

# Influences of granule-associated proteins on physicochemical properties of mungbean and cassava starches

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## Abstract

The architecture and physicochemical properties of mungbean starch (MB) and cassava starch (CS) granules were modified by calcium cross-linking under extreme alkaline conditions (pH ~ 12) and the addition of cysteine and calcium lactate at slightly acidic conditions (pH ~ 5–6). The presence of calcium lactate up to 100 mM at pH ~ 12 resulted in the low swelling of heated starch granules but high degree of starch leaching. Confocal laser scanning microscopy indicated that the formation of a rigid starch envelope induced by Ca<sup>2+</sup> cross-linking under extreme alkaline pH led to the massive leaching of starch content without collapsing the envelope and consequently lowered the final viscosity of both MB and CS ( $P < 0.05$ ). However, the addition of both cysteine and calcium lactate increased gelatinisation temperature of both starches. Results suggested that the protein-containing envelope plays an important role in determining the granule's ability to retain the starch content after heat treatment and subsequent pasting and thermal characteristics of MB and CS.

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## 1. Introduction

Starch gelatinisation can be defined as the disruption of the ordered-structure in starch granules, at a supramolecular level, due to the loss of crystallinity and swelling of the granules (Donovan, 1977). This process involves a hydration of starch, heat absorption, loss of crystallinity and disruption of granular structure if shear is involved (Hermansson & Svegmarm, 1996). The granule envelopes are formed during gelatinisation and they could degrade into amylopectin-rich ghost remnants after releasing all the starch content (Atkin, Abeysekera, & Robards, 1998). In addition, we have recently shown that the heat treatment in excess water could generate the protein-containing granule envelope encasing the mungbean and cassava starch content within the deformed granules due to the redistribution of the granule-associated proteins inside the granules

to the surface (Hongsprabhas, Israkarn, & Rattanawat-anaprakit, 2006). These granule-associated proteins could be involved in determining the viscoelastic characteristics of the ghost remnants, and consequently the heated starch granules.

By definition, starch granule-associated proteins are defined as the proteins naturally positioned in and on starch granules (Baldwin, 2001). They are different from storage proteins and are bound tightly on the surface and/or as integrated constituents within the granule structure. These proteins are mainly starch biosynthetic enzymes and have molecular weight around 5–149 kDa (Baldwin, 2001). Although present in minute quantities, the starch granule-associated proteins influence the rheological properties of starch paste as shown in maize starch (Han, Campanella, Guan, Keeling, & Hamaker, 2002). The limited swelling power of legume starch is also influenced by the existence of the peptide bridges in the starch granules that maintains the structure of the starch granule ghost (Oates, 1990).

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Mungbean (*Vigna radiata* (L.) Wilczec) and cassava (*Manihot esculenta* Crantz) are major starch crops in Thailand. Mungbean starch has the unique characteristics of having a low glycemic index carbohydrate and containing a potentially high resistant starch that have been attributed to its high amylose content and the molecular structure of the amylopectin (Biliaderis, Grant, & Vose, 1981; Biliaderis, Maurice, & Vose, 1980; Kasemsuwan, Bailey, & Jane, 1998). A comparative study among legume starches carried out by Zhou, Hoover, and Liu (2004) suggested that the rate and the extent of starch hydrolysis by  $\alpha$ -amylase was also affected by the structural organisation of the starch chains within the granule and by the extent of hydrolysed amylose chain interactions. Cooked legume starches are usually prone to retrograde and generate resistant starch (Tovar, Björck, & Asp, 1992) and this leads to their low glycemic index carbohydrate for human consumption (Juli-ano, Perez, Komindr, & Banphotkasem, 1989; Komindr, Ingsriswang, Lerdvuthisophon, & Boontawee, 2001; Madar & Stark, 2002). It is apparent that the glycemic index is regulated by the botanical source of starch and the downstream processes used in food manufacturing.

In this study, we focused on the contribution of the granule-associated proteins on the structural organisation of the starch granules before and after heat treatment. We hypothesized that the starch constituents of mungbean and cassava starch are compartmentalized within the deformed granules after heat treatment in excess water. The mechanisms involved in maintaining starch content within the deformed heated granules could be affected by the minute amount of protein present in the granules; and the viscoelastic properties of the granule envelope that helps retaining the starch content. The objective of this study was to further demonstrate the significance of the protein-containing starch envelope on the physicochemical properties of heated mungbean and cassava starches. The roles of protein–protein, starch–starch and protein–starch associations in maintaining the integrity of starch envelope were altered by alkaline treatment, calcium lactate and cysteine addition. A comparative study between mungbean and cassava starches towards the characterization of the protein-containing envelope may enhance understanding of the structural factors governing the starch functionalities for both nutritional and food processing challenges.

## 2. Materials and methods

### 2.1. Materials

Food grade mungbean starch (MB, Pine Brand, SithiNan, Thailand) and cassava starch (CS) (Jade Leaf Brand, Bangkok Interfood, Thailand) were obtained from a local supermarket. Congo Red (Ajax Finechem, Australia) and Rhodamine B (Invitrogen, USA) were used to stain starch and protein, respectively. The starches were proximately analysed for moisture, protein, lipid, ash and crude fibre (AOAC, 2000).

