

Influences of Waxy Rice Protein Network on Physical Properties of Waxy Rice Flour Composites

Kamolwan Israkarn and Parichat Hongprabhas*

ABSTRACT

The roles of waxy rice protein network as a binder on thermal, pasting and microstructural characteristics of waxy rice flour (WF)-mungbean starch (MB) were demonstrated. The presence of WF, even at one-tenth of the total solid, lowered both enthalpy of gelatinisation and enthalpy for retrogradation reversal of the WF-MB flour composite compared to those of MB ($P < 0.05$). The time-dependent shear-thinning characteristics at 95°C of WF, WF-MB and MB indicated that the composites with high ratio of WF to MB resisted shear the most ($P < 0.05$) during Rapid Visco Analysis (RVA). Confocal Laser Scanning Microscopy (CLSM) showed the swollen MB granules were bound by the protein network of WF after heat treatment. The resistance to shear during pasting of WF-MB composites, compared to those of WF or MB alone, were likely to be due to the binding of the swollen MB granules *via* interfacial interactions between WF protein network and the protein at the envelope of MB granules. This study highlighted the influences of microstructural alteration, through network formation of proteins, on the bulk pasting profiles and thermal properties of both starch and flour.

Key words: flour, microstructure, mungbean, protein, rice

INTRODUCTION

Rice (*Oryza sativa* L.) and mungbean (*Vigna radiata* (L.) Wilczec) are the first and the third major starch crops in Thailand. The native forms of rice flour and mungbean starch have been used extensively in the starchy foods in Asia such as noodles, snack and desserts. The textural properties of starch pastes and gels can range from soft and sticky waxy rice flour-based desserts to firm and chewy mungbean starch vermicelli. It is a general practice to use mix flour in creating different texture and appearance of foods. Traditionally, adding slight waxy rice flour to maintain soft texture of starch is quite common.

This is to minimise retrogradation of starch during cold storage.

The processing of foods containing starch and flour such as noodles, baked products, etc. involves phase transitions of the biopolymers and structure formation of the cooked ingredients to be ready for consumption. Most work has been carried out to understand the roles of phase transitions of starch fraction, particularly the molecular structure of amylose and amylopectin, on the texture of starch/flour-based foods. Gelatinisation and retrogradation characteristics of starch and flour have been explained by the ratio of amylose and amylopectin, degree of branching of amylopectin and degree of polymerisation of

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both amylose and amylopectin. This was due to their presence as the major ingredient in the formulations. Little attention has been paid in understanding the roles of protein, from polymer standpoints, on structure-forming process of starch/flour-based formulation during and after heating.

The significance of proteins on functional properties of starch granules has been reported only recently. Although present in minute quantities (i.e., less than 0.2% db), the starch granule-associated proteins influence the rheological properties of starch paste as shown in maize starch (Han *et al.*, 2002), as well as mungbean and cassava starches (Hongsprabhas *et al.*, 2007; Israkarn *et al.*, 2007). Our recent reports suggested that these granule-associated proteins redistributed to the envelope of the swollen granules and involved in the permeability of the envelope and amylose leaching out from the swollen granules (Hongsprabhas and Israkarn, 2008).

On the other hand, rice storage proteins, which present for 6-8% db, formed a continuous network with honeycomb structure when rice grain was cooked (Likitwattanasade and Hongsprabhas, 2007). Although proteins in rice endosperm exist in the form of protein bodies, they underwent heat-denaturation under thermal process and formed three dimensional networks, which later encased the gelatinised rice starch within the protein network. The fact that the proteins forms continuous phase after rice grain cooking suggests their significance in determining the sensory hardness, which correlated with the mechanical properties of cooked grains (Likitwattanasade and Hongsprabhas, 2007). To date, there is no report on such roles of rice storage proteins as the binder when rice flour is present with other starch.

The formation of a composite network of starch and protein is a multi-step process which occurs in the presence of excess water. The network formation includes phase transitions of

starch and protein fractions known as gelatinisation and protein denaturation, respectively. Starch gelatinisation is defined as a phase transition from semi-crystalline structure of amylopectin into a disordered hydrated state. Protein denaturation is the unfolding of the ordered structure, which sometimes followed by aggregation and network formation. When starch and protein were present together, the constitutes with lower phase transition temperature determine the continuous phase, regardless of the volume fraction of each phase (Hongsprabhas and Ditudom-po, 2006). The objective of this study was to further demonstrate the roles of storage rice proteins on structure-forming process of the composite network of waxy rice flour and mungbean starch. This was to get more insights in the mechanisms governing the textural characteristics of the flour composites.

