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# Polymorph and Crystallinity Disruption of Sago Starch during Gelatinization

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**Abstract:** Sago starch samples were collected from Port Moresby of Papua New Guinea (PNG) and Baybay, Leyte, of the Philippines (PLP) to study the polymorph and crystalline disruption of sago starch during gelatinization using corn and potato starch as references. Starch granule suspension was transferred onto a glass slide covered with a cover glass, sealed with nail polish to prevent moisture loss, mounted on a micro-heatplate, and observed using an optical microscope equipped with cross polarizers and a λ-plate. The behavior of sago starch granules during gelatinization was shown by the granular birefringence (Maltese cross) observed under polarized light. The initial, middle, and end gelatinization temperatures determined by a hot stage were, respectively, 78°C, 80°C, and 83°C for PNG sago starch and 76°C, 81°C, and 89°C for PLP sago starch in water. Sago starch still exhibited C-type crystallinity from 60 to 75°C. However, the X-ray diffraction peak at 5 to 6° for 2θ was observed to decrease, disappearing with increased heating temperature. At 18°, the peaks of PNG and PLP starches began to appear and slightly increase, although the intensity was decreased as a whole. The disappearance of B-type crystallinity from sago starch occurred during gelatinization.

**Keywords:** gelatinization, micro-heatplate, optical microscope, sago starch

## Introduction

Starch granules consist of mainly linear amylose and highly branched amylopectin. The branch chain lengths of amylopectin differ between A-type and B-type crystalline starches (Hizukuri, 1985). Starch with longer branch chains displays the B type, and starch with shorter branch chains displays the A-type. Furthermore, Hizukuri (1986) reported the polymodal distribution of the lengths of the amylopectins of waxy rice, tapioca, kuzu, and potato. Jane et al. (2003) showed that A-type starches possessed more short branch chains and were more easily digestible by amylases than were B-type starches. The amylopectin molecules of B-type starches have smaller molecular weights and less dense dispersed molecules than do those of A-type starches (Jane et al., 2003).

Sago starch, a C-type starch, is composed of polymorph structures, A and B types, that occur within the same granules (Sair, 1967). It has intermediate characteristics of gelatinization between those of A and B types. However, there have been few reports on this polymorph and the disruption of the structure of sago starch that accompanies gelatinization, although the observation of crystallinity disruption patterns during gelatinization for C-type starches was carried out using the pea (Bogracheva et al., 1998) and lotus rhizome starches (Cai and Wei, 2013). The behavior of starch granules during gelatinization was shown by the granular birefringence observed under a polarizing microscope with a λ-plate in combination with a hot stage. Bogracheva et al. (1998) concluded that A- and B-type polymorphs were present in the same pea granules, and that B polymorphs were situated in the center of all granules. However, the disruption of the crystalline structure of lotus rhizome C-type starch first occurred on the distal surface of the eccentric hilum and propagated along granules from the distal surface of the proximal surface of the eccentric hilum (Cai and Wei, 2013). Cai et

al. (2014) proposed a model of allomorph distribution and a gelatinization process of lotus rhizome in which the A-type allomorph was mainly located in the periphery region of the hilum end, and the central part of the granule had a mixed distribution of the A- and B-type allomorphs.

The objectives of this study are to show the polymorph of sago C-type starch and to determine the crystallinity disruption of sago starch during gelatinization.

## Materials and Methods

### 1. Sago starch samples

Sago starch samples were collected from Port Moresby of Papua New Guinea (PNG) and Baybay, Leyte, of the Philippines (PLP). PNG samples were obtained from a sago starch shop in the Koki Market, Port Moresby, in 2001. PLP samples were extracted in Baybay by the authors in 2011. The annual mean temperatures of Port Moresby and Baybay are 27.3°C and 28.0°C (the annual mean temperature of Mactan Island, which is located in 150 km west of Baybay), respectively. The moisture content of sago starch stocked in the laboratory was 12% for PNG and 13% for PLP. Corn (Kosakai Pharmaceutical Co., Tokyo, Japan) and potato (Miyazawa Pharmaceutical Co., Tokyo, Japan) starch samples were used as references.

### 2. Microscopic observation of starch granules

A starch granule suspension was prepared by dissolving 10 mg starch in 1.0 mL distilled water, and was transferred onto a glass slide covered with a cover glass and sealed with nail polish to prevent moisture loss (Cai et al., 2014). The specimen was mounted on a micro-heatplate (Kitazato MP-1000H, Shizuoka, Japan) and observed using a polarizing microscope (Meiji MT 9000, Saitama, Japan) equipped with cross polarizers and a λ-plate.