### 2.2. Granule morphology before and after heating

One milliliter of MB and CS suspensions were prepared in distilled water or NaOH dispersants containing different concentrations of cysteine and/or calcium lactate using the stock solutions of 1 M NaOH, 100 mM cysteine and 200 mM calcium lactate. The suspensions contained the final concentrations of 0.67% (w/v) starch, 0–100 mM NaOH, 0–25 mM cysteine and/or 0–100 mM calcium lactate. They were shaken vigorously and allowed to stand for 10 min at room temperature to absorb water, heated in a water-bath at 80 °C for 30 min in a quiescent condition, cooled down at room temperature (27 °C) for 1 min and centrifuged at 14,000 rpm for 5 min (Spectrafuge 16 M, USA) as described by Hongsprabhas et al. (2006). The supernatant liquid was discarded. The sediment obtained after centrifugation was suspended in a 0.5 mL of distilled water and stained with Congo Red solution (1% in distilled water). The microstructure was examined under a Leica DME Light Microscope (USA) before and after heating at 80 °C for 30 min.

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. Pasting characteristics

Rapid Visco Analyzer (RVA, Newport Scientific, Warriwood, Australia) was used to characterise pasting properties of starch suspensions containing different modifiers. The starches were prepared in the canisters using distilled water or NaOH solution containing different concentrations of cysteine and/or calcium lactate as dispersants. The stock solutions of 1 M NaOH, 100 mM cysteine and 200 mM calcium lactate were used. The starch suspensions contained the final concentrations of 12% (w/v) starch, 0–100 mM NaOH, 0–25 mM cysteine and/or 0–100 mM calcium lactate. Twenty-five milliliter of starch suspensions was held at 50 °C for 1 min, heated from 50 to 95 °C at the rate of 13 °C/min, held at 95 °C for 2.70 min, cooled to 50 °C at the rate of 11.5 °C/min and finally kept at 50 °C at 160 rpm for 1.24 min according to the AACC method 61-02 (AACC, 1995). Pasting characteristics were described using amylograms including 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).

## 2.4. Thermal properties

Differential scanning calorimetry (DSC) was used to determine the thermal properties of food grade starches. Briefly, a Pyris1 DSC (Perkin Elmer, USA) was used to characterize the thermal properties of 12% (w/w) of starch in different dispersants. The starches were dispersed in distilled water or NaOH solutions containing different concentrations of cysteine and/or calcium lactate. The stock solutions of 200 mM NaOH, 100 mM cysteine and 200 mM calcium lactate solution were used to prepare starch suspensions in 0–100 mM NaOH, 0–25 mM cysteine and 0–100 mM calcium lactate. The suspension was incubated at 25 °C for 10 min in a stainless steel pan and hermetically sealed prior to the measurement.

The samples were heated at a rate of 5 °C/min from 25 to 95 °C to determine the transition temperature and enthalpy of gelatinisation. The transition temperatures reported were the onset ( $T_o$ ), peak ( $T_p$ ) and end ( $T_e$ ) temperatures of the gelatinisation endotherm. The enthalpy of the gelatinisation ( $\Delta H$ ) was estimated by integrating the area between the thermogram and a base line connecting the points of onset and end temperature and expressed in J/g starch (db).

## 2.5. Statistical analysis

The experiments were carried out in two separated trials. Each trial was run in duplicates. The data were analyzed by analysis of variance (ANOVA) with significance at  $P < 0.05$ . Significant differences among mean values were determined by Duncan's multiple range test. All statistical analyses were performed using the SPSS Software Version 12. Non-linear regression was performed using the GraphPad Prism Software Version 4.00(Trial) (2003).

## 3. Result and discussion

Both commercial MB and CS starches had a very low protein, fat, fibre and ash content (Table 1). The MB starch granules were round to oval and irregular in shape. The damaged starch is shown in red when stained with Congo Red (Fig. 1a). Upon heating at 80 °C for 30 min, the gran-

Table 1  
Chemical composition (mean  $\pm$  standard deviation) of food grade mungbean and cassava starches

Constituent	Mungbean starch	Cassava starch
Moisture (% wb)	11.35 <sup>b</sup> $\pm$ 0.03	11.95 <sup>a</sup> $\pm$ 0.02
Protein (% wb)	0.16 <sup>a</sup> $\pm$ 0.01	0.16 <sup>a</sup> $\pm$ 0.01
Fat (% wb)	0.00 <sup>a</sup> $\pm$ 0.00	0.02 <sup>a</sup> $\pm$ 0.01
Ash (% wb)	0.08 <sup>b</sup> $\pm$ 0.01	0.19 <sup>a</sup> $\pm$ 0.01
Fibre (% wb)	0.08 <sup>a</sup> $\pm$ 0.04	0.15 <sup>a</sup> $\pm$ 0.01
Carbohydrate (% wb – by difference)	88.34 <sup>a</sup> $\pm$ 0.00	87.54 <sup>b</sup> $\pm$ 0.07

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

ules swelled and the absorption of water allows Congo Red to react with the residual amylose (Carroll & Cheung, 1964) shown in red (Fig. 1c). Note that some of the amylose was retained in the heated MB granules. Fig. 1b shows alkaline swelling of the unheated MB granules at pH 12. The granules disintegrated after heating at 80 °C for 30 min (Fig. 1d). However, the presence of calcium lactate (50 and 100 mM) in the alkaline suspensions maintained the ghost remnant structure although the starch content was leached out after heating (Fig. 1e and f, respectively).

