

## Changes in physicochemical properties and morphology of canna starches during rhizomal development

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### Abstract

Canna rhizomes aged seven months were harvested, and their segments were classified into four groups: mother, immature, premature and mature segments, according to the sequence of development. Based on weight of the segments, the mature segment was the greatest part (70%), whereas the immature and premature were of comparable amounts (14% and 15%, respectively). The average weight of one rhizome was 5324 g. Starch content increased from the immature segment (13.7%) to the premature segment (19.5%), and slightly decreased in the mature segment (18.6%), while the mother segment had the lowest starch content (7.9%). The size of the starch granules increased with progressive development of segments from immature to premature and mature stages. Starch from the mother segment contained a higher proportion of small size granules than the other segments and unusual features were observed on the surface of some granules. The appearance of granules from the different samples subjected to the same period of hydrolysis by porcine pancreatic  $\alpha$ -amylase was similar, although differences in the degree of hydrolysis were found. Signs of degradation were more readily seen as the period of hydrolysis was extended. The mode of attack by  $\alpha$ -amylase on canna starch was principally digestion through surface corrosion. Starches from different segments showed similar crystalline structures, and thermal and pasting properties; however, some aspects of the chemical composition of starch from the mother segment were different from the others.

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**Keywords:** Edible canna; Starch; Rhizome; Physicochemical properties; Granule morphology; Amylase

### 1. Introduction

Edible canna (*Canna edulis* Ker.) is a rhizomatous perennial herb of the family Cannaceae, native to the Andean region of South America. This plant has large starchy rhizomes, which have been used as a traditional staple food for Andean people for more than 4000 years. This

crop is now cultivated for starch production in small-scale factories in China, Taiwan, and Vietnam. There have been reports on the physicochemical and rheological properties (Hung & Morita, 2005; Pérez, Breene, & Bahnassey, 1998; Santacruz, Koch, Svensson, Ruales, & Eliasson, 2002; Santacruz, Ruales, & Eliasson, 2003; Soni, Sharma, Srivastava, & Gharia, 1990; Thitipraphunkul, Uttapap, Piyachomkwan, & Takeda, 2003a), molecular structure (Thitipraphunkul, Uttapap, Takeda, & Piyachomkwan, 2003b), chemical modification (Chuenkamol, Puttanlek, Rungsardthong, & Uttapap, 2007; Saartrat, Puttanlek,

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Rungsardthong, & Uttapap, 2005) and utilization (Chansri, Puttanlek, Rungsadthong, & Uttapap, 2005) of edible canna starch. Canna starch was characterized by very large granules, high amylose content, clear paste, high viscosity, low breakdown, high retrogradation and high resistance to hydrolysis by  $\alpha$ -amylase.

In general, the edible canna forms a branching rhizome which orients parallel to the surface of the ground. In mature plants, the canna rhizome consists of several segments (or generations) developed consecutively. Each of these segments varies in size and age. Starch granules accumulated in the different stage-segments, even in the same rhizome, are supposed to have differences in their state of development, chemical compositions and physicochemical properties. Until now the properties of starch in the underground stem/portion of starchy plants have been focused solely on tuber and root crops. Changes in chemical compositions and functional properties of potato and cassava starches during growth have been reported. Peak viscosity and breakdown of starch increased with increasing tuber size (Madsen & Christensen, 1996). Liu, Weber, Currie, and Yada (2003) also found that starch obtained from potatoes with a shorter growth time had higher gelatinization and pasting temperatures, lower peak viscosity and higher final viscosity. In the case of cassava starch, the granule size of cassava starches from six varieties increased up to the sixth month and thereafter remained almost constant, whereas the amylose content and reducing values did not vary much at different stages of growth (Moorthy & Ramanujam, 1986).

It is believed that the tuberization process in potato/cassava is initiated when the supply of assimilated carbon from photosynthesis exceeds the minimum level required for the growth of leaves and stems (Cock, Franklin, Sandoval, & Juri, 1979; El-Sharkawy, 2003; Tan & Cock, 1979). The pattern of tuber/root formation is roughly characterized by the almost synchronous development of all elements of tubers or roots. Therefore, the starches accumulated in each of the tubers/roots at various stages of development are assumed to have similar properties. In contrast, as mentioned previously, rhizome formation in canna plants is much more complicated, and the starches accumulated might be affected by the sequence of rhizome development. However, no information regarding this event is available in the existing literature.

