, and e) and heated (b, d, and f) starches. Food grade mungbean starch (a and b), cassava starch (c and d) and rice flour (e and f). Starch/flour suspensions (0.67% (w/v) in distilled water) were heated at 80 °C for 30 min under quiescent condition. Amylose was stained blue with Lugol's iodine solution. Bars = 20 μm.

14% (w/v), and a fractal dimension around 2.63 (Hongsprabhas, 2006). However, both CS and RF cannot form self-supporting gel within that concentration range. The different ability of MB, compared with CS and RF, to form a firm and elastic network could be due to the difference in the granular characteristics of a cooked starch, particularly the strength of the deformed granules after cooking.

We further investigated the influence of hydrocolloid addition on the pasting characteristics of MB. The addition of solubilised alginate to MB was to competitively absorb water against MB during cooking; thus further restricted the swelling of MB granules. It has been known that the addition of hydrocolloid gum to starch suspensions is to modify and control the rheological properties of starches by influencing their gelatinization, retrogradation and gelation characteristics (Alloncle et al., 1989; Christianson et al., 1981; Funami et al., 2005a, 2005b, 2005c; Sae-kang & Suphantharika, 2006; Shi & BeMiller, 2002). The influences of gums on overall viscosity of starch–gum mixture could be attributed to the retardation of viscosity increase due to the decreased amount of leached amylose. Moreover, the association of leached amylose with gums could lead to a decrease in retrogradation, phase separation of starch and gums and/or the enhancement of amylose gelation. The RVA pasting characteristics of the MB added with various concentrations of commercial alginate were summarized in Table 1 and Fig. 2.

The pasting temperature of MB and MB-alginate mixed suspensions were not significantly different ($p \geqslant 0.05$). However, the time required for the suspensions to reach peak viscosity increased in the presence of alginate ($p < 0.05$). Nevertheless, the addition of alginate drastically increased the peak viscosity of MB-alginate mixed suspension during the heating stage almost twice when the alginate was present at the level of 1.0%. The ability of alginate to retard the viscosity increase of MB starch conforms the observation reported by Shi and BeMiller (2002) on the influences of hydrocolloid addition on pasting characteristics of mixed starch-hydrocolloid suspension.

However, the restriction of amylose leaching due to the presence of alginate could consequently affect further physical characteristics of cooked paste during holding stage at 95 °C. Although both cooked MB and MB-alginate mixed pastes showed time-dependent shear-thinning at 95 °C, raising the alginate concentration resulted in an increase of the holding strength ($p < 0.05$, Table 1). This suggests that the presence of alginate assisted the cooked MB-alginate mixed paste to be more resistant to shear. We hypothesizes that the more rigid deformed MB granules with increasing the alginate concentration are responsible for shear-resistant characteristics. This is probably due to the retained amylose within the swollen granules at high alginate level.

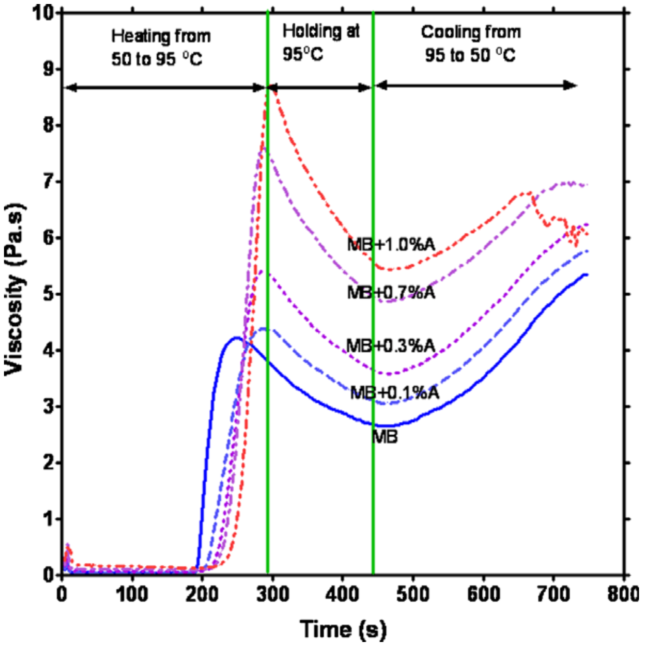

Fig. 2. RVA pasting characteristics of mixed mungbean starch-alginate suspension at different alginate concentration.