The purpose of calcium lactate addition to the MB suspension at pH  $\sim$  12 was to cross-link the negatively charged molecules, which could be the ionized OH<sup>-</sup> of amylose and amylopectin at extremely high pH (Suortti, Gorenstein, & Roger, 1998), as well as the charged proteins in the starch granules. Fig. 2a and b illustrate the arrangement of the protein fractions in the unheated MB and CS granules, which fluoresced in red under the CLSM. Heat treatment (80 °C, 30 min under quiescent condition) applied to MB and CS granules at pH  $\sim$  12 induced the disintegration of granule remnants and dispersed the proteins (Fig. 2c and d). Although the presence of calcium lactate helped stabilise the structure of ghost remnants of both MB and CS after heating under extreme alkaline conditions (Fig. 2e–h) by maintaining the proteins within the remnant structure, some proteins still present outside the remnants due to the alkaline solubilisation shown as diffused red colour in the CLSM (Fig. 2).

Both light micrographs and confocal laser scanning micrographs clearly showed that the presence of proteins within starch granule, which redistributed to the granule envelope upon heating in excess water, was involved in the preservation of the ghost remnant structure. This protein fraction could help maintaining the integrity of the envelope after heat-treatment apart from the amylopectin fraction reported by Atkin et al. (1998). It is also possible that these granule-associated proteins are responsible for the determination of granular swelling characteristics of starch. Upon heating the MB and CS in the presence of calcium lactate at pH  $\sim$  12, the Ca<sup>2+</sup>-crosslinking among the macromolecules could lead to reduced flexibility of the envelope and the decreased ability to retain the hydrated starch content within the granules. This resulted in the low swelling of heated MB and CS granules observed as a smaller size of both starches (Figs. 1–3) under both light microscope and CLSM. The discharged starch content of MB and CS showed aggregation or clumping of red inclusions (Figs. 1 and 3). The formation of these amorphous inclusions in the presence of Ca<sup>2+</sup> under extreme alkaline conditions suggested the Ca<sup>2+</sup>-crosslinks induced the formation of the insoluble inclusions. These microstructural elements were not observed in heated MB and CS starches in the distilled water and NaOH solution.

The formation of the inextensible envelope of heated MB and CS ghost when calcium lactate was present at extreme alkaline condition, as well as the massive leaching of starch content, could be responsible for different RVA

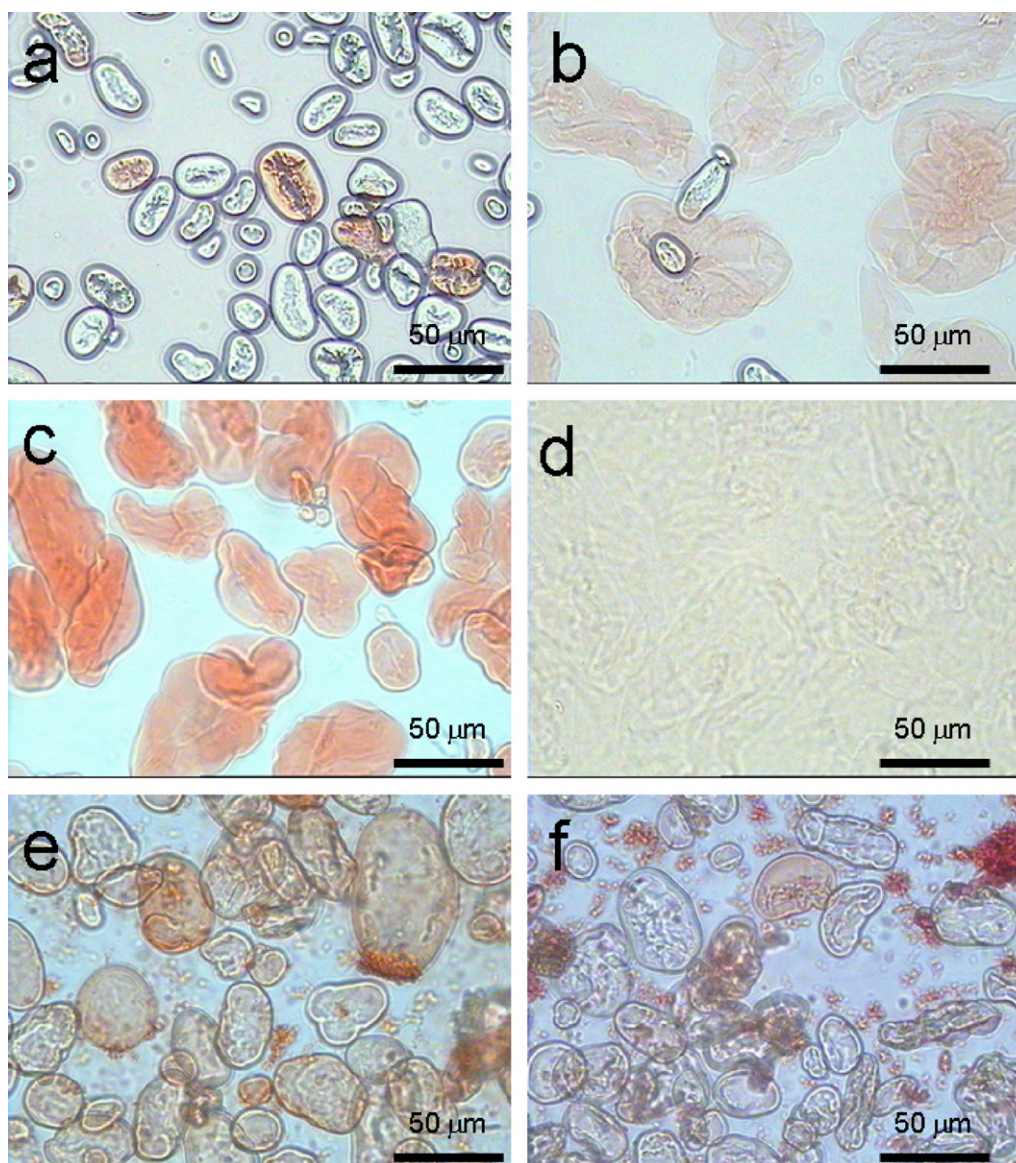


Fig. 1. Light micrographs of food grade mungbean starch (MB) stained with Congo Red. Starch suspensions (0.67% w/v) were heated at 80 °C for 30 min in a quiescent condition. (a) Unheated MB in distilled water, (b) unheated MB in 100 mM NaOH, (c) heated MB in distilled water, (d) heated MB in 100 mM NaOH, (e) heated MB in 100 mM NaOH and 50 mM calcium lactate, (f) heated MB in 100 mM NaOH and 100 mM calcium lactate. Bar = 50 μm.