MATERIALS AND METHODS

Food grade waxy rice flour (WF) (Jade Leaf Brand, Bangkok Interfood, Bangkok, Thailand) and mungbean starch (MB, Pine Brand, SithiNan, Bangkok, Thailand) were obtained from a local supermarket. The WF contained 10.57% moisture content and 6.56% protein while MB had 12.01% moisture content and 0.17% protein (AOAC, 2000). Potassium iodide (Ajax Finechem, Seven Hills, NSW, Australia) and Rhodamine B (Molecular Probes, Eugene, OR, USA) were used to stain starch and protein, respectively.

Thermal properties

A Pyris1 DSC (Perkin Elmer, Norwalk, CT, USA) was used to characterise the thermal properties of 15% (w/w) of starch/flour in distilled water (n = 2/treatment/trial). The suspension was prepared in a stainless steel pan and hermetically sealed. The samples were heated at a rate of 12.5 °C/min from 30 °C to 95 °C to determine the transition temperature and enthalpy of

gelatinisation. The heated pans were kept at 4°C for 48 h to induce retrogradation and rescanned from 30°C to 95°C to determine the transition temperature and enthalpy of retrogradation reversal. The transition temperatures reported were the onset (T_o), peak (T_p) and end (T_e) temperatures of the endotherm. The enthalpy (ΔH) 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 (db).

Pasting characteristics

Rapid Visco Analyzer (RVA; Newport Scientific, Warriwood, Australia) was used to characterise pasting properties of WF, MB and their composites containing different mass ratio of WF:MB as 1:0, 0.75:0.25, 0.5:0.5, 0.25:0.75 and 0:1. Briefly, 25 mL of flour suspension containing 3 g of total solid (i.e., 12% w/v) was heated from 50°C to 95°C at the rate of 12°C/min, held at 95°C for 2.5 min, cooled to 50°C at the rate of 12°C/min and finally kept at 50°C at 160 rpm ($n = 2/\text{treatment/trial}$). Amylogram describing pasting characteristics included peak viscosity (the maximum viscosity developed soon after the heating cycle ended), holding strength (viscosity after holding at 95°C for 2.5 min) and final viscosity (viscosity after cooling at 50°C for 2 min).

Curve-fitting – heating stage

The pasting profiles during the heating stage were characterised by a Non-linear regression model. The half-time values (t_{half}) of each peak viscosity were compared by fitting the curves from time zero (t_0 , at 50°C) to the time that the peak viscosity was achieved (t_{end} , at 95°C) using the Boltzmann Sigmoidal equation below (Hongprabhas *et al.*, 2007).

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

This equation describes the viscosity increase (η_t) as a function of time (t) when the temperature was increased during the heating stage. 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 (i.e., peak viscosity). Half-time (t_{half}) is the time halfway between t_0 and t_{end} . Slope describes the steepness of curve, with a larger value denoting a shallow curve.

Curve fitting-holding stage

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 (Hongprabhas *et al.*, 2007). The following exponential decay equation was used to describe the kinetics of time-dependent shear-thinning of the heated starch/flour. The viscosity started at η_0 and decreased to plateau ($\eta_{plateau}$) with the rate constant K and the half-time (t_{half}) of the decay is $0.693/K$.

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

Granule morphology

One mL of WF, MB and their composite suspensions (0.67% w/v in distilled water) were prepared in 1.5 mL Eppendorf tube. 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 16M, Labnet International, Edison, NJ, USA) (Hongprabhas, 2007). The sediment was resuspended in a 0.5 mL of distilled water and stained with 10% Lugol's iodine solution (Autio and Salmenkallio-Marttila, 2001). The microstructure was examined under a light microscope (Leica DME, Leica Microsystems, Buffalo, NY, USA) before and after heating the suspension at 80°C for 30 min.

A solution of Rhodamine B (0.01% in 95% ethanol) was added to the unheated, heated and retrograded starch suspensions (4°C, 48 h) 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). A HeNe laser with an excitation wavelength of 543 nm was used. CLSM digital image were acquired using the LSM 5 PASCAL program.

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.