A native starch suspension (2.5% in distilled water) was shaken by hand to resuspend the sediment starch. The behavior of sago starch granules during gelatinization was shown using the Meiji MT 9000 to observe granular birefringence (Maltese cross) under polarized light on a micro-heatplate. The step mode of heating was used: from room temperature to 50°C with a heating rate of 5°C $min^{-1}$, a holding temperature at 50°C for 30 sec, from 50°C to 90°C with a heating rate of 1°C $min^{-1}$, a holding temperature at 90°C for 5 min, and from 90°C to 25°C with a heating rate of 10°C $min^{-1}$. A process of cooling was required for the next specimen. The starch granules during heating were observed with a 400x objective lens and photographed with a camera (Cannon EOS KISS X5).

### 3. X-ray diffraction analysis of native and gelatinized starch samples

Native and heated starch suspensions (2.5% w/v) were shaken by hand to resuspend the sediment starch in accordance with the method of Bogracheva et al. (1998) and Cai et al. (2014). In a water bath, the starch samples were heated at 60 to 80°C for 3 min. The heated starch samples were immediately cooled in an ice bath and centrifuged (2140 g) for 10 min. The samples were washed four times with Milli-Q water and anhydrous ethanol and then dried at 40°C for 3 days using a Shibata BV-001 under 0.065 MPa. The dried starch samples were ground into powder in a mortar and pestle. After pretreatment, the moisture contents of the PNG and PLP samples were 4.56 % and 4.61 %, respectively. X-ray diffraction analysis showed that the moisture contents of the corn and potato samples were 3.68 % and 5.05 %, respectively.

X-ray diffraction (XRD) analysis was performed using a Rigaku MiniFlex. Copper (Cu) Kα radiation was used with nickel filter. The scanning speed was recorded as 2° $min^{-1}$, and the operation angle was 3° to 45° for 2θ. The samples were held on an antireflection silicon sample holder because of its exclusion of external X-ray intensities (Katsumi et al., 2015).

## Results

### 1. Morphological characteristics of sago starch

Native sago starch granules had medium oval and temple bell shapes, with the eccentric hila at one end of the granules (Figs. 1 and 2, normal, polarized, and λ-plate); these appearances were similar to those observed by Kobayashi (1993).

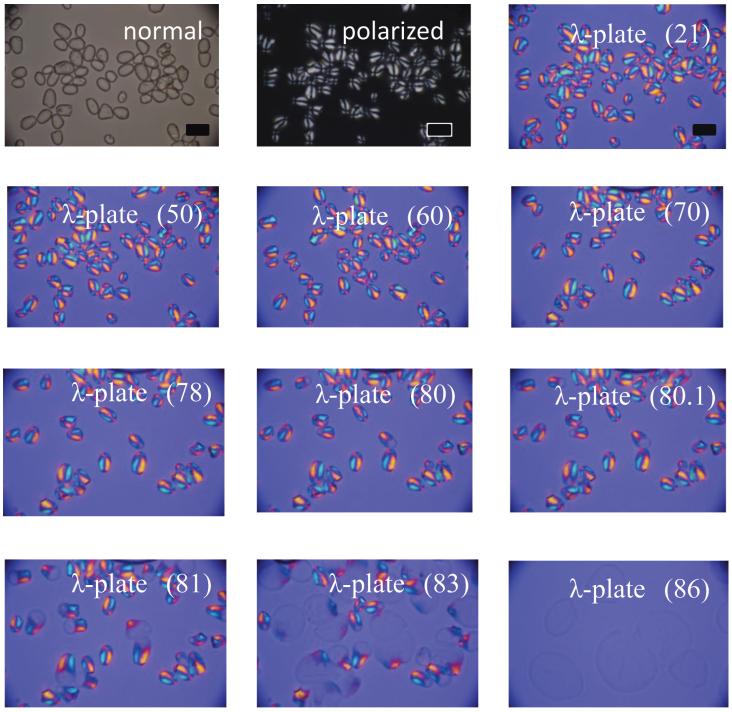


**Fig. 1.** Micrographs of PNG sago starch under normal and polarized light in conjunction with a λ-plate during gelatinization
Bar is 50 μm.
The number in parentheses shows the temperature during gelatinization.