pasting profiles of both starches (Fig. 4a and b). The native MB starch in distilled water showed high peak viscosity, followed by a moderate thinning during holding stage and a high final viscosity. The CS suspension exhibited high peak viscosity, a fast and excessive thinning during holding, followed by a low final viscosity. It should be noted that the addition of 100 mM NaOH, which led to pH 12, gave rise to the low signal to noise ratio in the RVA pasting profiles of both MB and CS after peak viscosity was reached. This was possibly due to the solubilisation of proteins, which caused excessive foaming of the cooked starch pastes under shear. The addition of calcium lactate at pH 12 altered the viscosity profiles of both MB and CS drastically. The presence of calcium lactate at extreme alkaline condition increased the peak viscosity of MB starch, dras-

tically thinned the MB paste during holding stage and lowered the final viscosity substantially. The addition of calcium lactate under extreme alkaline conditions, however, lowered the peak viscosity of CS, thinned the CS paste during holding and lowered the final viscosity. Overall, MB and CS displayed low final viscosity when treated with 100 mM NaOH, both in the absence or presence of calcium lactate.

The aim of cysteine addition was to reduce the disulfide bond of the granule-associated proteins and made the protein-containing envelope more extensible. T-test revealed that cysteine slightly increased RVA pasting parameters; namely, peak viscosity, holding strength, breakdown, final viscosity and setback of MB starch (Fig. 4c;  $P < 0.05$ ). However, the addition of cysteine reduced holding strength

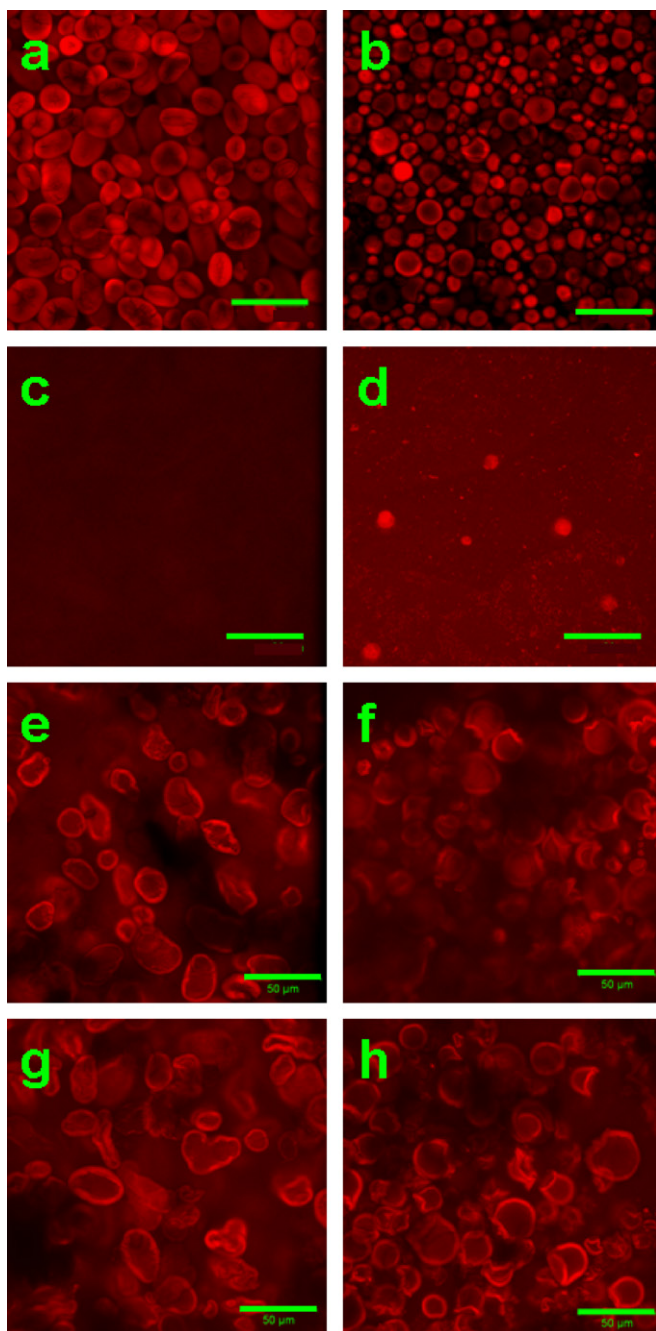


Fig. 2. Confocal laser scanning micrographs of food grade mungbean starch (MB) and cassava starch (CS). Starch suspensions (0.67% w/v) were heated at 80 °C for 30 min in a quiescent condition. Proteins were stained with Rhodamine B and are shown by red fluorescence. (a) Unheated MB in distilled water, (b) unheated CS in distilled water, (c) heated MB in 100 mM NaOH, (d) heated CS in 100 mM NaOH, (e) heated MB in 100 mM NaOH and 50 mM calcium lactate, (f) heated CS in 100 mM NaOH and 50 mM calcium lactate, (g) heated MB in 100 mM NaOH and 100 mM calcium lactate, (h) heated CS in 100 mM NaOH and 100 mM calcium lactate. Bar = 50 μm.