During cooling stage, where the temperature was lowered from 95 to 50 °C, the cooked MB-alginate pastes containing 0.7–1.0% alginate showed cooling stage viscosity peak. In addition, they had lower setback values than the cooked MB and MB-alginate pastes containing less alginate. The higher final viscosity of the cooked MB-alginate mixed paste, at elevated alginate concentration, indicated the rheopectic characteristics of the cooked MB-alginate paste under constant shear rate during cooling. The peak viscosity during cooling stage corroborated the assumption

Table 1
Effect of commercial alginate concentration on RVA pasting characteristics of mungbean starch-alginate mixed suspensions

Pasting characteristics	Alginate concentration (% w/v)				
	0.0	0.1	0.3	0.7	1.0
Pasting temperature (°C)	64.93^{a}	63.30^{a}	64.07^{a}	66.82^{a}	65.25^{a}
Peak time (min)	4.23^{b}	4.84^{a}	4.86^{a}	4.87^{a}	5.00^{a}
Peak viscosity (Pa s)	4.21^{e}	4.38^{de}	5.40^{c}	7.60^{b}	8.73^{a}
Holding strength (Pa s)	2.64^{e}	3.05^{d}	3.57^{c}	4.87^{b}	5.43^{a}
Breakdown viscosity (Pa s)	1.57^{cd}	1.32^{d}	1.82^{c}	2.73^{b}	3.30^{a}
Final viscosity (Pa s)	5.34^{d}	5.77^{c}	6.22^{b}	6.94^{a}	6.08^{bc}
Setback (Pa s)	2.70^{a}	2.72^{a}	2.66^{a}	2.07^{b}	0.64^{c}

Means in the same row followed by different superscript are significantly different ($p < 0.05$).

that the three-dimensional network, formed at high alginate concentration, is broken up under prolonged shearing.

The structure-forming ability of the cooked MB-alginate mixed paste during cooling stage, shown as a second peak viscosity in the presence of 0.7% and 1.0% alginate, suggests the interactions of the cooked starch and alginate. This could be due to the entanglement of the alginate and the leached amylose in the bulk phase surrounding the swollen starch granules. Another possibility is the formation of a close-packed amylose-containing swollen granule. It is likely that the swollen granules are structurally maintained by the constituents retained within the granular structure, particularly at the high concentration of alginate (i.e., above 0.7% (w/v)). The final viscosity of MB-alginate pastes were in the range of 5.77–6.08 Pa s, which is higher than those of alginate (5–180 mPa s, Fig. 3) and the cooked MB paste (5.34 Pa s). The result suggests the synergistic effects of alginate addition, at high level as 0.7–1.0% (w/v), on the structure-forming process of the cooked MB-alginate mixed paste during cooling.

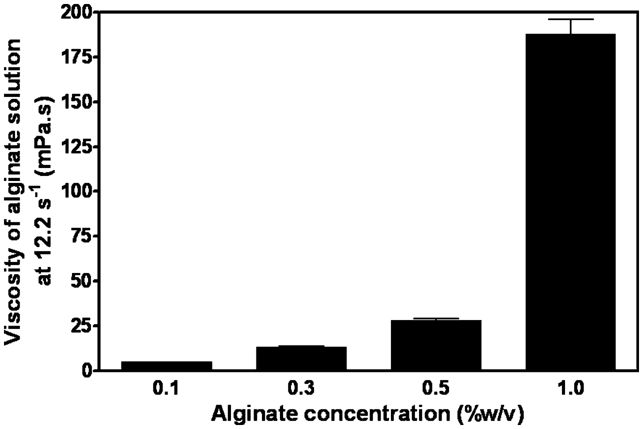

Fig. 3. Effect of alginate concentration on solution viscosity of alginate at 25 °C. Bars represent standard deviation.