RESULTS AND DISCUSSION

The gelatinisation temperature range of WF was around 61-75°C and its enthalpy of

gelatinisation was 11.82 J/g; while MB gelatinised at the temperature range of 73-80°C with the gelatinisation enthalpy of 18.27 J/g (Figure 1). These results were corroborated by the results previously reported by Hongprabhas (2007). The presence of WF lowered the enthalpy of gelatinisation of MB from 18.3 to 12.5 J/g (db) although it was substituted for only one-tenth of MB fraction.

The cooked WF did not show detectable retrogradation after being kept at 4°C for 48 h. The high amylose starch like MB starch (Kasemsuwan *et al.*, 1998), showed retrogradation. The retrogradation can be reversed at the transition temperature ranging from 43-67°C with the enthalpy of retrogradation reversal of 3.22 J/g (Table 1). This suggested that they were recrystallised amylopectin (Lin *et al.*, 2001). WF lowered the enthalpy of retrogradation reversal of the composite for almost 40% though it made only one-tenth of total solid. However, it did not affect the transition temperatures ($P > 0.05$). The ability of WF substitution on the reduction of enthalpy of gelatinisation and enthalpy of retrogradation reversal of WF-MB requires further kinetic study on the co-operative effects of protein and starch on phase transitions.

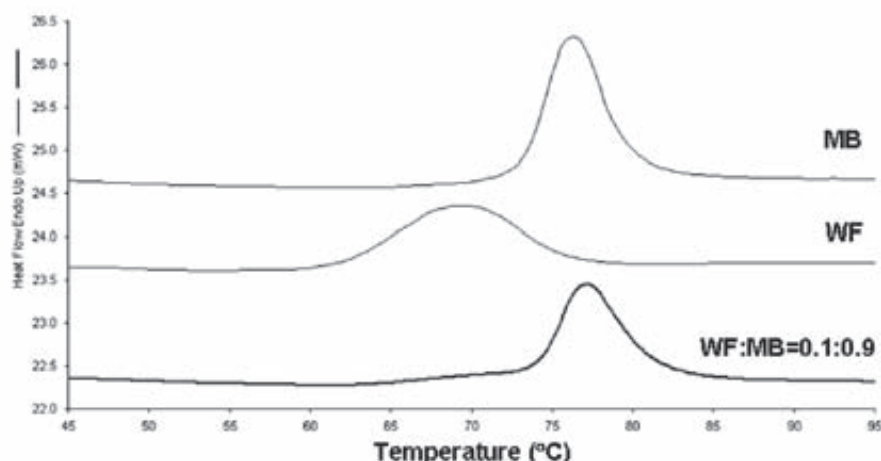


Figure 1 DSC thermograms during gelatinisation of mungbean starch (MB), waxy rice flour (WF) and the composite containing WF:MB at the mass ratio of 0.1:0.9.

Table 1 Thermal transition of the heated and refrigerated waxy rice flour (WF), mungbean starch (MB) and their composite (15% solid w/w).

Thermal properties	WF:MB		
	1:0	0.1 : 0.9	0:1
T _o (°C)	ND	50.46 ^a	43.37 ^a
T _p (°C)	ND	57.67 ^a	57.47 ^a
T _e (°C)	ND	68.41 ^a	67.03 ^a
Enthalpy of retrogradation reversal (J/g db)	ND	1.87 ^b	3.22 ^a

ND = thermal transition not detected.

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

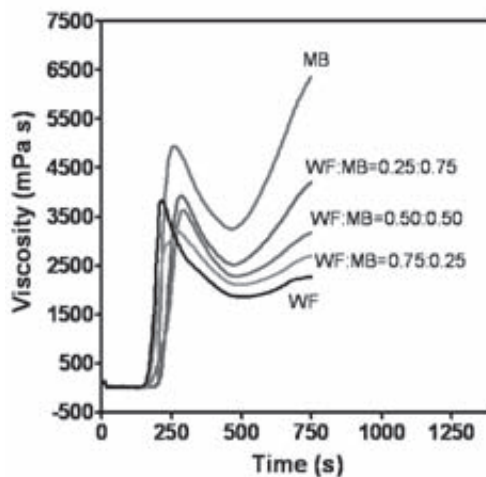
The transition temperature range of the retrogradation reversal from 43 to 67°C indicated the recrystallisation of amylopectin during refrigeration. Nonetheless, the thermal transition of the refrigerated WF was not detected although the WF was rich in amylopectin. This also suggested that the ratio of amylose and amylopectin alone could not fully explain the retrogradation or recrystallisation of starch/flour after refrigeration. The ability of WF to reduce the enthalpy of retrogradation reversal was noted. This evidence supported the traditional formulation of adding waxy rice flour to reduce retrogradation of high amylose starch.