of CS starch and slightly increased the peak viscosity, breakdown, final viscosity and setback (Fig. 4d;  $P < 0.05$ ). The increase in peak viscosity of both starches, in the presence of cysteine, suggested that the disulfide-reduced swollen granules could maintain their granular

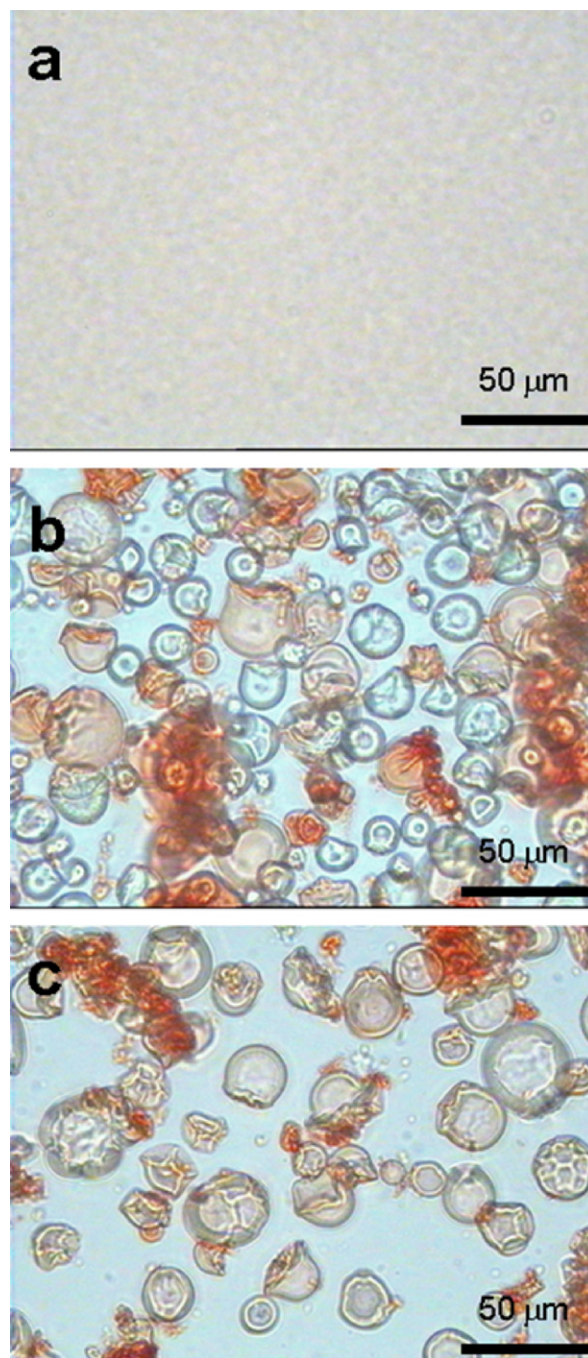


Fig. 3. Light micrographs of food grade cassava starch (CS) stained with Congo Red. Starch suspensions (0.67% w/v) were heated at 80 °C for 30 min in a quiescent condition. (a) Heated CS in 100 mM NaOH, (b) heated CS in 100 mM NaOH and 50 mM calcium lactate, (c) heated CS in 100 mM NaOH and 100 mM calcium lactate. Bar = 50 μm.

structure. Subsequently, the temperature-dependent dilatancy of the granules was maintained by alteration of the viscoelastic properties of the granule envelope.

Due to the difference in both the viscosity increased and the magnitude of the peak viscosity between MB and CS, their viscosity profiles during the heating stage were characterized mathematically by a Non-linear regression model and their half-time values ( $t_{\text{half}}$ ) were summarized in