The effects of alginate and carrageenan solutions, at a low concentration (0.1% w/v) on the RVA pasting profile of CS, RF and their mixture were summarized in Tables 2 and 3, as well as Fig. 4. The addition of both hydrocolloids had no significant effect on pasting temperature and peak time of each starch/flour suspension ($p \geqslant 0.05$). The difference between the peak time values was rather dependent on the type of starch/flour than the presence of hydrocolloids. The addition of alginate and carrageenan, at 0.1% (w/v), slightly increased the peak viscosity and holding strength of CS ($p < 0.5$). However, it had no significant effect on breakdown viscosity, final viscosity and setback of CS ($p \geqslant 0.05$). In contrast, the addition of both hydrocolloids drastically increased holding strength and lowered the setback of RF ($p < 0.5$) but had no effect on its peak viscosity ($p \geqslant 0.05$). The effects of either alginate or carrageenan addition (at low concentration as 0.1% w/v) on the RVA pasting parameters of CS+RF mixed flour were in between those of CS and RF.

Table 2

RVA pasting characteristics of cassava starch (CS), rice flour (RF) and their mixture (CS+RF at the ratio of 1:1) added with 0.1% commercial alginate (A)

Pasting characteristics	Mixed flour sample					
	CS	CS+0.1%A	CS+RF	CS+RF+0.1%A	RF	RF+0.1%A
Pasting temperature (°C)	63.28^{a}	64.88^{a}	65.70^{a}	63.63^{a}	67.25^{a}	62.43^{a}
Peak time (min)	3.97^{c}	3.87^{c}	5.57^{b}	5.57^{b}	6.50^{a}	7.00^{a}
Peak viscosity (Pa s)	4.79^{b}	5.34^{a}	3.50^{de}	4.03^{c}	2.97^{e}	3.26^{e}
Holding strength (Pa s)	1.77^{c}	1.90^{c}	2.23^{b}	2.86^{a}	2.39^{b}	3.16^{a}
Breakdown viscosity (Pa s)	3.02^{a}	3.45^{a}	1.28^{b}	1.17^{b}	0.58^{c}	0.10^{c}
Final viscosity (Pa s)	2.53^{b}	2.54^{b}	3.36^{c}	3.91^{c}	5.56^{a}	5.49^{a}
Setback (Pa s)	0.76^{d}	0.64^{d}	1.14^{cd}	1.05^{d}	3.17^{a}	2.34^{b}

Means in the same row followed by different superscript are significantly different ($p < 0.05$).

Table 3

RVA pasting characteristics of cassava starch (CS), rice flour (RF) and their mixture (CS+RF at the ratio of 1:1) added with 0.1% commercial carrageenan (C)

Pasting characteristics	Mixed flour sample					
	CS	CS+0.1%C	CS+RF	CS+RF+0.1%C	RF	RF+0.1%C
Pasting temperature (°C)	62.45^{a}	63.73^{a}	62.90^{a}	62.53^{a}	64.16^{a}	64.05^{a}
Peak time (min)	5.47^{d}	5.60^{cd}	3.90^{f}	4.04^{ef}	6.37^{b}	6.90^{a}
Peak viscosity (Pa s)	3.70^{c}	3.74^{b}	4.92^{a}	5.04^{a}	3.10^{de}	3.05^{e}
Holding strength (Pa s)	2.18^{d}	2.47^{bc}	1.75^{f}	1.90^{ef}	2.35^{cd}	2.75^{a}
Breakdown viscosity (Pa s)	1.53^{bc}	1.27^{c}	3.17^{a}	3.15^{a}	0.76^{d}	0.30^{e}
Final viscosity (Pa s)	3.36^{d}	3.46^{cd}	2.61^{ef}	2.53^{f}	5.73^{a}	4.99^{b}
Setback (Pa s)	1.18^{cd}	0.98^{de}	0.86^{de}	0.64^{e}	3.38^{a}	2.24^{b}

Means in the same row followed by different superscript are significantly different ($p < 0.05$).