In this study, the canna rhizome segments were divided into four groups based on the stage of development: immature, premature, mature and mother segments. Number- and weight-based distributions and starch content of the segments in each group were determined. Starches isolated from these segments were examined in order to determine whether there was any variation in granule morphology, susceptibility towards  $\alpha$ -amylase hydrolysis, chemical composition and physicochemical properties among these segments.

## 2. Materials and methods

### 2.1. Materials

Edible canna (Vietnam-purple) was grown on experimental plots at the Rayong Field Crops Research Center, Rayong, Thailand. Seven-month-old rhizomes were harvested, and the rhizome segments were separated into four groups based on the stage of development: immature, premature, mature and mother segments.

### 2.2. Starch preparation

Approximately 1-cm cubes of cleaned rhizomes in water were ground in a blender. The slurry was screened through a bolting cloth, and the suspension obtained was filtered through a 106  $\mu$ m sieve. The filtrate was allowed to settle until a dense, firm starch layer was deposited. The supernatant was decanted, and the starch cake was rewashed at least three times. The starch cake was then dried in an oven at 50 °C for 15 h.

### 2.3. Starch and moisture content of rhizome

Standard AOAC method (1990) was used for the measurement of moisture content. For the starch content, the slurry of the rhizome segment was digested with  $\alpha$ -amylase at 90 °C, pH 6.0 for 1 h, followed by digestion with amyloglucosidase at 60 °C, pH 4.5, for 48 h. The supernatant was then separated from the residue by centrifugation, and glucose concentration in the supernatant was determined by the GOD-PAP method (Barham & Trinder, 1972) with some modifications. The starch content of rhizome was calculated according to the following equation,

$$\begin{aligned} \text{Starch (\% wet weight basis)} = & \text{glucose concentration (mg/ml)} \\ & \times \frac{162}{180} \times \frac{100}{\text{sample weight (mg)}} \\ & \times \text{dilution factor} \end{aligned}$$

### 2.4. Chemical composition analysis

Standard AOAC methods (1990) were used for the measurement of moisture, nitrogen, lipid and ash. Protein was determined from estimates of total nitrogen using a conversion factor of 6.25. For measurement of inorganic constituents, the starch sample (500 mg) was firstly digested with 30% hydrogen peroxide (1 ml) and 65% nitric acid (6 ml) in Microwave Digester (Milestone, model MLS-1200 MEGA). Ca, Fe and Na were analyzed by inductively coupled plasma (ICP) using JY124 model (HORIBA Jobin Yvon Inc., NJ, USA). The phosphorus content was determined by a colorimetric chemical method (Smith & Caruso, 1964). Apparent amylose content was determined by a procedure described by Jayakody and Hoover (2002).

The amylose content was calculated from a standard curve prepared by using mixtures of pure amylose and amylopectin fractionated from edible canna starch (over the range of 0–100% amylose).

### 2.5. Scanning electron microscopy (SEM)

Starch granules were prepared by sprinkling the starch onto double-sided adhesive tape attached to a circular specimen stub and coated with gold using a Balzers SCD 004 sputtering coater. The samples were viewed using a JEOL JSM-5800 scanning electron microscope at an accelerating voltage of 20 kV.

### 2.6. Granule size distribution

Starch samples were suspended in 80% sucrose solution to minimize the effects of refractive index and stained with 0.2% I<sub>2</sub>/KI. Two slides of each sample were analyzed separately, with 1000 granules measured from each slide, to give a total of 2000 starch granules analyzed per sample. The granules were viewed under a light microscope using an Olympus BX51 series microscope (Olympus Optical Co. Ltd., Tokyo, Japan) at 100× magnification. Images were taken with an Olympus DP70 series color video camera and the granule sizes were then analyzed by image analysis software (Image-Pro Plus 3.0, Media Cybernetics, LP, USA). The coefficient of variation was calculated by using the formula: (standard deviation/mean sample value) × 100%.

