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New insights on the characteristics of starch network

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ABSTRACT

This study investigated the roles of granule-associated proteins (0.16% w/w d.b.) in mungbean starch (MBS, *Vigna radiata* (L) Wilczec) on the microstructural and thermo-mechanical properties of MBS gel and thermoplastic. Gelatinization at high water content (9–13% w/w) and intermediate water content (40–60% w/w) ranges generated microscopic multi-phase structure of swollen MBS granules, separated starch-rich phase and separated protein-rich phase. The presence of 50 mM calcium lactate plus 25 mM cysteine lowered the compressive stress of MBS gels at high water content range, increased T_o, T_p and T_e of MBS slurries at intermediate water contents, and lowered the storage modulus of the protein-rich phase in thermoplastic MBS at its T_g ($P < 0.05$). This study revealed the new insights on the significance of granule-associated proteins in the regulation of the envelope permeability, the leaching of starch and the thermo-mechanical characteristics of starch network over a wide range of water contents.

1. Introduction

Gelatinization of starch is defined as the phase transition of starch granules from an ordered to a disordered state in the presence of water (Donovan, 1977). During gelatinization the swollen granules bind with the available water, leading to an increase in the viscosity of starch paste during heating. The outermost surface surrounding the swollen granules or the envelope, which is composed of amylopectin, is responsible for holding the integrity of the swollen granules (Atkin, Abeysekera, & Robards, 1998) while amylose leaching out of the swollen granules, in the presence or absence of shear, is responsible for a generation of a two-phase structure (Hermansson & Svegmark, 1996). Such structure is consisted of the swollen granules and a continuous amylose phase, which is responsible for the overall rheological properties of starch paste and gel (Hermansson & Svegmark, 1996).

The uniqueness of legume starch is the limitation of swelling during gelatinization. In the case of mungbean (*Vigna radiata* (L) Wilczec) starch, it has been reported that the degree of swelling is influenced by the existence of peptide bridges in starch granules that maintain the structure of the starch granule ghost (Oates, 1990). These proteins, apart from amylopectin, are also responsible for the structure of the envelope (Hongsprabhas, Israkarn, & Rattanawattanaprakit, 2007; Israkarn, Hongsprabhas, & Hongsprabhas, 2007). By definition, starch granule-associated proteins are defined as the proteins naturally positioned in and on starch granules (Baldwin, 2001). These proteins 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). The existence of the granule-associated proteins, their locations, and their influences on starch functionality have been recently demonstrated (Fannon, Gray, Gunawan, Huber, & BeMiller, 2004; Han, Benmoussa, Gray, BeMiller, & Hamaker, 2005; Han, Campanella, Guan, Keeling, & Hamaker, 2002; Han & Hamaker, 2002a; Han & Hamaker, 2002b; Huber & BeMiller, 2000). Although present in minute quantity, the starch granule-associated proteins affect significantly the rheological properties of starch paste as shown in the case of maize starch (Han et al., 2002).

Despite its importance, the influence of the granule-associated proteins has received little attention since they generally present in starch granules in a very small amount. Nonetheless, some previous works indicated that the gelatinization temperatures of mungbean and cassava (*Manihot esculenta* Crantz) starches could be increased in the presence of calcium lactate and cysteine mixture, which promoted the alterations of the envelope (Israkarn et al., 2007).

In this study, it was further hypothesized that the granule-associated proteins also involved in the controlling of the envelope permeability and subsequent leaching of amylose out of the swollen granules. Thus, the overall physical characteristics, e.g., microstructure and thermo-mechanical properties, of gelatinized starch network would be modified. The alteration of the protein fractions were carried out in the presence of cysteine and calcium lactate over a wide range of water content in this study. The former was used to reduce disulfide bond while the latter assisted the cross-linking of the negatively charged groups present in starch granules.

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The insights may help understanding the influence of the polydispersed characteristics of gelatinized starch network at the concentration practical to foods and bio-based thermoplastics.

2. Materials and methods

Food grade mungbean starch (MBS, Pine Brand, SithiNan, Bangkok, Thailand) was obtained from a local supermarket. Rhodamine B (Invitrogen, Caelsbad, USA) was used to label protein. The MBS contained 11.35% moisture, 0.16% protein, 0.08% ash and 0.08% crude fiber (d.b.) with no detectable lipid (AOAC, 2000).

2.1. Mungbean starch gel preparation

In the presence of excess water, one hundred mL of MBS suspension (9–13% w/w) was prepared. The dispersants included distilled water and modifiers at the final concentrations of cysteine of 25 mM cysteine and calcium lactate of 50 mM. The suspension was mildly agitated using a magnetic stirrer in a 250 mL beaker and heated at 73 ± 1 °C for 7 or 10 min in a waterbath. A polycarbonate tube (internal diameter of 20 mm) was then filled with the obtained hot paste, left at 27 °C for 3 h and incubated at 25 °C for 1 h prior to starch gel property and microstructure analyses.