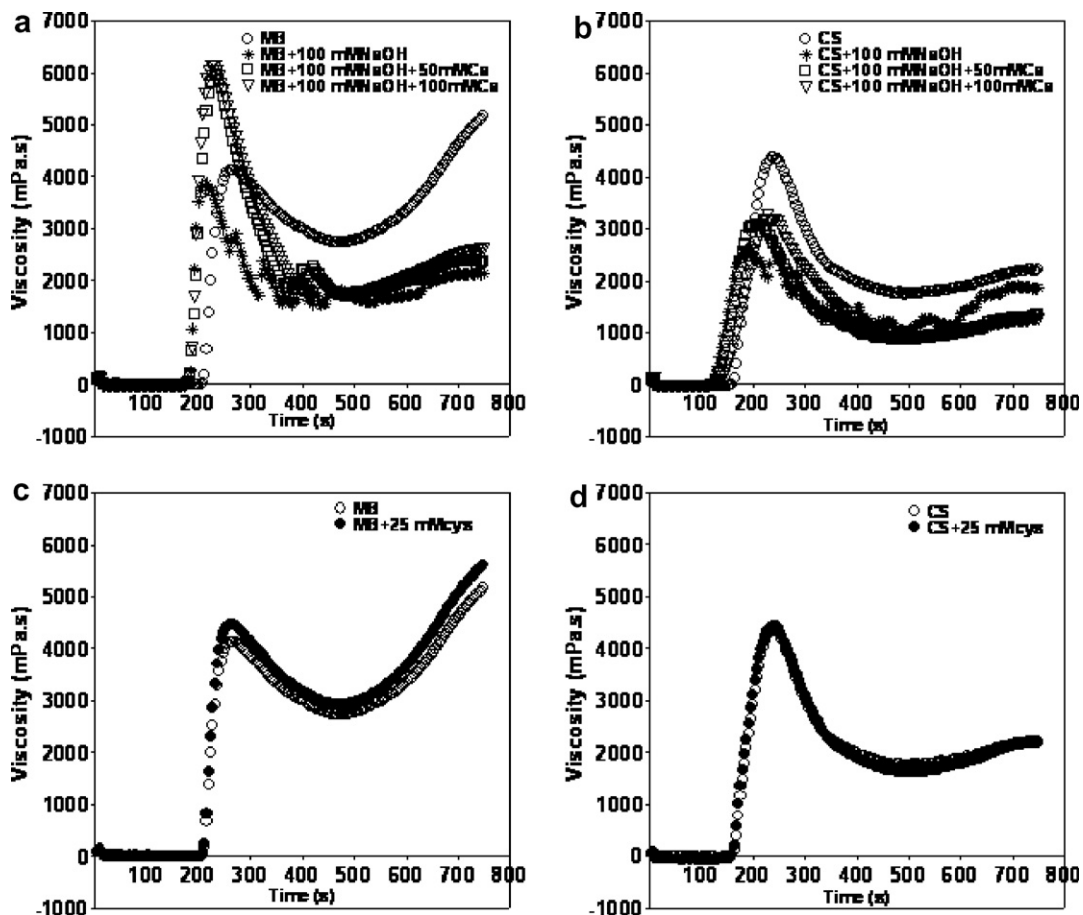


Fig. 4. RVA pasting profile of (a,c) mungbean starch (MB) and (b,d) cassava starch (CS) containing 12% (w/v) total solid.

Table 2 using the Boltzmann sigmoidal equation below. The viscosity data during heating stage were fitted from time zero ( $t_0$ , at 50 °C) to the time that the peak viscosity was achieved ( $t_{end}$ , at 95 °C) as described by Hongsprabhas et al. (2006).

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

This equation describes the viscosity increase ( $\eta_t$ ) as a function of time ( $t$ ) when the temperature was increased during

the heating stage (Fig. 5). The viscosity varied from time zero ( $t_0$ ) to the viscosity at the terminal time selected ( $t_{end}$ ), at which the maximum value of the sigmoidal curve was achieved and designated as fitted peak viscosity in Table 2. Half-time ( $t_{half}$ ) is the time at which the viscosity 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.

During the heating stage, starch granules undergo microstructural changes including the absorption of water,

Table 2

Non-linear curve fitting of viscosity (dependent variable) and time (independent variable) of heated mungbean (MB) and cassava (CS) starches during heating stage

Modifiers	Heating from 50 to 95 °C, up to peak viscosity (Boltzmann sigmoidal equation)							
	MB				CS			
	Half-time (s)	Fitted peak viscosity (mPa.s)	$R^2$	Standard deviation of residuals (mPa.s)	Half-time (s)	Fitted peak viscosity (mPa.s)	$R^2$	Standard deviation of residuals (mPa.s)
None	225.1	4004.0	0.9931	106.1	190.1	4358.0	0.9928	128.5
100 mM NaOH	191.3	3822.0	0.8739	72.7	150.3	2632.0	0.9812	107.1
100 mM NaOH + 50 mM Ca lactate	200.8	5951.0	0.9667	452.2	163.3	3187.0	0.9910	137.5
100 mM NaOH + 100 mM Ca lactate	197.2	6056.0	0.9929	345.3	182.6	3379.0	0.9812	112.0
25 mM Cysteine	224.2	4359.0	0.9974	175.2	187.9	4414.0	0.9957	151.9

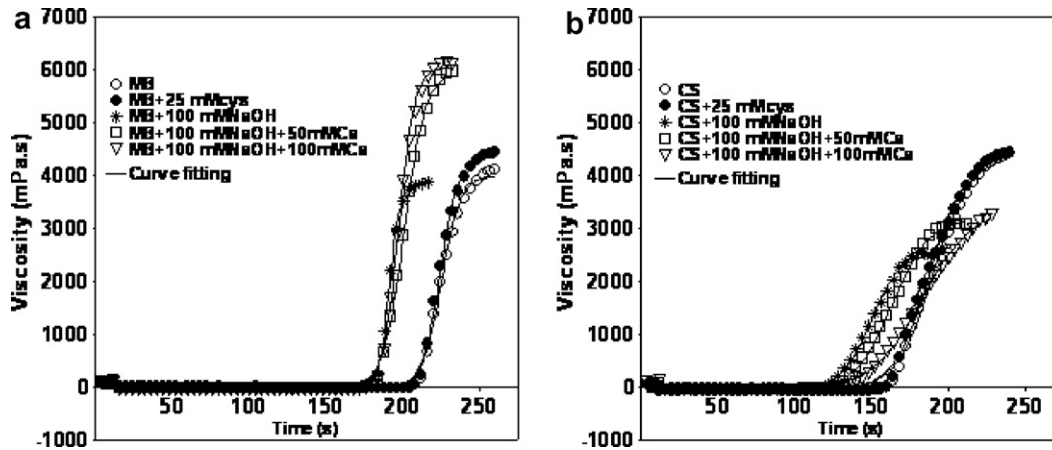


Fig. 5. Sigmoidal curve fitting of viscosity (dependent variable) and time (independent variable) of (a) heated mungbean (MB) and (b) cassava (CS) starches during heating stage.

granular swelling, loosening crystalline structure within the granules and leaching of amylose (Rao, Okechukwu, Da Silva, & Oliveira, 1997; Rao & Tattiyakul, 1999). This viscosity–time relationship describes the lag phase at the early stage of heating and the rapid increase of viscosity as time proceeds. Under extreme alkaline conditions, the viscosity of the heated starch suspensions started to increase within a shorter time than did either the untreated starch or the starch treated with 25 mM cysteine. This suggested that in the presence of 100 mM NaOH, the suspension reached peak viscosity faster than the untreated and the cysteine-added ones. However, the addition of calcium lactate to MB starch suspension caused an increase in the peak viscosity even at pH 12 while this influence was not observed in the CS starch.