### 2.7. Enzymatic hydrolysis

One hundred milligrams of starch was suspended in 10 ml of 0.05 M phosphate buffer (pH 6.9) containing 0.003 M CaCl<sub>2</sub> with vigorous mixing, and 10 µl of porcine pancreatic α-amylase (40 units/mg starch) was added. The suspension was incubated at 45 °C and 500 rpm in a water bath shaker. One milliliter of sample was removed at 3, 6, 24, 48 and 72 h and centrifuged (10,000 rpm) for 5 min. The supernatant was analyzed for reducing sugar by the Dinitrosalicylic colorimetric method (Miller, 1959) using maltose as a standard. Percentage of hydrolysis was calculated as the amount of maltose released per 100 mg of dry starch. The residue was washed with distilled water (2×) and absolute ethanol (2×), and then dried at 40 °C. The dried sample was used for morphology examination by SEM. The experiment was carried out with two replications.

### 2.8. Pasting properties

Pasting properties of starch slurry at a concentration of 6% (w/w) were determined using a Rapid Visco Analyzer (RVA-3D, Newport Scientific, Narrabeen, Australia) with a paddle rotating at a fixed speed of 160 rpm. The starch slurry was heated from 40 to 92.5 °C at the rate of 3 °C/min,

maintained at 92.5 °C for 15 min, and then cooled to 40 °C at the same rate.

### 2.9. Differential scanning calorimetry (DSC)

Thermal properties of starches were determined by differential scanning calorimeter (DSC-Pyris 1, Perkin Elmer, Norwalk, CT). Starch (3 mg) was weighed in a DSC pan and water (6 mg) was added. The pan was sealed and allowed to stand for 24 h at 4 °C. The scanning temperature range and the heating rate were 30–120 °C and 5 °C/min, respectively. Water (6 mg) was used as a reference. The transition temperatures reported are the onset temperature ( $T_o$ ), peak temperature ( $T_p$ ) and conclusion temperature ( $T_c$ ). The enthalpy of gelatinization ( $\Delta H$ ) was estimated by integrating the area between the thermogram and a base line under the peak, and was expressed in terms of joules per gram of dry starch. The retrogradation study was performed following the same method, using the same gelatinized starch samples that had been stored at 4 °C for seven days.

### 2.10. X-ray diffraction pattern

X-ray diffraction patterns of wet specimens were obtained with an X-ray diffractometer (Rotaflex RV-20013, Rigaku Denki Co., Tokyo, Japan) using the conditions described by Hizukuri et al. (1988).

## 3. Results and discussion

### 3.1. Characteristics of canna rhizome

#### 3.1.1. Rhizome development and classification of rhizome segments

In this experiment, each canna plant originated from one segment called the “mother segment”. That is, one canna plant would possess only one mother segment throughout its life. These segments contained several nodes and internodes which could be more clearly seen when the segments were enlarging. About two months after planting, the mother segment started to bud a number of new segments. These new segments emerged from the internodes in an alternating arrangement on the opposite sides of the mother segment. The newly emerged segments gradually enlarged until reaching a stage when shooting had just started to be observed. The segments up to this stage were all classified as “immature segments”. Further development of these segments resulted in the “premature segments”, which included the segments up to a stage that their stems were no more than 5 in. long. Finally, these segments would develop to a mature stem and inflorescence, respectively. The rhizome segments at this stage of development were called “mature segments”.

Fig. 1 shows a physical feature of edible canna rhizome harvested at the fourth month after planting. The rhizome had a yellowish skin, and the tip of each segment was