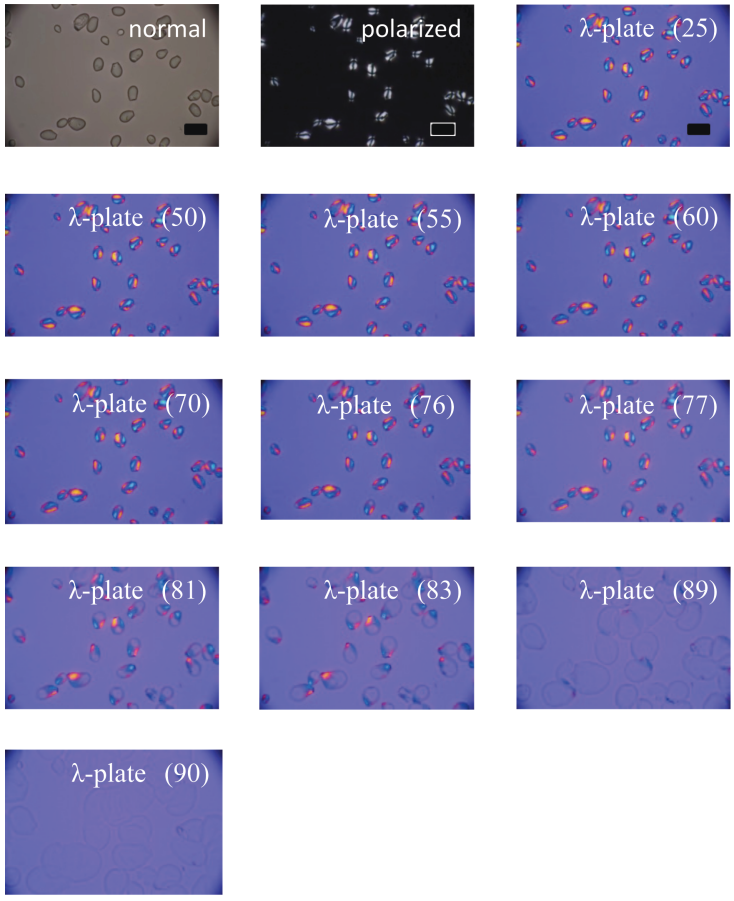


**Fig. 2.** Micrographs of PLP sago starch under normal and polarized light in conjunction with a λ-plate during gelatinization
Bar is 50 μm.
The number in parentheses shows the temperature during gelatinization.

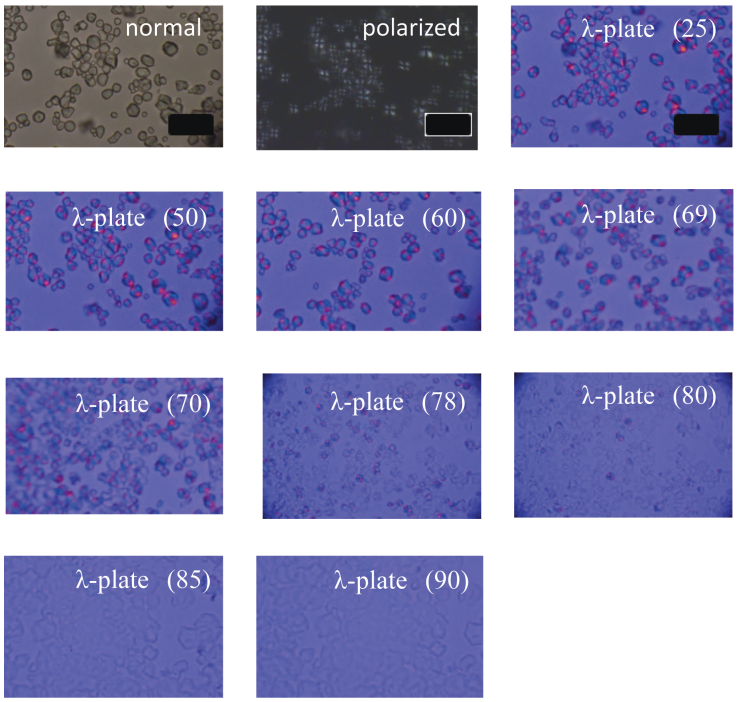


**Fig. 3.** Micrographs of corn starch under normal and polarized light in conjunction with a λ-plate during gelatinization
Bar is 50 μm.
The number in parentheses shows the temperature during gelatinization.

