



# Physicochemical properties of starches and proteins in alkali-treated mungbean and cassava starch granules



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## ABSTRACT

This study explored the influences of envelope integrity of cooked starch granules on physicochemical and thermophysical properties of mungbean and cassava starches. Alkali treatment was used to selectively leach amylose from the amorphous region of both starches and partially fragmented starch molecules into lower-molecular-weight polymers. It was found that despite the loss of 40% of the original content of amylose, both mungbean and cassava starches retained similar crystallinities, gelatinization temperature ranges, and pasting profiles compared to the native starches. However, the loss of granule-bound starch synthases during alkali treatment and subsequent alkali cooking in excess water played significant roles in determining granular disintegration. The alterations in envelope integrity due to the negative charge repulsion among polymers within the envelope of swollen granules, and the fragmentation of starch molecules, were responsible for the alterations in thermophysical properties of mungbean and cassava starches cooked under alkaline conditions.

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## 1. Introduction

The structure-forming process of starch paste and gel is a multi-step process which occurs in the presence of excess water. The process involves phase transition from a semi-crystalline structure into a disordered hydrated state and the swelling of starch granules, followed by leaching of (mainly) linear chain amylose and the packing of swollen granules. This results in a structure of granular starch particles embedded in a continuous amylose matrix, a so-called close-packing structure, particularly at high starch concentration. Such a composite structure is dominated by swollen granules in a continuous amylose matrix (Hermansson & Svegmarm, 1996). Starch gel characteristics are thus mainly governed by the volume fraction of the dispersed swollen granules, their rigidity and surface interactions. The integrity of the envelope encasing swollen granules is therefore responsible for the degree of amylose/amylopectin leaching, as well as the capacity of the swollen granules to dissipate force during shearing or compressing (BeMiller, 2011; Debet & Gidley, 2006, 2007; Hongsprabhas & Israkarn, 2008; Hongsprabhas, Israkarn, & Rattanawattanaprakrit, 2007).

Phase separation in the continuous phase may also play an important role in determining the properties of starch pastes and gels. The microscopic phase separation involves demixing of macromolecular amylose and amylopectin after the granular structure disintegrates. As a consequence, amylopectin usually forms the continuous phase due to its higher mass and volume ratio compared to amylose and is responsible for starch paste characteristics (Hermansson & Svegmarm, 1996).

Our previous investigation showed that the gelatinization temperatures of native mungbean starch (N-MB) and native cassava starch (N-CS) granules were lowered during alkali cooking. This resulted in drastic leaching of starch constituents out of the granules and disintegration of swollen starch granules (Israkarn, Hongsprabhas, & Hongsprabhas, 2007). As a consequence, the mechanical and thermophysical properties of demixed amylose-amylopectin paste and gel were entirely different from those governed by a close-packed structure.

Alkali treatment or nixtamalization has been used in starch processing to remove the pericarp of cereal grains. For example, alkali soaking of maize in NaOH or Ca(OH)<sub>2</sub> is part of tortilla and taco shell production (Flores-Morales, Jiménez-Estrada, & Mora-Escobedo, 2012; Mestres, Colonna, & Buleon, 1988; Toro-Vazquez & Gómez-Aldapa, 2001). In Asia, yellow alkali noodles and certain desserts are made under alkaline conditions to give

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a soft but cohesive texture (Karim et al., 2008). Starch extraction, as well as starch modification processes – etherification, esterification and crosslinking – are often performed under alkaline conditions. Sui, Shah, and BeMiller (2011) recently reported that subjecting a starch to the conditions of derivatization at pH 11.2 or 11.3 using 1 M NaOH without added reagent could result in greater changes to pasting profiles and gelatinization temperature ranges.

Nevertheless, the current understanding of the effects of alkali cooking on starch properties is limited mainly to maize grain, flour and starch. Most research work has been carried out on the traditional nixtamalization or alkali cooking of maize with  $\text{Ca}(\text{OH})_2$  during the production of tortillas. Industrial nixtamalization of maize was shown to have both melting and annealing effects on maize starch (Mestres et al., 1988; Toro-Vazquez & Gómez-Aldapa, 2001). It was also found that nixtamalization of maize resulted in an increase in crystallinity of the starch fraction, alteration of the protein structure, and enhancement of protein–starch–lipid interactions (Flores-Morales et al., 2012).

Recently, Karim et al. (2008) found alterations of sago starch characteristics after alkali treatment. They reported that modification of sago starch by alkaline soaking at 30 °C for 30 d, followed by neutralization to pH 7 and oven-drying at 40 °C for 3 d, increased the swelling power and solubility of the starch. Amylose leaching, endodepolymerization, and oxidation of starch molecules could occur during soaking, resulting in lower peak viscosity and reduced breakdown of modified sago starch compared with the native starch.