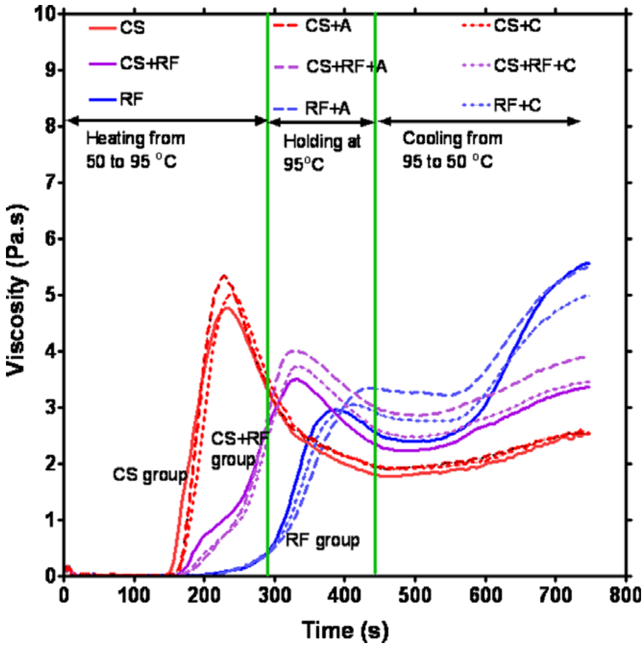

Fig. 4. RVA pasting characteristics of mixed cassava starch (CS), rice flour (RF) and their mixture (CS+RF) in the presence of 0.1% alginate (A) or 0.1% carrageenan (C).

Nevertheless, the shape of each RVA pasting profile, during heating and holding stages, merits further comparative characterization mathematically. This is due to the influences of the swollen granules on the viscosity profiles in both stages. The pasting characteristics of cooked mixed pastes during cooling stage were not included in this study. This is because after shear-induced disruption of the swollen granules during the holding stage at 95 °C, the polydispersed characteristics of the sheared and cooked pastes would be even so complicated that the simple mathematical equation cannot describe the viscosity of such water-in-water (w/w) mixed pastes. The mixed pastes during cooling stage would contain swollen granules, the granule ghosts, helical leached amylose, randomly oriented and opened chain amylose, amylose-rich starch remnants, granular amylopectin in the remnants and disrupted remnants (Hermansson & Svegmark, 1996).

The viscosity profiles of MB, CS and RF, in the absence and presence of hydrocolloids, during heating and holding stages were characterized mathematically by a Non-linear regression and their half-time values (t_{half}) at each stage were summarized in Table 4. The viscosity data during heating stage were fitted from time zero (t_0, at 50 °C) to the time that peak viscosity was achieved (t_{end}, at 95 °C). The temperature-dependent dilatancy characteristics of each amylogram during heating stage were fitted using Boltzmann Sigmoidal curve as follows:

$$\eta_t = \eta_{t_0} + \frac{\eta_{t_{\mathrm{end}}} - \eta_{t_0}}{1 + \mathrm{e}^{\frac{t_{\mathrm{half}} - t}{\mathrm{slope}}}}$$

This equation describes the viscosity increase (η_t) as a function of time (t) when the temperature was increased during heating stage. The viscosity varied from time zero of each stage (t_0) to the viscosity at the terminal time selected (t_{end}), at which the maximum value of the sigmoidal curve was achieved. Half-time (t_{half}) is the time at which the viscosity value of each curve, is halfway between t_0 and t_{end}. *Slope* describes the steepness of curve, with a larger value denoting a shallow curve.