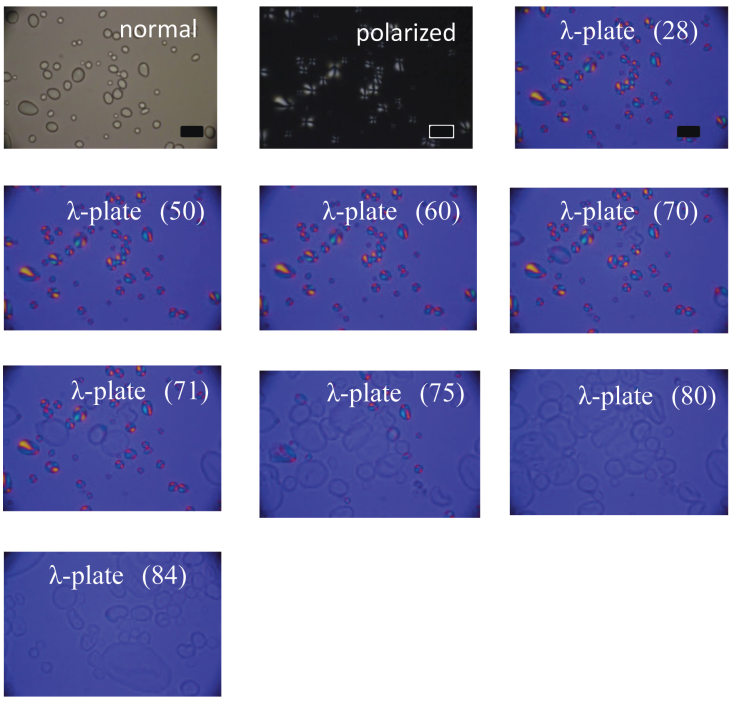


**Fig. 4.** Micrographs of potato starch under normal and polarized light in conjunction with a λ-plate during gelatinization
Bar is 50 μm.
The number in parentheses shows the temperature during gelatinization.

**2. Crystalline structure disruption in sago starch granules during gelatinization**

The behavior of sago starch granules was shown by the granular birefringence (Maltese cross) observed under polarized light before gelatinization (Fig. 1, polarized and λ-plate). The blue and yellow areas, indicating radial alignments, represent the regions of crystallinity of sago starch. The loss of colors upon heating indicates the disappearance of the radial alignment of crystallinity. The disruption of crystallinity occurred on the proximate surface of the eccentric hilum and was propagated from the proximate surface to the distal surface of the eccentric hilum. The initial, middle, and end gelatinization temperatures determined by a hot stage were, respectively, 78.0°C, 80.0°C, and 83.0°C for the PNG sago starch (Fig. 1), and 75.9°C, 80.9°C, and 89.1°C for the PLP sago starch (Fig. 2). Figures 3 and 4 show microscopic images of corn and potato starch during gelatinization, which reveals lower starting temperatures of gelatinization : 70.0°C for corn and 69.8°C for potato.

X-ray diffraction (XRD) showed C-type allomorphs containing different proportions of A- and B-type polymorphs in PNG and PLP sago starches, as seen in Fig. 5, as compared to corn (A type) and potato (B type) starches. The peak intensity of PNG sago starch at 60°C from 3° to 40° was lower than that at 21°C, which is caused by the gradual progression of gelatinization (Fig. 1, λ-plate (21) to λ-plate (60)). This kind of peak-lowering observation resembled that of the potato sample. The corn sample showed strong X-ray diffraction peaks at about 17°, 18°, and 23° for Cu Kα, and the potato sample exhibited strong diffraction peaks at about 5.6°, 18°, 22°, and 23° for Cu Kα. Sago starch samples still showed C-type crystallinity from 60°C to 75°C. However, the corn sample's peak intensity decreased with increasing heat from 65° C. The B-type crystallinity disappeared from 70°C. At 17° to 18°, the double sharp peaks began to be single clearly, and the clear single peak gradually disappeared. This kind of alteration also showed that the crystallinity of sago starch changed from C-type (mixture of A-type and B-type) to the A-type only, which caused the