The difference in the architecture and viscosity profiles of each starch, in the presence or absence of calcium lactate, could be a result of both starch and protein phase transition characteristics as summarized in Figs. 6, 7, Tables 3 and 4. At an extremely alkaline pH, the phase transition temperatures ( $T_o$ ,  $T_p$  and  $T_e$ ) of both MB and CS were lower than those of the untreated ones. However, the presence of calcium lactate under extreme alkaline conditions increased the phase transition temperatures (except  $T_p$  and  $T_e$  for CS) although these temperatures were still lower than the untreated ones ( $P < 0.05$ ). The addition of 25 mM cysteine did not cause significant changes on the phase transition temperatures ( $P > 0.05$ ). However, the presence of calcium lactate and cysteine could increase  $T_o$ ,  $T_p$  and  $T_e$  of MB starch and  $T_o$  and  $T_p$  of CS starch ( $P < 0.05$ ).

Starch gelatinisation has been described as the loss of crystallinity of the granules. The increased solubility of starch is enhanced in extreme alkaline treatment due the charge repulsion among chains, which causes the rupture of intermolecular hydrogen bonds and the favourable random coil conformation of amylose, as well as the initiation of starch degradation (Han & Lim, 2004). The swelling behaviour of both starch molecules and granules was

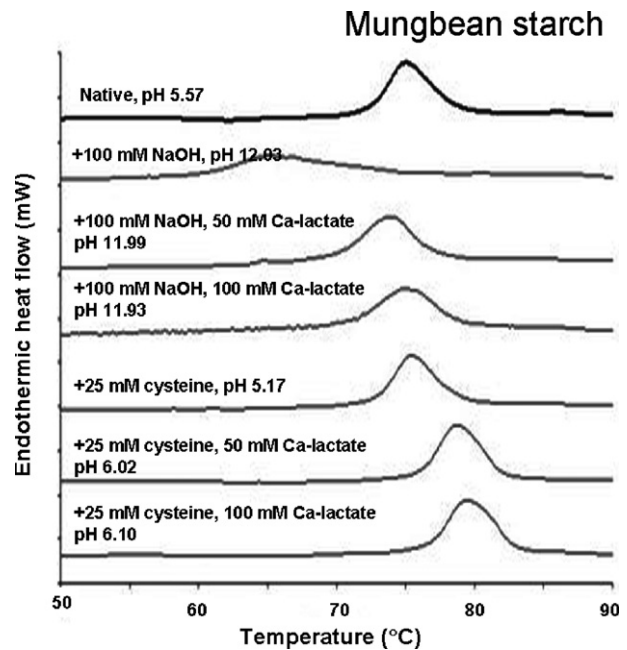


Fig. 6. Thermograms of mungbean starch suspensions (12% w/w) in the absence or presence of modifiers at different pH.

altered by the increased solubility of starch molecules and the permeability of the envelope, respectively. This resulted in the lower  $T_o$  of MB and CS at extreme alkaline pH, and the consequent leaching of starch out of the granule, both in the presence or absence of calcium lactate. Nevertheless, the calcium lactate addition could further modify the permeability of the envelope by altering the nature of pores in the envelope chemically and physically. It is likely that both starch and protein molecules in the pores, and possibly the channels (Fannon, Gray, Gunawan, Huber, & BeMiller, 2004; Han, Benmoussa, Gray, BeMiller, & Hamaker, 2005; Han & Hamaker, 2002b; Huber & BeMiller, 2000), could aid the permeation of starch content from the interior to the environment. Nevertheless, the existence

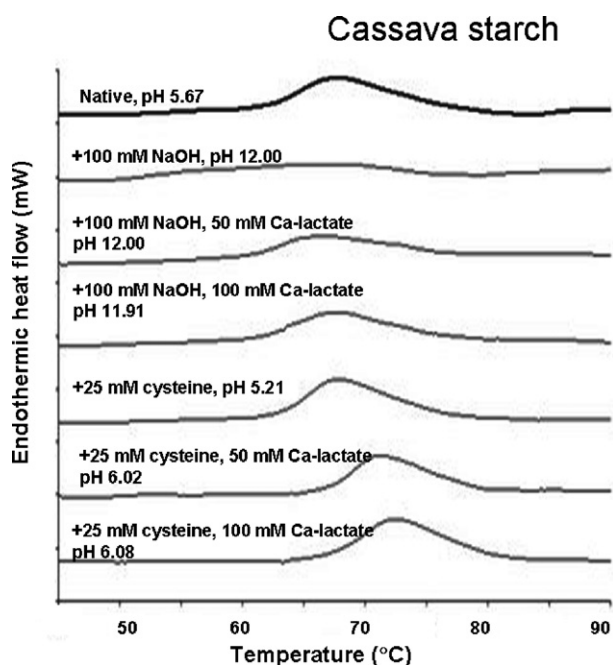


Fig. 7. Thermograms of cassava starch suspensions (12% w/w) in the absence or presence of modifiers at different pH.

and behaviour of channels in MB and CS granules still need further investigation.