The amylogram during holding stage, representing time-dependent shear-thinning characteristics at 95 °C, was fitted between the time that the peak viscosity started to drop and the time that cooling process began. The following exponential decay equation was used to describe the kinetics of time-dependent shear-thinning of cooked starch/flour, in the absence and presence of hydrocolloids. The viscosity starts at η_0 and decreases to plateau with the rate constant K and the half-time (t_{half}) of the decay is $0.693/K$.

$$\eta_t = \eta_0 * \mathrm{e}^{-K*t} + \eta_{\mathrm{plateau}}$$

The microstructural changes of starch granule during heating stage include absorption of water, granular swelling, loosening crystalline structure within the granules and leaching of amylose (Rao, Okechukwu, Da Silva, & Oliveira, 1997; Rao & Tattiyakul, 1999). Although the addition of 0.1% (w/v) of hydrocolloids had no significant effect

Table 4
Non-linear curve fitting of viscosity (dependent variable) and time (independent variable) of cooked mungbean (MB), cassava starch (CS), rice flour (RF) in the absence and presence of commercial alginate (A) or carrageenan (C) during heating and holding stages

Mixed flour and hydrocolloid	Heating from 50 to 95 °C, up to peak viscosity (Boltzmann sigmoidal equation)			Holding at 95 °C (Exponential decay equation)		
	Half-time (s)	R^2	Absolute sum of square	Half-time (s)	R^2	Absolute sum of square
MB	207.3	0.9975	0.7281	97.9	0.9957	0.0816
MB+0.1% alginate	236.0	0.9975	0.7169	145.8	0.9947	0.0676
CS	183.4	0.9971	0.6496	51.7	0.9940	0.4675
CS+0.1% alginate	190.7	0.9873	3.6680	46.0	0.9940	0.6207
CS+0.1% carrageenan	197.2	0.9936	1.1350	46.6	0.9942	0.4907
RF	325.8	0.9963	1.2890	NA	NA	NA
RF+0.1% alginate	352.2	0.9984	0.5247	NA	NA	NA
RF+0.1% carrageenan	339.4	0.9976	0.8315	NA	NA	NA

NA = not available, the data did not fit with exponential decay equation or linear equation.

on pasting temperature of all strach/flour (Tables 2 and 3), Table 4 shows that the addition of 0.1% (w/v) alginate slightly increased the half-time values of MB and RF. Both starch/flour had lower water absorption than the CS (Hongsprabhas, 2006) and were likely to maintain more rigid granular structure at the end of heating stage in the presence of hydrocolloids due to the retardation of amylose leaching. High water absorption ability of CS (Hongsprabhas, 2006), however, could lead to the less influence of hydrocolloid addition, at the level of 0.1% (w/v), during heating stage. Thus, the rate of viscosity increase and the magnitude of peak viscosity of the cooked pastes during heating stage could arise from the friction of the swollen starch granules possessing different rigidity.

However, the influence of hydrocolloid addition on the time-dependent shear-thinning characteristics at 95 °C of MB, CS and RF appeared to be different. Although the addition of 0.1% (w/v) alginate extended the half-time values of cooked MB-alginate mixed paste during holding stage, it had no significant effect on CS-hydrocolloid mixed pastes (Fig. 4 and Table 4). The slight decrease in viscosity of the cooked RF paste and the cooked RF-hydrocolloid mixed pastes, during holding stage (Fig. 4), suggests that the RF and RF-hydrocolloid mixed pastes were the most shear-resistance.

A difference in the pasting profile of starch and flour from different botanical sources, in the absence or presence of hydrocolloids, could be due to the differences in the microstructural elements responsible for the overall paste viscosity. The CLSM is quite a powerful technique in characterizing and positioning some constituents in starch granule such as protein (Han & Hamaker, 2002). Fig. 5a and c illustrate the arrangement of the protein fractions in MB and CS unheated granules fluoresced in red colour under