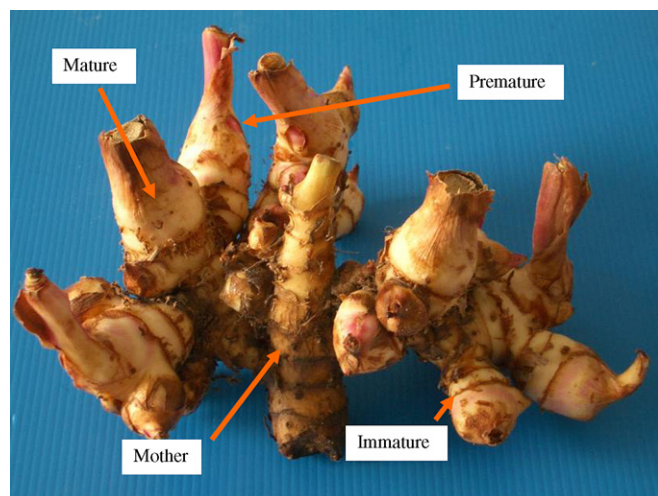


Fig. 1. Physical features of edible canna rhizome harvested at fourth month after planting.

covered with purple-colored scales. The rhizome at this period had three or four generations of segment development and was expanded to five or six generations at the seventh month. Ten canna plants at the seventh month were collected, and their rhizome segments were separated into four groups: mother, immature, premature and mature segments. Average weight and number of segments are presented in Table 1. One rhizome was comprised of 52 segments on average. The immature segment was in the majority (56%) by number of segments, followed by mature (27%), premature (15%) and mother segment (2%). Based on the weight of segments, however, the mature segment was the greatest part (70%), whereas the immature and premature had comparable amounts (14% and 15%, respectively). The average weight of one rhizome was 5324 g. The relative quantities of each group of segments may affect the physicochemical properties of the pooled starch.

Fig. 2 shows the parenchyma cells of immature, premature, mature and mother segment located approximately 0.5 mm away from the rim of the vascular bundle. The starch granules in the parenchyma cells of the mother segment had the smallest size, and the number of granules contained in these cells seemed to be higher than those of the other segments. The starch granules of immature segment had a disk or elliptical shape and were slightly smaller than those of the premature and mature segments. The starch granules looked rounder in the cells of the mature segment.

### 3.1.2. Starch and moisture content

The data on starch and moisture content of each segment are presented in Table 1. The starch content increased from the immature segment (13.7%) to the premature segment (19.5%) and slightly decreased in the mature segment (18.6%), while the mother segment had the lowest starch content (7.9%). Low content of starch in the mother segment reflects an assimilation of starch for maintenance during dormancy and for propagation of the new segments two months after planting. In contrast, the moisture content was highest in the mother segment. The moisture contents of the premature and mature segments were relatively lower and comparable, while that of the immature segment was a bit higher. In total, one rhizome contained approximately 1 kg of starch (956 g). The higher starch content in premature and mature segments could be attributable to the increase in the number and/or size of the starch granules. At seven months after planting, starch from the mature segments was in the majority and would have the most influence on the properties of the whole starch.

### 3.2. Characteristics of canna starch

Starches were isolated from immature, premature, mature and mother segments of canna rhizomes, and their morphology, susceptibility towards  $\alpha$ -amylase hydrolysis, chemical composition, and thermal, pasting and gel properties were investigated. The results obtained were as follows.

#### 3.2.1. Granule morphology

Fig. 3 shows scanning electron micrographs of starch granules from immature, premature, mature and mother segments. Similar to the microscopic observation of fresh tissue, the size of the starch granules increased with progressive development of segments from immature to premature and mature stages. Most of the starch granules were in a disk or oval shape with a smooth surface. The full size granules seemed to have a rounder shape. For the mother segment, the small and medium size granules were in the majority. Moreover, it could be noticed that the surface of some granules was not smooth, and some defects were observed. A clearer view of these defects could be seen when the micrograph was magnified 2000–3500 times as shown in Fig. 4. The unusual features of granules included: parallel-striations, wave-like patterned surface (Fig. 4A), holes/pores on surface (Fig. 4B), collapsed granule

Table 1  
Average weight and number of segments per canna plant, starch and moisture content of each group of canna segments

Attribute	Immature segment	Premature segment	Mature segment	Mother segment
Average number of segments per plant (segments/%)	29/56.5	8/14.6	14/27.0	1/1.9
Average weight of segments per plant (g/%)	746.9/14.0	796.9/15.0	3735.2/70.2	44.7/0.8
Starch content (% based on wet weight)	13.7	19.5	18.6	7.9
Total starch per plant (g)	102.3	155.4	694.7	3.5
Moisture content (% based on wet weight)	74.5	71.5	71.4	82.6



