Characteristics of Thai Yam (Dioscorea alata L.) and Spherulitic Structure in Starch Film

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ABSTRACT

This study investigated the properties of greater yam (Dioscorea alata L.) tuber and starch. Yam starch granules were spade-shaped with truncated end with the size range of 20 to 50 µm. The apparent amylose content in yam starch was around 36 % (d.b.). The gelatinisation temperature range determined by differential scanning calorimetry was 75.8 – 83.3 ºC. Heating yam starch in alkali solution enhanced solubilisation of granule-bound proteins and most likely ionised the –OH group in starch molecules. Subsequent Ca²⁺-crosslinking of the cooked ionised starch at 25 ºC for 16 h, followed by drying at 45 ºC in a tray-dryer, resulted in starch films having spherulitic structure distributed in the rubbery matrix of ionised starch polymers at room temperature. The size of Ca²⁺-containing spherulites fabricated under alkali condition was around 3-5 µm. This study reported the novel method of using alkali-treated yam starch to form spherulitic structure containing Ca²⁺ after drying in a tray-dryer.

Key words: gelatinisation, Dioscorea, spherulite, starch, yam

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INTRODUCTION

Yam (*Dioscorea* sp.) is one of the world’s starch sources from tubers consumed in tropical and sub-tropical countries. Among yam tuber varieties, greater yam, *Dioscorea alata* L., is the most widely distributed species of the genus *Dioscorea* in the humid and semi-humid tropics (Jayakodi et al., 2007). The characteristics of *D. alata* L. flour and starch have received attention recently due to its high amylose content. *D. alata* L. varieties usually have apparent amylose contents around 29 – 36% (Mali et al., 2002; Freitas et al., 2004; Jayakodi et al., 2007), which is fairly high, compared to that of tuber and root starches. The characteristics of *D. alata* L. starch and modified yam starch have been investigated in-depth for the last ten years (Freitas et al., 2004; Jayakodi et al., 2007; 2009).

Alkali and alcoholic-alkali treatments have been reported to increase water absorption capacity, solubility and freeze-thawed stability of starch from *D. alata* L. (de Delahaye and Techeira, 2009). We have also reported that alkali cooking of starches from mungbean and cassava enhanced water uptake, granular swelling and leaching of amylose and amylpectin when starch granules are disintegrated (Hongsprabhas et al., 2007; Israkarn et al., 2007; Hongsprabhas and Israkarn, 2008). Alkali cooking not only lowered gelatinisation temperature range of starches (Hongsprabhas et al., 2007), but also enhanced the solubilisation of granule-bound proteins (GBPs), which are mainly granule-bound starch synthases immobilised within the granules during granule development (Hongsprabhas and Israkarn, 2008). The loss of GBPs could alter viscoelastic properties of starch granular ghost membrane (Israkarn et al., 2007; Hongsprabhas and Israkarn, 2008), resulting in the effective disintegration of swollen starch granules and leaching of starch molecules to the aqueous phase.

In the present study, it was hypothesised that alkali cooking could help solubilise GBPs in *D. alata* starch granules and the helical starch molecules could uncoil due to the negatively charged repulsion. The ionised starch molecules would be susceptible to subsequent crosslinking by divalent cation for nucleation and crystal growing processes upon drying. The objectives of the present study were to characterise starch isolated from the tubers of *D. alata* L., the purple yam grown in the north-eastern and eastern parts of Thailand; and to explore the potential technique for the fabrication of Ca$^{2+}$-containing spherulites using salt-induced structure-forming process. The insights would help better understanding on the fundamentals of the self-assembly of Ca$^{2+}$-containing crystalline structure.
MATERIALS AND METHODS

1. Materials

Yam tubers (*Dioscorea alata* L.) accession Khon Kaen Field Crop Research Center (acc. KKFCRC) were harvested in January, 2009. They were transported from Khon Kaen to the Department of Food Science and Technology, Kasetsart University in Bangkok, two days after being harvested and kept at room temperature (27 °C). Yam flour and starch were prepared within 2 weeks after harvest.

2. Characteristics of yam tuber and starch

Yam tubers were cleaned with tap water, peeled, sliced and ground with distilled water containing 0.1% sodium azide. Flour suspension was allowed to settle at 4 °C for 16 h. The supernatant liquid was discarded, washed with distilled water twice, filtered through sinter glass filter and dried in an oven at 40 °C for 16 h. The flour had 11.58% moisture and 0.79 % protein determined by Kjeldahl method (AOAC, 1995).

The starch was extracted from yam flour by mixing the flour with 0.1% NaOH at the ratio of flour to NaOH of 1:3 (w/v) and starch granule was allowed to sediment at 4 °C for 16 h. The supernatant liquid was discarded and starch was washed with distilled water three times, filtered, centrifuged at 3000×g, and dried in an oven at 40 °C for 16 h. Excessive washing resulted in whitish starch powder, which had amylose content of 35.99 % (d.b.) determined using potato starch as standard amylose (Chrustil, 1987). Protein was not detected when determined by Kjeldahl method.