The RVA pasting profiles of WF, MB and their composites were illustrated in Figure 2. The MB had the highest peak viscosity, holding strength and final viscosity. The substitution of MB with WF for 25-75% of total solid lowered the peak viscosity of the composites, which were lower than that of the WF alone as well. The presence of WF also decreased the holding strength and the final viscosity of the composites.

The shape of each RVA pasting profile, during heating and holding stages, merited further comparative characterization mathematically. The viscosity profiles of both stages may be influenced by the swollen granules of both starch sources. The viscosity change profiles during cooling stage, however, were not included in this study. 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 and Svegmarm, 1996) after shear-induced disruption of the swollen granules during the holding stage at 95°C. Thus, 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 during cooling storage.

Boltzmann sigmoidal equation and exponential decay equation were used to

**Figure 2** Amylograms of waxy rice flour (WF), mungbean starch (MB) and their composites (WF-MB) determined by the Rapid Visco Analyser.

distinguish the pasting curve of each composite during heating stage and holding stage, respectively. This was because each sample possessed different magnitude and viscosity change rate. This was due to the nature of water absorption of the granules, the events occurred during heating, and the ability of the swollen granules to withstand shear at high temperature. The microstructural changes of starch granule during heating stage included absorption of water, granular swelling, loosening crystalline structure within the granules and leaching of amylose (Atkin *et al.* 1998). Figure 3 and Table 2 indicated that upon heating, the peak viscosity values of the composites containing WF and MB at the ratio of 0.25:0.75 and 0.50:0.50 were reached slower than those of WF, MB and the composite containing higher ratio of WF.

The exponential decrease in the viscosity of heated WF, MB and their composites under shear at 95 °C was described as t_{half} during holding stage (Figure 3, Table 2). The composite containing a mixture of WF to MB at a ratio of 0.75:0.25 appeared to resist shear the most since it required longer half-time for viscosity to drop compared to the others.

Nevertheless, the ability to resist shear of such ratio required further study in order to prove that it was via shear-induced aggregation of the microstructural elements under shear at high temperature.

Upon cooling, where the temperature was lowered from 95 to 50 °C, the higher final viscosity of the heated MB showed rheopectic characteristics under constant shear rate and the final viscosity was higher than the peak viscosity value. The heated WF and WF-MB composites, however, had lower final viscosity than the heated MB. In addition, their final viscosity values were lower than their peak viscosity values. This suggested that the presence of WF hinder structure-forming process of the composite during cooling. Our previous report confirmed that the mixed WF-MB gel (within similar concentration range) were much softer and more extensible than the MB gel (Hongprabhas, 2007).

The difference in pasting profiles of MB, WF and their composites could be due to the differences in the microstructural elements responsible for the overall paste viscosity. The light micrographs of WF and MB, before and after heating under quiescent condition, were illustrated