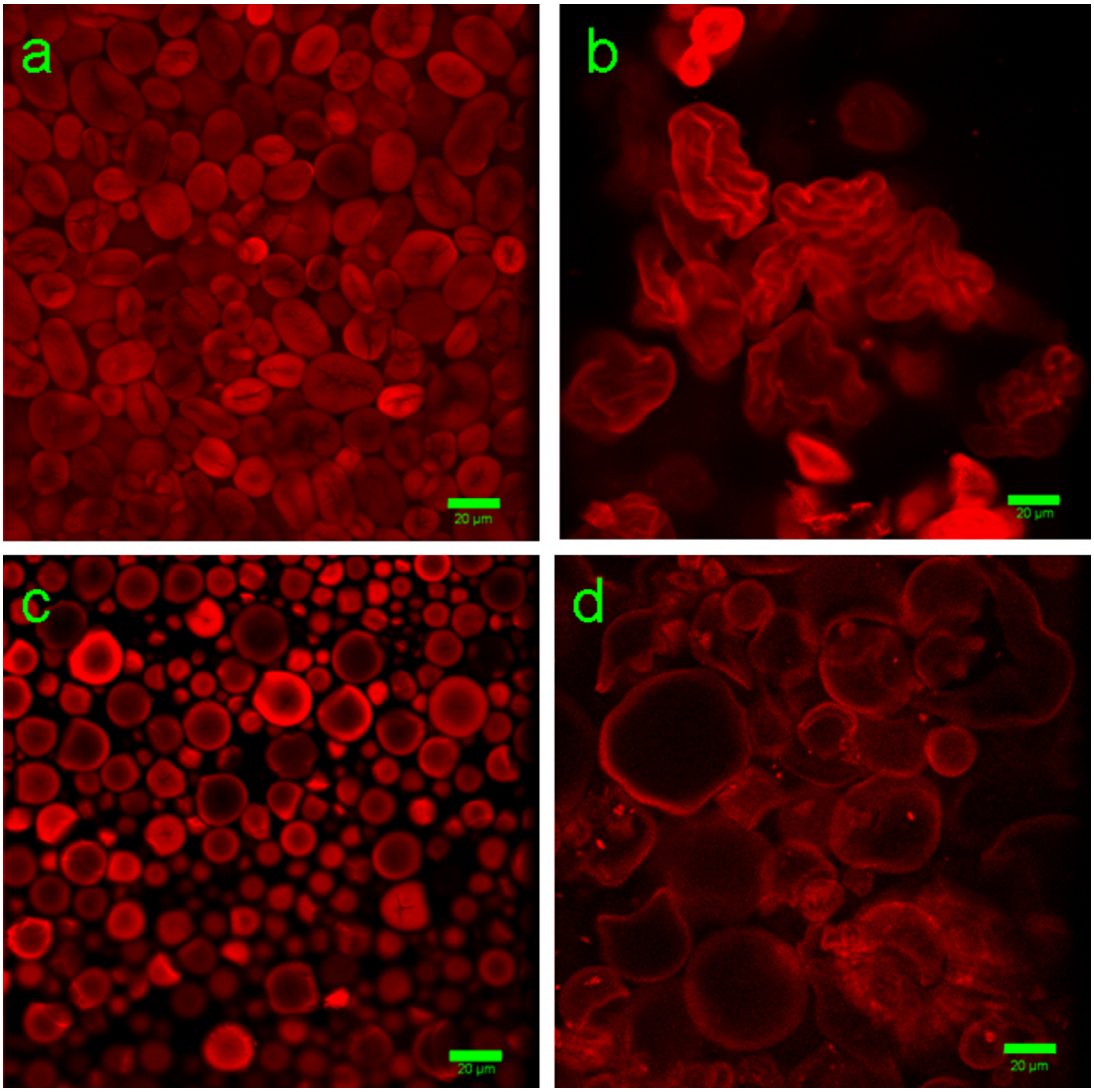

Fig. 5. Confocal laser scanning micrographs of unheated and heated mungbean and cassava starch. Starch suspensions (0.67% (w/v) in distilled water) were heated at 80 °C for 30 min under quiescent condition. Proteins were stained with Rhodamine B, appeared in red fluorescence. (a) unheated mungbean starch, (b) heated mungbean starch, (c) unheated cassava starch and (d) heated cassava starch. Bars = 20 µm.