2.2. Stress relaxation test

Starch gels were cut into 10 mm long cylinders and compressed for 20% of their original height for 60 s between a lubricated stationary bottom plate and a moving upper plate using a TA-XTi texture analyser (Stable Micro Systems Ltd., Surrey, UK) at the rate of 100 mm/min (n = 4/treatment/trial). The stress (σ) and strain (ϵ) were calculated as described by Tang, Lelievre, Tung, and Zeng (1994) as follows:

$$\sigma = \frac{F(L-\Delta L)}{\pi r^2 L} \tag{1}$$

$$\varepsilon = -\ln\left[1-\frac{\Delta L}{L}\right] \tag{2}$$

where F is the compressive force, L is the original sample length, ΔL is the corresponding deformation, and r is the original radius.

Assuming the sample had reached equilibrium at the completion of the test, each stress relaxation curve was fitted to the Maxwell models as follows:

$$\text{One Maxwell element}: \sigma_0 = A\exp\left(\frac{-t}{\lambda_{\text{rel}}}\right)+\sigma_e \tag{3}$$

$$\text{Two Maxwell elements}: \sigma_0 = A_1\exp\left(\frac{-t}{\lambda_{\text{rel1}}}\right)+A_2\exp\left(\frac{-t}{\lambda_{\text{rel2}}}\right)+\sigma_e \tag{4}$$

The relaxation time was designated as λ_{rel}. Both equations describe that the stress decays from the initial stress (σ_0) to the plateau (σ_e) at a rate of $1/\lambda_{\text{rel}}$ in the case of one Maxwell element in parallel with a spring. In the case of two Maxwell elements in parallel with a spring, two different decay rates, namely $1/\lambda_{\text{rel1}}$ and $1/\lambda_{\text{rel2}}$ are present (Steffe, 1992).

2.3. Microstructure evaluation

One mL of MBS slurries at the concentrations of 40% and 50% (w/w) was prepared by dispersing the starch in dispersants containing distilled water, 25 mM cysteine and 50 mM calcium lactate plus 25 mM cysteine in a 1.5 mL Eppendorf tube, incubated at 27 °C for 24 h and autoclaved at 121 °C for 15 min. The starch gels were sectioned (approximately 1 mm thick) using a razor blade. A solution of rhodamine B (0.01% in 95% ethanol) was added to the MBS gel section. The excess dye was washed off with distilled water after incubation for 5 min. Each sample was observed for a location of fluorescent-labelled protein using a confocal laser scanning microscope or CLSM (Axio Imager MI, Carl Zeiss, Göttingen, Germany). A HeNe laser with an excitation wavelength of 543 nm was used as the light source. The CLSM image was acquired using LSM 5 PASCAL program. The light micrograph was observed by staining with 10% Lugol's iodine solution (Autio & Salmenkallio-Marttila, 2001) and the image was acquired using Image Pro Plus software version 6.0 (MediaCibernetics, Bethesda, USA).

2.4. Differential scanning calorimetry

Differential scanning calorimeter (DSC822^e/400W, Mettler Toledo, Columbus, USA) was used to determine the thermal properties of MBS (40% and 60% w/w) in different dispersants. The MBS was dispersed in distilled water, 25 mM cysteine solution or 50 mM calcium lactate plus 25 mM cysteine solution prepared as described by Israkarn et al. (2007). The glycerol effect was also investigated at the same MBS contents using the above-mentioned dispersants at the ratio of dispersant:glycerol of 1:1. Each suspension was incubated at 25 °C for 24 h in a hermetically sealed stainless steel pan prior to the measurement.

The samples were heated at a rate of 5 °C/min from 25 °C to 210 °C to determine the transition temperature and enthalpy of gelatinization. The transition temperature was reported in terms of the onset (T_o), peak (T_p) and end (T_e) temperatures of the gelatinization endotherm. The enthalpy of gelatinization (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 starch (d.b.).

2.5. Dynamic mechanical analysis

Ten grams of MBS slurries in distilled water, 50 mM calcium lactate and 50 mM calcium lactate plus 25 mM cysteine containing MBS:dispersant:glycerol at the ratio of 4:3:3 was first equilibrated at 27 °C for 24 h in a polypropylene tube with an internal diameter of 10 mm. The samples were then heated at 90 °C for 5 min and autoclaved at 121 °C for 15 min and cooled in an ice-bath for 30 min after being taken out from the autoclave. The solid rod (11.38 mm in diameter) of starch gel was sectioned into 1.5 mm thick discs and equilibrated at 25 °C for 4 days in a controlled relative humidity chamber. The final water activity of the starch discs was maintained at around 0.215 prior to the dynamic mechanical measurement.

The starch discs were analysed by the dynamic mechanical analyzer DMA/SDTA861^e (Mettler Toledo, Columbus, USA) under the shear mode between 25 °C to 145 °C at a heating rate of 4 °C/min at 1 Hz and the displacement amplitude of 30 μm. The storage modulus (E'), loss modulus (E'') and loss tangent ($\tan\delta$) at each temperature were recorded.

2.6. Statistical analysis

The experiments were carried out in two separated trials. Each trial was run in duplicate unless stated otherwise. The data were analyzed by means of analysis of variance (ANOVA) at 95% significance level. Significant differences among mean values were determined by Duncan's multiple range test. All statistical analyses were performed using SPSS Software Version 12.