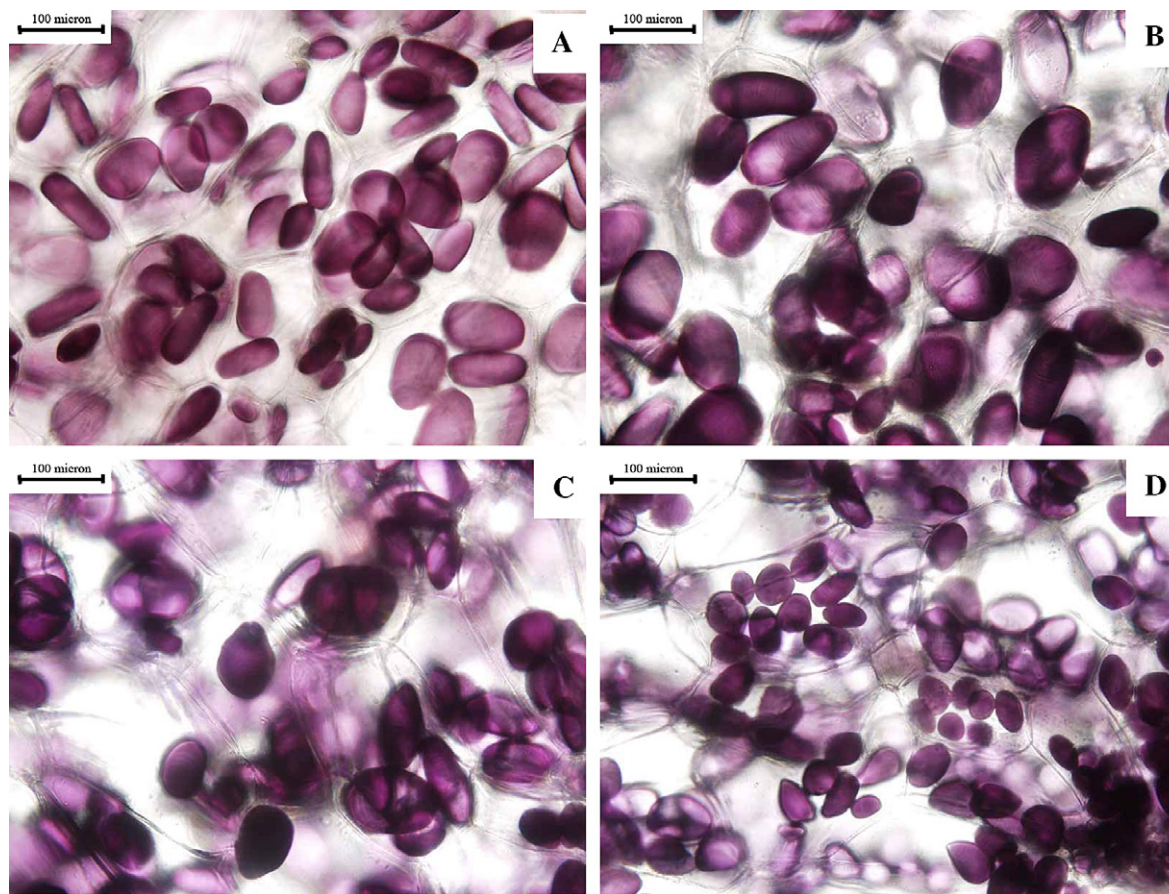


Fig. 2. Parenchyma cells of immature (A), premature (B), mature (C), and mother (D) segments stained with iodine solution (400 $\times$ , bar as 100  $\mu$ m).