CLSM. The resulting intrinsic deformability of each starch from different botanical origins might be due to the different assembly of protein within the granules. Note that both heated MB and CS granules contained amylose (Fig. 1) but CS had lower degree of shear resistance during holding stage (seen as shorter half-time, Table 4). Figs. 1 and 5 show that in the unheated MB starch, amylose and protein aligned themselves fairly evenly in the array of growth rings, respectively. Unlike the MB, the presence of amylose and protein in the unheated CS granules appeared to be less ordered. The protein fraction of the unheated CS concentrated at the edge of most granules. Both food grade MB and CS were obtained from wet-milling process. The soaking process of both MB and CS during wet-milling is usually more than 12 h. At this stage, the water-soluble proteins could diffuse into the granules and be present at the perimeter of the granules during drying process.

The heated MB and CS granules, however, show different locations of protein (Fig. 5b and d) compared to the unheated ones. After heat treatment at 80 °C for 30 min, the protein fraction progressed to the circumference of the granules. This protein fraction could help maintaining the integrity of the granule envelope after heattreatment apart from the amylopectin fraction reported by Atkin et al. (1998). The brighter red protein fraction shown in the heated MB granules suggests the higher protein level in the envelope compared to that of CS. The ability of the protein-containing envelope to maintain the starch con-