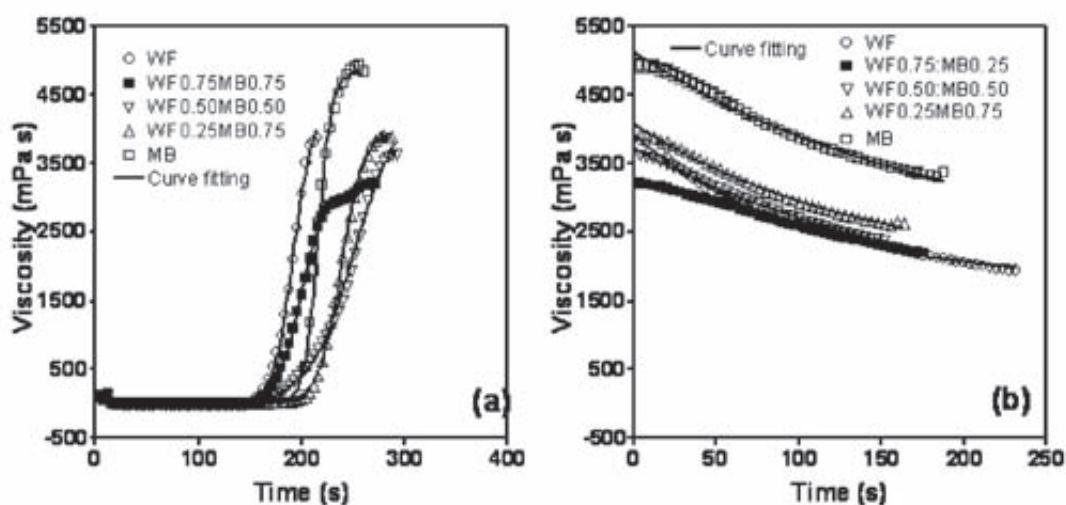
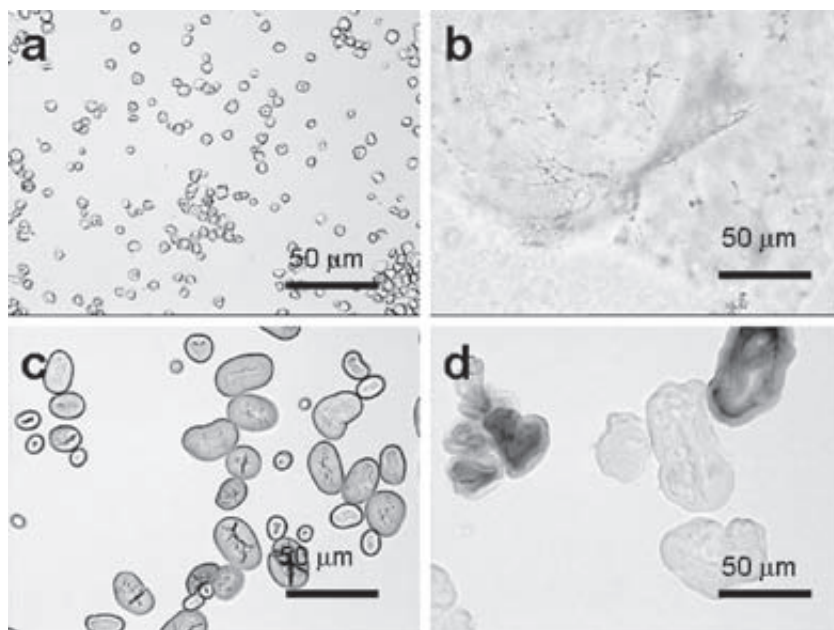


Figure 3 Effect of waxy rice flour substitution on the pasting characteristics of waxy rice-mungbean composite during (a) heating stage from 50 °C to 95 °C and (b) holding stage at 95 °C.

Table 2 Non-linear curve-fitting of viscosity (dependent variable) and time (independent variable) of cooked waxy rice flour (WF) and mungbean starch (MB) during heating and holding stages.

WF:MB mass ratio	Heating from 50 to 95°C, up to peak viscosity (Boltzmann sigmoidal equation)			Holding at 95°C (Exponential decay equation)		
	Fitted half-time (s)	R ²	Standard deviation of residuals (mPa.s)	Fitted half-time (s)	R ²	Standard deviation of residuals (mPa.s)
1.00 : 0.00	215.75 ^b	0.9850	205.7	131.15 ^{ab}	0.9761	84.08
0.75 : 0.25	199.20 ^c	0.9987	44.2	353.00 ^a	0.9964	19.23
0.50 : 0.50	251.00 ^a	0.9963	70.6	134.85 ^{ab}	0.9934	32.55
0.25 : 0.75	238.75 ^a	0.9895	132.2	103.01 ^b	0.9800	61.94
0.00 : 1.00	192.10 ^c	0.9976	53.5	71.50 ^b	0.9964	32.05

Means followed by different superscript are significantly different ($P < 0.05$).

**Figure 4** Light micrographs of (a) unheated waxy rice flour; (b) heated rice flour; (c) unheated mungbean starch and (d) heated mungbean starch. Heating was carried out at 80°C for 30 min under quiescent condition. Amylose was stained in blue by Lugol's iodine solution.

in Figure 4. WF did not show amylose-iodine complex due to its low content of amylose (Figure 4a). After heating, the granules disintegrated and there was a structure periodically observed under light microscope (Figure 4b). MB

granules were blue due to the amylose-iodine complex in both unheated and heated ones. After heating, the MB granules swelled, collapsed and some granules showed empty ghost while some retained amylose within the granules (Figure 4d).