Yam starch was kept at -20 °C prior to analyses for microstructure, gelatinisation and pasting characteristics.

Yam tubers were thin-sectioned and stained with Lugol’s iodine solution; while yam starch (0.5%, w/v) suspension was dispersed in distilled water prior to observation under microscopes. The starch samples were stained by 1 μL of Lugol’s iodine solution and incubated for 5 min before each sample was loaded onto a slide and observed under a light microscope (LM) (Axio Imager MI, Carl Zeiss Pte Ltd, Jena, Germany) with and without polarised filter. The image was acquired using Image Pro Plus software version 6.0 (Media Cybernetics, Bethesda, MD, USA).

Confocal laser scanning microscope (CLSM; Axio Imager MI) was used to study protein distribution in yam starch granules. One mL of yam starch suspensions was prepared in either distilled water or 100 mM NaOH at a concentration of 0.5% (w/v). A solution of rhodamine B (0.01% in 95% ethanol) was added to starch suspensions. After incubation for 5 min, each sample was loaded into a well and observed for a location of fluorescent-labelled protein. A HeNe laser with an excitation wavelength of 543 nm was used. CLSM digital image was acquired using the LSM 5 PASCAL program.
Differential scanning calorimetry (DSC) was used to determine the thermal properties of yam starches. Briefly, a Pyris1 DSC (Perkin Elmer, Norwalk, CT, USA) was used to characterise the thermal properties of 12% (w/w) starch suspended in distilled water. The suspension was incubated at 25 °C for 24 h 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 gelatinisation temperature and enthalpy of gelatinisation. The gelatinisation temperatures reported were the onset (T_o), peak (T_p), end (T_e) temperatures of the gelatinisation endotherm and gelatinisation temperature range (ΔT or T_e - T_o). The enthalpy of the gelatinisation (ΔH) was estimated by integrating the area between the thermogram and a baseline connecting the points of onset and end temperature and expressed in J/g starch (d.b.).

3. Effect of CaCl₂ concentration on yam starch spherulite films

Yam starch suspension (2 % in 100 mM NaOH) was heated in a water bath at 75 °C for 30 min, cooled to 25 °C in a water bath (5 °C) using the cooling rate of 2-3 °C/min. After that, distilled water or CaCl₂ solutions was added to the cooked starch suspensions to obtain the final concentration of 0, 6, 12 and 18 mM CaCl₂ and mixed thoroughly. The mixture was poured into a polypropylene plastic box and dried in a tray-dryer at 45 °C for 16 h. The dried starch films were kept at -20 °C prior to analyses for microstructural and thermal properties.

The films were observed under a light microscope (Axio Imager MI) with or without polarised filter by fixing starch films in glycerol to avoid dissolution of the films. DSC (Perkin Elmer) was used to characterise the glass transition temperature (T_g) of starch films. Sample of 1-3 mg was frozen in a hermetically sealed stainless steel pan at (−)40 °C, then it was heated from (−)40 °C to 130 °C at a rate of 15 °C/min, cooled from 130 °C to (−)40 °C at a rate of 15 °C/min and heated from (−)40 °C to 130 °C at a rate of 15 °C/min.

4. Statistical analysis

The experiments were carried out in two separate trials. Each trial was run in duplicate. The data were analysed by using analysis of variance (ANOVA) at 95% significance level. Significant differences among mean values from ANOVA were determined by Duncan’s multiple range tests. All statistical analyses were performed using SPSS Software Version 12 (SPSS Inc., Chicago, IL, US).

RESULTS AND DISCUSSION

Yam tubers cultivated at KKFCRC station in Khon Kaen had various size and shape (Figure 1a) with varying weight of tubers from 300 to 800 g. Starch granules showed dark blue colour when stained with Lugol’s iodine. They were spade-shaped with truncated end and packed densely in the cytoplasm (Figure 1b). The isolated starch granules had the size ranged from 20 to 50 μm (Figure
1c). Under cross-polarised light, the birefringence was observed in starch granules with the intersection of Maltese cross close to the tip of the spade-shaped granular structure (Figure 1d).

The size and shape of the tubers of *D. alata* acc. KKFCRC was different from those tubers of Sri Lanka *D. alata* cultivars (Jayakodi et al., 2007), suggesting that the Thai yam and Sri Lanka yam may be of different cultivars. The remarkable morphological variation of *D. alata* tubers has been reported to exist among cultivars (Jayakodi et al., 2007). However, the size and shape of starch granules from Thai *D. alata* reported in this study were in agreement with the characteristic size and shape of starch granule from *D. alata* commonly consumed in Sri Lanka, which had truncated and spade-shaped of 30–45 μm (Jayakodi et al., 2007).