(Fig. 4C), indented surface (Fig. 4D), peel/spongy flake on surface (Fig. 4E), and a loaf-of-bread-like granule (Fig. 4F). Other features such as cracked surface, scratched surface, curve-like, loop-like granules and the remnants of granules were also found. These features indicate the degradation of starch granules in the mother segments. Santa-cruz et al. (2002) reported the presence of pores along the equatorial region of granules of *Arracacha xanthorrhiza*. However, no such pores were observed on granules of *C. edulis* (Fannon, Hauber, & BeMiller, 1992; Santacruz et al., 2002). Granules of corn, sorghum and millet starches have small pores randomly distributed over their surfaces. Pores are also found along the equatorial groove of large granules of wheat, rye and barley starches, but not on rice, oat, potato or tapioca starches (Fannon et al., 1992). The holes/pores found on the intact granules, a wave-like pattern on granule surfaces, as well as other defects, suggested that the granule degradation that occurred in mother segments was caused by virtue of both direct attack of hydrolytic enzymes on the surface of starch granules and by access of the enzymes to the inner part of the granules via pores, allowing digestion to take place from within.

### 3.2.2. Granule size distribution

Size distribution profile of canna starch granules is shown in Fig. 5. Coefficients of variation of the data were

approximately in a range of 5–20%. A broad range of granule size, from 10 to 140  $\mu$ m, was evident. Starches from immature, premature and mature segments exhibited a similar profile, with a noticeable peak at a granule size of 50–60  $\mu$ m. However, slight shifts in the proportion of starch granules towards the larger size were found as the segments progressed from immature to premature and mature segments. The mean granule sizes of starches from immature, premature and mature segments were 54, 53 and 60  $\mu$ m, respectively. In contrast, starch from the mother segment displayed a unique distribution pattern. There was another peak of small size granule, and the profile was characterized as a bimodal distribution pattern. The two distinct populations of granule were centered at about 20 and 50/60  $\mu$ m, with mean granule size of 46  $\mu$ m. Compared with the starches from other segments, the number of large granules in mother segment (80–140  $\mu$ m) was much lower. The existence of the small size granule in a substantial amount agreed well with what was observed from the cross-section of mother segment.

### 3.2.3. Susceptibility towards $\alpha$ -amylase hydrolysis

Digestibility of raw canna starches by porcine pancreatic  $\alpha$ -amylase was evaluated to better understand the nature of starch granules at different stages of development. As has always been found in digestion of other raw starches,