Drastic changes of starch granule characteristics and in the content of constituents leached from the granules (particularly amylose and granule-bound proteins) during alkali cooking of N-MB and N-CS have been reported by our group (Hongsprabhas & Israkarn, 2008; Hongsprabhas et al., 2007). Both the –OH groups on starch molecules, which are ionized at alkaline pH above 12.5 (Suortti, Gorenstein, & Roger, 1998), and the negatively charged granule-bound proteins are likely involved in the formation of the envelope encasing starch contents within the swollen granules (BeMiller, 2011; Debet & Gidley, 2006, 2007). Nevertheless, the influence of alkali treatment on the physicochemical changes of starch molecules and proteins within the treated granules has received little attention. The characteristics of alkali-treated granules thus merit further investigation.

We hypothesized that the granular integrity of swollen granules during cooking under alkaline pH (pH ~ 11) was determined by the viscoelastic properties of the starch granule envelope. Disintegration of this envelope, particularly by charge repulsion, could enhance disintegration of the swollen granules. The objectives of this study were therefore to investigate the influences of alkali treatment on the characteristics of dried starch granules and subsequent cooking of the alkali-treated starches. The insights on the alterations of physicochemical characteristics of starch and protein fractions during alkali cooking may help in better controlling the textural properties of demixed amylose–amylopectin–protein composites.

## 2. Materials and methods

### 2.1. Materials

Food-grade mungbean starch (“Pine” brand; Sithinan, Bangkok, Thailand) and cassava starch (“Fish” brand; E.T.C. International Trading, Bangkok, Thailand) were purchased from a local supermarket. All chemicals used were of reagent grade.

### 2.2. Methods

#### 2.2.1. Preparation of alkali-treated starches

Native mungbean starch (N-MB) and native cassava starch (N-CS) were soaked in 100 mM NaOH solution for 18 h at a concentration of 25% (w/v). During the first 3 h of soaking, starch suspensions were stirred using a magnetic stirrer at 25 °C, and then left for 15 h. After that, the supernatant liquid was discarded.

The alkali-treated starches were prepared using two washing regimes. Starches in the first group, designated as AN-MB and AN-CS, were prepared by washing the alkali-treated starches with distilled water until the alkali-treated starches were neutralized to pH 7.0. Starch in the second group, designated as A-MB, was prepared by washing the alkali-treated starch once with distilled water, resulting in alkali-treated starch having high pH (pH ~ 11) after drying. All modified starches were dried at 40 °C in a hot-air oven (model 400; Memmert, Schwabach, Germany) for 18 h until the final moisture content was about 10%. Then they were ground, sifted through a 100-mesh sieve, sealed in plastic bags and stored in a freezer at –20 °C.

#### 2.2.2. Chemical composition

N-MB, N-CS, AN-MB and AN-CS starches were analyzed for moisture (AOAC, 2000), protein (AOAC, 2000), phosphorus (AOAC, 2000) and amylose (Chrastil, 1987) contents, using potato amylose as a standard.

#### 2.2.3. Thermal properties

The thermal properties of native and treated starches were determined by differential scanning calorimetry (DSC). Briefly, a Pyris 1 DSC (PerkinElmer, Waltham, MA, USA) was used to characterize the thermal properties of a 12% (w/w) slurry of starch in distilled water. Each suspension was incubated at 25 °C for 24 h in a stainless steel pan and hermetically sealed prior to 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 gelatinization. The transition temperatures reported were the onset ( $T_o$ ), peak ( $T_p$ ) and end ( $T_e$ ) temperatures of the gelatinization endotherm, and the gelatinization temperature range ( $\Delta T$ , or  $T_e - T_o$ ). The enthalpy of gelatinization ( $\Delta H$ ) was estimated by integrating the area between the thermogram and a baseline connecting the points of onset and end temperatures, and was expressed in J/g starch (dry basis).

#### 2.2.4. Crystallinity

The native and modified starches were equilibrated at 25 °C and 86% relative humidity (RH) in KCl for 18 d before being examined by wide-angle X-ray diffractometry (XRD). Analyses were conducted using an JDX-3530 X-ray diffractometer (JEOL, Tokyo, Japan) and Jade software version 6.5 for data collection. The samples were analyzed between 3° and 50°  $2\theta$ , at a step angle of 0.02°, a count time of 1 s, tube voltage of 30 kV, and tube current of 40 mA. The % relative crystallinity (% RC) of the samples was calculated as the proportion of crystalline area to total area multiplied by 100.

#### 2.2.5. Pasting characteristics

A rapid visco analyzer (RVA) (Newport Scientific, Warriewood, NSW, Australia) was used to characterize pasting properties of native and treated starches. The starch suspensions (12%, w/v) were prepared in individual canisters using distilled water as a dispersant. Each starch suspension (25 mL) was held at 50 °C for 1 min, heated from 50 to 95 °C at a rate of 13 °C/min, held at 95 °C for 2.50 min, cooled to 50 °C at a rate of 13 °C/min, and finally kept at 50 °C at 160 rpm for 1.24 min. Pasting characteristics were described using amylograms, and included pasting temperature

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