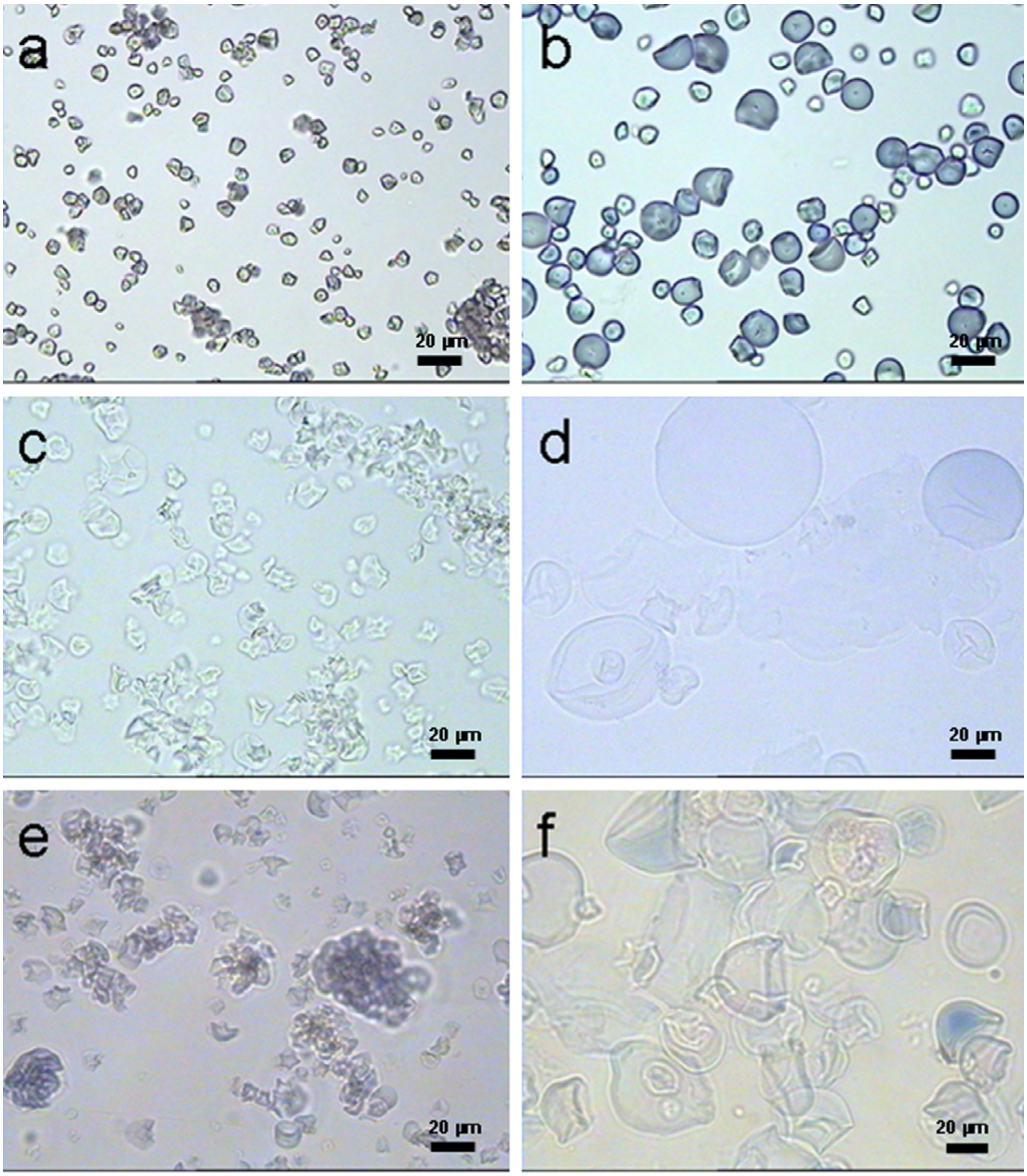

Fig. 6. Light micrographs of food grade rice flour (a, c, and e) and cassava starch (b, d, and f). Starch/flour suspensions (0.67% (w/v)) were prepared in distilled water before heating (a and b); after heating at 80 °C for 30 min (c and d); and in 0.07% (w/v) carrageenan solution and heated at 80 °C for 30 min (e and f). Bar = 20 µm.

stituents, including amylose, within the deformed granules may be responsible for the low swelling power (Oates, 1990), low water absorption ability (Hongsprabhas, 2006) and the more shear-resistant characteristics of MB in the holding stage at 95 °C, compared to the CS (characterized by the RVA) as previously described.

The influences of anionic hydrocolloids in preventing the time-dependent shear-thinning of RF, compared to CS, could be described by the aggregation of heated amylose-rich RF swollen granules induced by the presence of carrageenan (Fig. 6). Heating of the RF in distilled water resulted in the leaching of amylose out of the swollen ghosts; while retaining the amylose within the CS ones (Fig. 1). However, in the absence of hydrocolloids, the heated RF paste was more shear-resistant than the heated CS, observed as the higher magnitude of holding strength and the lower value of breakdown (Tables 2 and 3, Fig. 4). This could be due to the much smaller size of RF ghosts, compared with the CS ones, which resulted in the denser RF ghost particles in the cooked paste containing similar concentration as the CS. In addition, our previous report indicated that the lower water absorption ability of RF compared to CS (Hongsprabhas, 2006) could consequently contribute to the more rigid RF ghosts than the CS ones. The addition of carrageenan even further increased the resistance to shear of the heated RF pastes by inducing the aggregation of the amylose-rich RF fraction as shown in the light micrographs (Fig. 6).

4. Conclusions

The overall effects of hydrocolloid addition on the pasting characteristics of starch-hydrocolloid mixed systems in this study are in good agreement with the results reported by other investigators (Alloncle et al., 1989; Christianson et al., 1981; Funami et al., 2005a, 2005b, 2005c; Lai, Huang, & Lii, 1999; Sae-kang & Suphantharika, 2006 & Shi & BeMiller, 2002). This is most likely due to the interference of soluble starch, the molecular incompatibility, the exclusion effects of swollen granules and the combined interactions within the polydispersed composite cooked pastes. However, this study further demonstrates that the pasting characteristics of starch and flour suspensions also relied upon the restriction of amylose leaching, the maintenance of swollen granules' rigidity, and the aggregation of the amylose-rich fraction, particularly those involving granule-associated proteins. It is also the first report illustrating the redistribution of protein in unheated and heated MB and CS starch granules. Further investigations on the influences of granule-associated proteins on the architecture and granular strength of MB and CS are underway.

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