Proteins in WF, MB and their composites under CLSM were fluoresced in red when stained with Rhodamine B (Figure 5). The storage proteins of WF granules, which are present in the form of protein bodies in the exterior of the granule, were illustrated as scattered red colour (Figure 5a). After heating, the heated WF showed red fluoresced network (Figure 5b), which was also found in the refrigerated WF suspensions (Fig. 5c). The unheated MB showed red colour within the intact granule (Figure 5m) although the MB protein was present in a small amount (i.e., 0.17% db, mostly granule-associated proteins responsible for starch biosynthesis). After heating, the MB granules swelled and some protein existed in the granule envelope (Figure 5n). The protein-containing envelope of MB was also observed in the refrigerated collapsed granules (Figure 5o).

The micrographs of the unheated WF-MB composites show the WF protein fraction, which tended to associate to each other and formed a protein clump separated from the large MB granules (Figures 5d, 5g, 5j). After heating, however, the WF protein formed a continuous network surrounding the swollen MB (Figures 5e, 5h, 5k). Some WF proteins existed as a separated phase when the ratio between WF to MB was high. The collapsed MB granules bound by the WF network at the interface existed in the refrigerated WF-MB suspensions (Figures 5f, 5i, 5l).

Overall, the light and confocal laser scanning micrographs suggested polydispersed structure of WF, MB and their composites. The cooked starch/flour suspensions were composed of many microstructural elements; e.g. leached amylose, collapsed granules, protein network, etc. Although the suspensions were heated in excess water and the gelatinisation of starch was expected to be completed, these microstructural elements and their polydispersed characteristics could influence the bulk viscosity when the mixed flours were heated at a much higher concentration during pasting in the RVA.

Both storage proteins in WF and granule-associated proteins in MB generated the protein-microphase outside the granules and within the envelope of the granule, respectively. These microstructural elements might be a 'glue' that bound the swollen starch granules during heating, holding and cooling stages in the RVA. The denaturation temperature range of the isolated oryzenin, the major storage proteins in rice endosperm, was around 55-65 °C (Teo *et al.*, 2000). It is possible that both starch and protein fractions underwent phase transitions during heating. It was likely that the viscosity profiles (measured as t_{half}) during heating stage reflected progressive alteration events of starch and protein fractions.

The high values of t_{half} in the composite containing WF:MB of 0.50:0.50 and 0.25:0.75 during heating could be explained by the restriction of MB swelling as the aggregation of MB granules bound by WF protein network. In addition, these microstructural elements formed during heating stage are likely to be involved in the ability of the cooked paste to resist time-dependent shear-thinning at 95°C during holding stage. The composite containing WF:MB at the ratio of 0.75:0.25 was able to withstand shear better than the others, particularly the WF alone. It is likely that the swollen MB granules were reinforcing particles in the WF protein network under shear.

The structure-forming ability of the heated MB during cooling stage could be due to the formation of a close-packed amylose-containing swollen granule. It is likely that the swollen granules of MB are structurally maintained by the starch constituent retained within the granular structure and the integrity of the protein-containing envelope (Hongprabhas *et al.*, 2007; Israkarn *et al.*, 2007). The redistribution of granule-associated proteins to the granule's envelope could alter the surface properties of the envelope, particularly charge density and free sulfhydryl group. Both rice storage proteins and