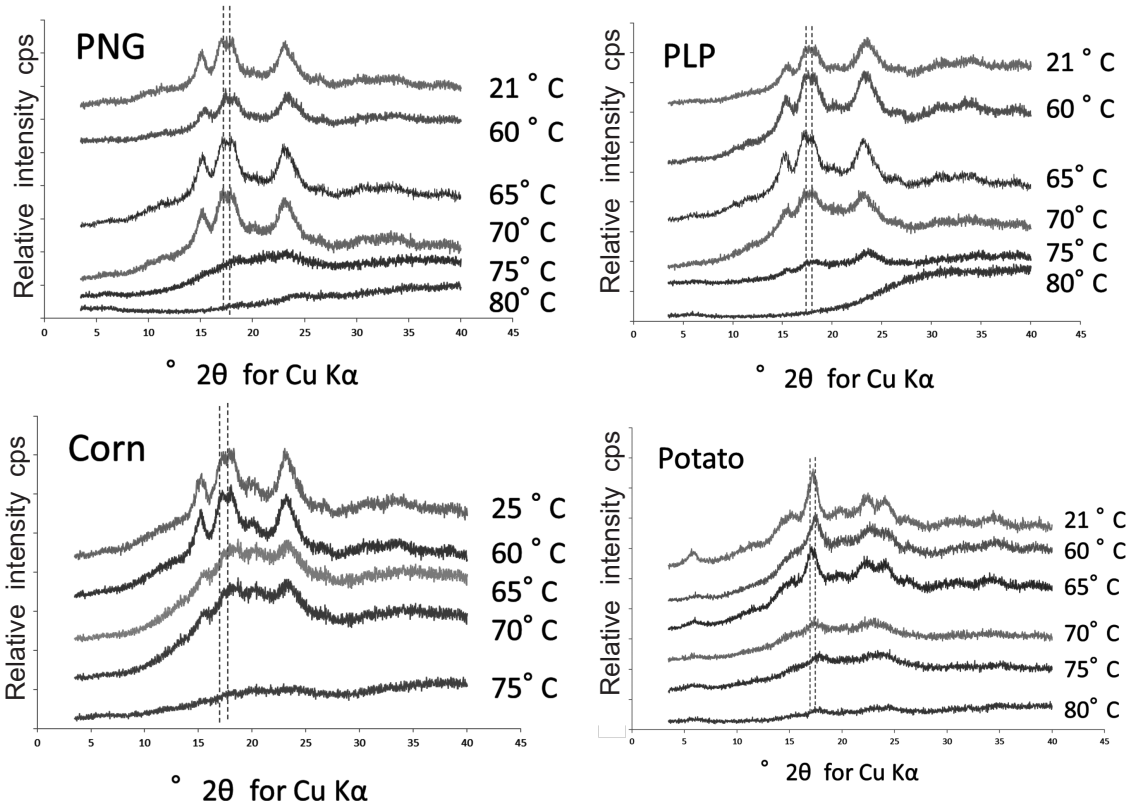


**Fig. 5.** Changes in the X-ray diffraction of starch samples during gelatinization

disappearance of water molecules in the cavity of B-type crystallinity, according to the X-ray diffraction pattern (Okazaki et al., 2015). The peak intensity at 5.6°, 17°, 18°, 22°, and 23° of all starches significantly decreased at more than 80°C.

## Discussion

### 1. Type classification of starch structure disruption using a polarized microscope

The structure disruption of sago starch (C type) during gelatinization has not yet been completely discussed. Cai and Wei (2013) proposed that during gelatinization, starch granules were classified into four patterns of crystallinity disruption (P1: on the proximate surface of the eccentric hilum, P2: on the distal surface of the eccentric hilum, P3: from the central hilum, and P4: on the surface of the central hilum). Sago starch granules were classified as P1, implying that the B type in sago starch grains was concentrated in the proximate surface of the eccentric hilum.

### 2. Polymorph accompanied to gelatinization

The gelatinization of sago starch caused the structure modification; X-ray diffraction showed the disappearance of peaks at 5.6°, 17°, 18°, and 23°, resulting in the presence of the A type only. Heat and moisture change the gelatinization temperature, swelling behavior, and paste translucency of root (B type) starches to those of cereal (A type) starches (Sair, 1967). Zobel et al. (1988) demonstrated the structure change of potato starch with 19 to 22% moisture content at 125°C and 148°C from B type to A type. These results were interpreted to be solid-state transitions.

Kainuma and Hatta (2003) reported a change in the X-ray diffraction intensity at 5.6° and in the value of the full width at half maximum (FWHM) of potato starch (B type) accompanied by heating from 84.1 to 84.9°C. The disruption of C-type starch (sago starch) with gelatinization, reported by Okazaki et al. (2015), represented the disappearance of the cavity in the hexagonal system of the B type. These facts suggest a polymorph in C-type starches.

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