The influence of cysteine study further elucidated that the gelatinisation of starch granules could be influenced by the granule-associated proteins as well. This is probably due to the ability of proteins to compartmentalize the starch content when the protein-containing envelope is

developed upon heating in excess water (Hongsprabhas et al., 2006). The alkaline treatment caused solubilisation of the granule-associated proteins, followed by the disintegration of starch granules and their ghosts. The swelling of starch granules are favoured and the transition temperature is consequently lowered.

$\text{Ca}^{2+}$  could stabilise the envelope integrity by cross-linking the negatively charged macromolecules and slightly increased the phase transition temperature, even under extreme alkaline conditions. However, the rigid envelope caused by electrostatic interactions could not hold the hydrated starch contents. They were discharged out of the starch ghosts, possibly through the pores or channels, and clumped together as insoluble amorphous inclusions.

Nevertheless, increasing the flexibility of the protein fractions in the envelope by reducing the disulfide bonds of the granule-associated proteins (Han & Hamaker, 2002a), together with the addition of calcium lactate at  $\text{pH} \sim 6$  could help maintain existence of the protein fractions in the envelope stabilised by salt bridges. As a result, the altered viscoelastic properties of the envelopes could maintain the granular structure and retain the contents efficiently. Consequently, the transition temperatures of the starch granules were increased when both cysteine and calcium lactate were present. Both modifiers were likely to influence only the protein fractions since the pH was not high enough to ionize the OH- of amylose and amylopectin.

However, the non-linear curve fitting during the heating stage and the thermal properties showed slightly different characteristics between MB and CS for the influence of the alkaline and calcium lactate treatments. It is possible that the macromolecular response to  $\text{Ca}^{2+}$  of MB at

Table 3

Effect of modifier on gelatinisation characteristics of food grade mungbean starch (MB) (12% w/w)

Modifiers	$T_o$ (°C)	$T_p$ (°C)	$T_e$ (°C)	$\Delta H$ (J/g db)
None	72.74 <sup>c</sup>	75.09 <sup>c</sup>	78.88 <sup>b</sup>	12.84 <sup>a</sup>
100 mM NaOH	61.19 <sup>f</sup>	65.85 <sup>c</sup>	69.95 <sup>d</sup>	10.11 <sup>a</sup>
100 mM NaOH + 50 mM Ca lactate	70.07 <sup>c</sup>	73.47 <sup>d</sup>	76.42 <sup>c</sup>	11.43 <sup>a</sup>
100 mM NaOH + 100 mM Ca lactate	71.32 <sup>d</sup>	75.05 <sup>c</sup>	78.52 <sup>bc</sup>	10.86 <sup>a</sup>
25 mM cysteine	72.98 <sup>c</sup>	75.30 <sup>c</sup>	78.77 <sup>b</sup>	13.02 <sup>a</sup>
25 mM cysteine + 50 mM Ca lactate	76.16 <sup>b</sup>	78.60 <sup>b</sup>	81.80 <sup>a</sup>	11.96 <sup>a</sup>
25 mM cysteine + 100 mM Ca lactate	77.28 <sup>a</sup>	79.76 <sup>a</sup>	83.05 <sup>a</sup>	12.65 <sup>a</sup>

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

Table 4

Effect of modifier on gelatinisation characteristics of food grade cassava starch (CS) (12% w/w)

Modifiers	$T_o$ (°C)	$T_p$ (°C)	$T_e$ (°C)	$\Delta H$ (J/g db)
None	62.50 <sup>bc</sup>	67.68 <sup>c</sup>	77.24 <sup>abc</sup>	18.37 <sup>a</sup>
100 mM NaOH	49.95 <sup>d</sup>	65.27 <sup>d</sup>	75.97 <sup>bc</sup>	13.34 <sup>a</sup>
100 mM NaOH + 50 mM Ca lactate	60.47 <sup>c</sup>	65.77 <sup>d</sup>	74.16 <sup>c</sup>	14.38 <sup>a</sup>
100 mM NaOH + 100 mM Ca lactate	61.94 <sup>bc</sup>	67.43 <sup>c</sup>	75.78 <sup>bc</sup>	14.56 <sup>a</sup>
25 mM cysteine	63.38 <sup>b</sup>	67.60 <sup>c</sup>	75.62 <sup>bc</sup>	14.78 <sup>a</sup>
25 mM cysteine + 50 mM Ca lactate	66.81 <sup>a</sup>	71.22 <sup>b</sup>	78.39 <sup>ab</sup>	13.77 <sup>a</sup>
25 mM cysteine + 100 mM Ca lactate	67.89 <sup>a</sup>	72.76 <sup>a</sup>	80.02 <sup>a</sup>	15.05 <sup>a</sup>

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



alkaline pH was different from that of CS in terms of the envelope permeability and the solubility of starch.

#### 4. Conclusion

The clarification of the roles of granule-associated proteins on the molecular interactions within the envelope demonstrated in this study could open the venue for understanding the influences of proteins on starch functionalities. Downstream food process to alter starch granular structure, as well as its integrity for a specific purpose have been investigated in our laboratory to exploit the physicochemically engineered functionality of starch granules in glycemic-controlled foods. Nevertheless, the different sensitivity to  $\text{Ca}^{2+}$ -addition between MB and CS under extreme alkaline conditions on heat-induced swelling and viscosity characteristics requires further investigation.

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