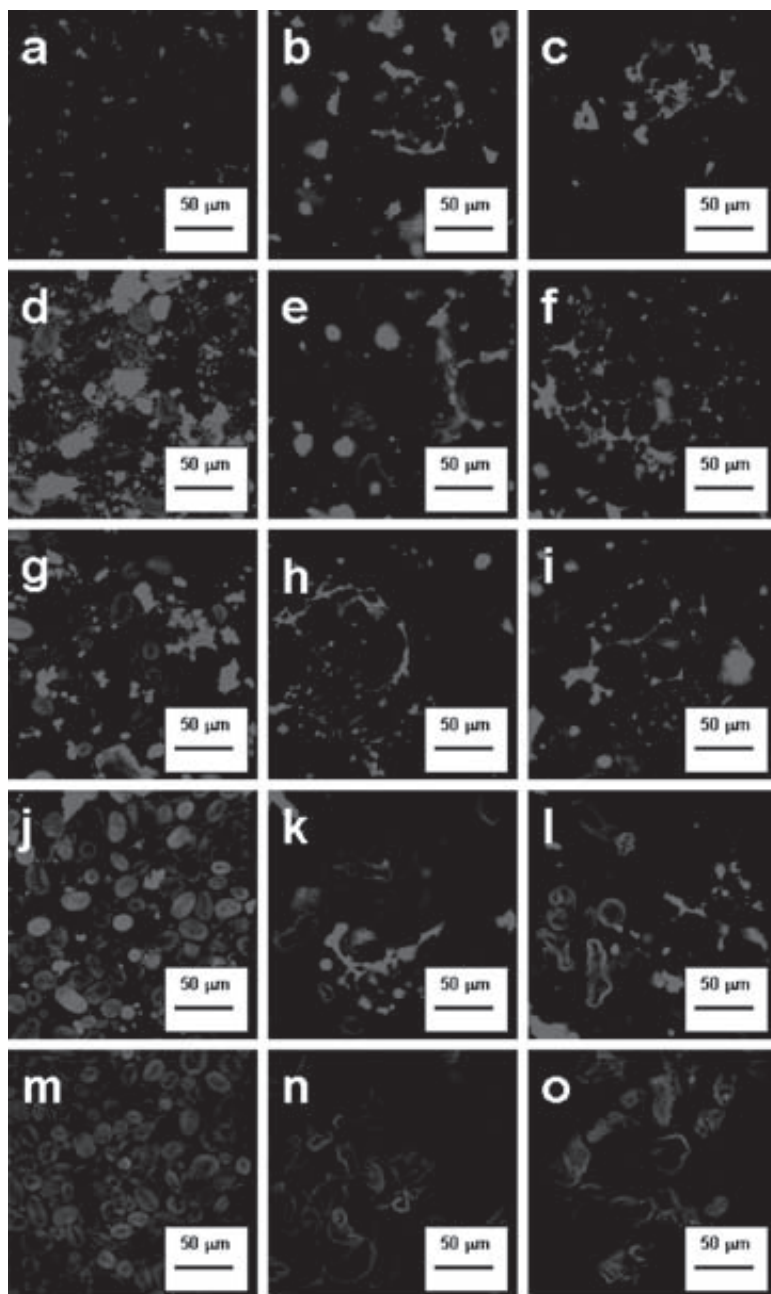


Figure 5 CLSM of waxy rice flour (WF) and mungbean starch (MB). a = unheated WF; b = heated WF; c = retrograded WF; d = unheated WF:MB (0.75:0.25); e = heated WF:MB (0.75:0.25); f = retrograded WF:MB (0.75:0.25) g = unheated WF:MB (0.5:0.5); h = heated WF:MB (0.5:0.5); i = retrograded WF:MB (0.5:0.5); j = unheated WF:MB (0.25:0.75); k = heated WF:MB (0.25:0.75); l = retrograded WF:MB (0.25:0.75); m = unheated MB; n = heated MB; o = retrograded MB. Starch/flour suspensions (0.67% w/v) were heated at 80°C for 30 min and retrograded at 4°C for 48 h under quiescent condition. Proteins are fluoresced in red by Rhodamine B.

granule-associated proteins contain sulfhydryl group and disulfide bond (Oates, 1990; Baldwin, 2001), both of which are likely to react at the interface between swollen MB granules and the exterior WF network.

However, the presence of WF, even at 25% of total solid, lowered the final viscosity of the composite and hindered structure-forming process of MB during cooling stage. It is possible that the continuous protein network is, in part, determined the overall mechanical properties of WF-MB pastes in addition to the leached amylose. The overall structure-forming process of the composite during cooling stage was hindered although the aggregated collapsed MB granules, bound by WF protein network, were able to resist shear during holding stage.

CONCLUSION

This study clearly showed that the formation of protein network influenced physical properties of the heated WF, MB and their composites in different manners. The presence of minute amount of granule-associated proteins in MB redistributed to the envelope and stabilised the granule structure. The storage proteins of WF, however, formed three-dimensional network as a separated phase in the heated suspension and they bound the collapsed MB granules. The insights in the microstructural alterations of the heated starch and flour determined pasting properties, which could be used to manipulate the functional properties of the mixed flours in food formulation and processes.

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