![Figure 1](image)

**Figure 1** Morphology of (a) yam (*Dioscorea alata* L.) tuber, (b) starch granules in cytoplasm stained dark with Lugol's iodine; (c) isolated starch granules stained dark with Lugol's iodine and (d) isolated starch granules under polarised light. Bars = 50 μm.

The gelatinisation temperature range of Thai yam starch was 75.8 – 83.3 °C and the enthalpy of gelatinisation was 23.8 J/g (d.b.) (Table 1). The high amylose content of yam; i.e. 36 %, was slightly higher than that of *D. alata* starch reported by other investigators (Mali et al., 2002; Freitas et al., 2004; Jayakodi et al., 2007).

Although the protein content in yam starch was not detected by Kjeldahl method, CLSM is quite effective in detecting the residual protein in yam starch granules (Figure 2). This is because the fluoresced rhodamine B bound specifically to the amine group of proteins in starch granules. The residual proteins in yam starch granules were likely GBPs, observed as red colour of fluoresced rhodamine B distributed within the granules dispersed in distilled water (Figure 2a).
Table 1  Gelatinization characteristics (mean values ± standard deviation) of Dioscorea alata L. starch.

<table>
<thead>
<tr>
<th>Gelatinisation characteristics</th>
<th>( T_0 ) (°C)</th>
<th>( T_p ) (°C)</th>
<th>( T_e ) (°C)</th>
<th>( \Delta T )</th>
<th>( \Delta H ) of gelatinisation (J/g (d.b.))</th>
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<tr>
<td>( T_0 ) (°C)</td>
<td>75.8 ± 1.5</td>
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<tr>
<td>( T_p ) (°C)</td>
<td>78.9 ± 0.2</td>
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<tr>
<td>( T_e ) (°C)</td>
<td>83.3 ± 0.2</td>
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<tr>
<td>( \Delta T )</td>
<td>7.6 ± 1.6</td>
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<tr>
<td>( \Delta H ) of gelatinisation (J/g (d.b.))</td>
<td>23.8 ± 2.7</td>
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Alkali soaking of yam starch, even for 5 min, solubilised the GBPs in yam starch granules, observed as the red colour in the aqueous phase in the background (Figure 2b). This influence of alkali in solubilising the GBPs was also observed in mungbean and cassava starches as reported previously (Israkarn et al., 2007; Hongsprabhas and Israkarn, 2008).

Figure 2  Confocal laser scanning micrographs of granule-bound protein in yam starch granules dispersed in (a) distilled water and (b) 100 mM NaOH. Protein phase fluoresced as red colour by rhodamine B. Bars = 50 μm.

After yam starch was cooked in alkali solution, cooled down to room temperature, allowed to react with CaCl\(_2\) solution and dried at of 45 °C, Ca\(^{2+}\)-induced spherulitic structure was formed in starch films. In the absence of CaCl\(_2\), such spherulites were not observed. This suggested that Ca\(^{2+}\)-crosslinking is essential for salt-induced structure-forming process of the spherulites upon drying. The spherulites having homogeneous size range of around 3-5 μm were distributed within the film (Figure 3c). It should be noted that the size of spherulites was much smaller than that of native starch granules (Figure 3a and Figure 3b). The birefringence observed in the spherulites also exhibited radial growth during spherulitic crystallization of such Ca\(^{2+}\)-containing structure.
The concentration of CaCl$_2$ did not show significant influences on phase transitions of the films (Table 2). The films containing spherulites had distinct phase transition temperatures including two transitions of glassy to rubbery states. The $T_{g1}$ of around $(-)10$ – $(-)13$ °C represented the glass transition temperature range of the unfreezable water phase. The $T_{g2}$, which was within the range of 23 – 25 °C, was likely the $T_g$ of starch matrices. Heating the film up to 130 °C did not melt the spherulitic structure fabricated.

![Image](image.png)

**Figure 3** Appearance of yam starch granules and spherulites in yam starch films under light microscope: (a) granules stained with Lugol's iodine; (b) birefringence of granule under polarised light; (c) appearance of spherulites in starch film; and (d) birefringence of spherulite under polarised light. Bars = 20 μm.

<table>
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<tr>
<th>[CaCl$_2$] mM</th>
<th>Glass transition temperature</th>
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<tr>
<td></td>
<td>$T_{g1}$ (°C)$^{ns}$</td>
</tr>
<tr>
<td>6</td>
<td>-11.9 ± 5.7</td>
</tr>
<tr>
<td>12</td>
<td>-11.5 ± 1.7</td>
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<tr>
<td>18</td>
<td>-10.8 ± 0.6</td>
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$^{ns}$ Not significantly different ($P \geq 0.05$)

**CONCLUSION**

This study has reported the existence of spherulitic structure in starch film made from alkali cooked yam starch and its method for preparation. However, the composition and the types of crystalline structure in spherulites need further characterisation. The salt-induced structure-forming process of spherulitic structures for the production of Ca$^{2+}$-containing spherulites from other starch sources is under way.
ACKNOWLEDGEMENTS

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REFERENCES