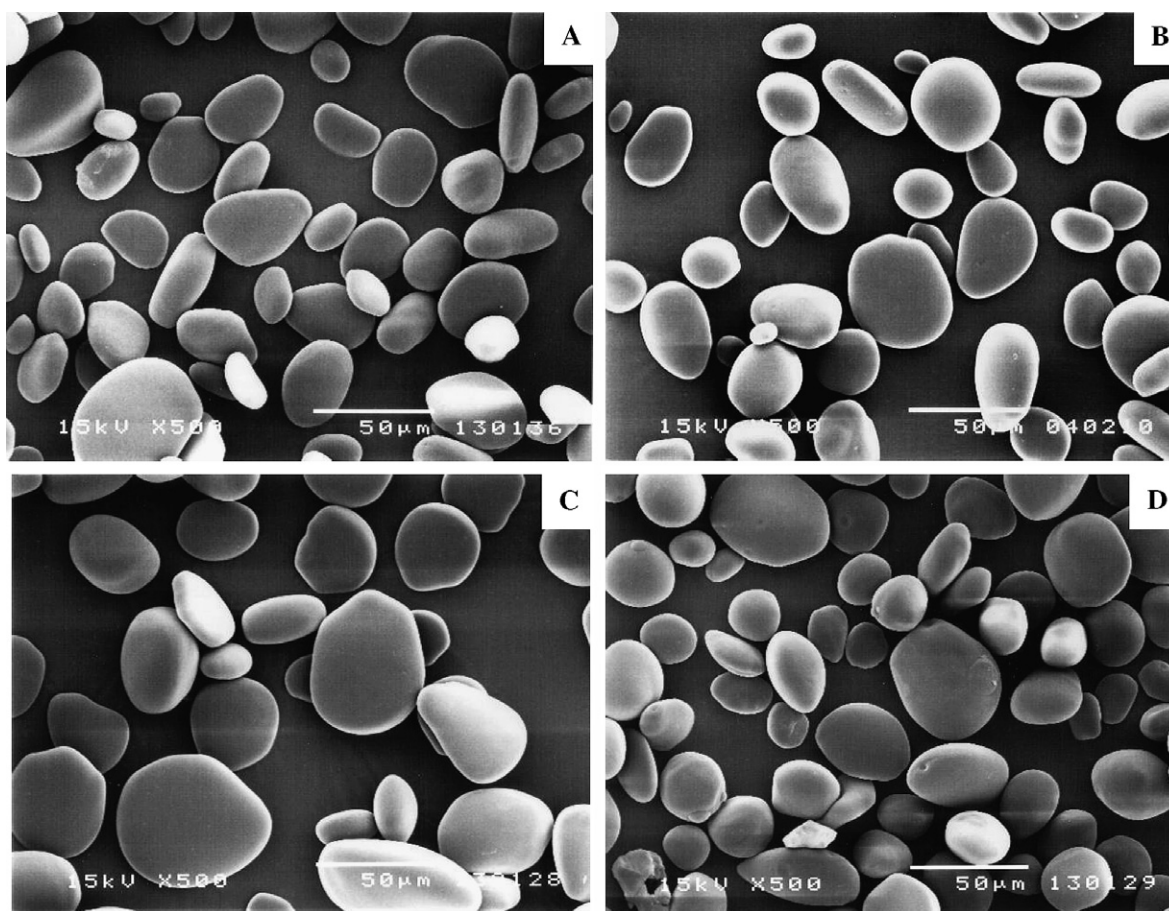


Fig. 3. Scanning electron micrographs of canna starches from immature (A), premature (B), mature (C) and mother (D) segments (500 $\times$ , bar as 50  $\mu$ m).

changes in the hydrolysis rate of canna starch samples were bi-phasic: a relatively rapid rate at the initial stage, followed by a progressively decreasing rate thereafter (Fig. 6). During the first 24 h, the hydrolysis rate of starches from immature and mother segments was notably higher than that of premature and mature segments. After 72 h, however, degree of hydrolysis of starch from immature, premature, mature and mother segments had reached the extent of 57.6%, 69.5%, 37.8%, and 48.3%, respectively. The rate of hydrolysis may be influenced by the surface features, morphology and/or internal structure of starch granules. It has been previously reported that small starch granules were more easily digested than large granules in barley (Kang, Sugimoto, Kato, Sakamoto, & Fuwa, 1985; MacGregor & Ballance, 1980) and potato (Cottrell, Duffus, Paterson, & Mackay, 1995; Kainuma, Yamamoto, Suzuki, Takaya, & Fuwa, 1978; Noda et al., 2005). Damaged starch granules had more susceptibility than non-damaged ones (Frias, Fornal, Ring, & Vidal-Valverde, 1998; Noda et al., 2004). Also, microscopic observation has shown that polyhedral-shaped granules of tropical tuber starches (tannia, sweet potato and cassava) are hydrolyzed to a greater extent than spherical granules by  $\alpha$ -amylases (Valetudie, Colonna, Bouchet, & Gallant, 1993). As evidenced by SEM, a certain amount of small

size granules, and some starch granules from the mother segment, were damaged. For the starch from immature segments, the majority of starch granules were in the small to medium size. Additionally, the granules of premature and mature starches were bigger and had assumed a rounder shape. These factors would contribute to the higher susceptibility of starches from mother and immature segments towards hydrolysis than the starches from premature and mature segments at the initial stage. Differences in hydrolysis rates at the latter stage would be related to the characteristics of the remaining starch granules. It was likely that resistance of the remaining starches was associated with the stage of segmental development. More-developed starch granules in mature and mother segments had higher resistance to  $\alpha$ -amylase hydrolysis.

Appearance of the hydrolyzed granules was also studied with SEM (Fig. 7). Scanning of several hundred granules over several frames revealed that differences in appearance of granules among the samples subjected to the same period of hydrolysis were barely observable, although differences in the degree of hydrolysis were found. However, signs of degradation were more obvious as the period of hydrolysis was extended. After 12 h of hydrolysis, the surface of most of the starch granules was still smooth, but some had small shallow holes and/or stretch marks on part



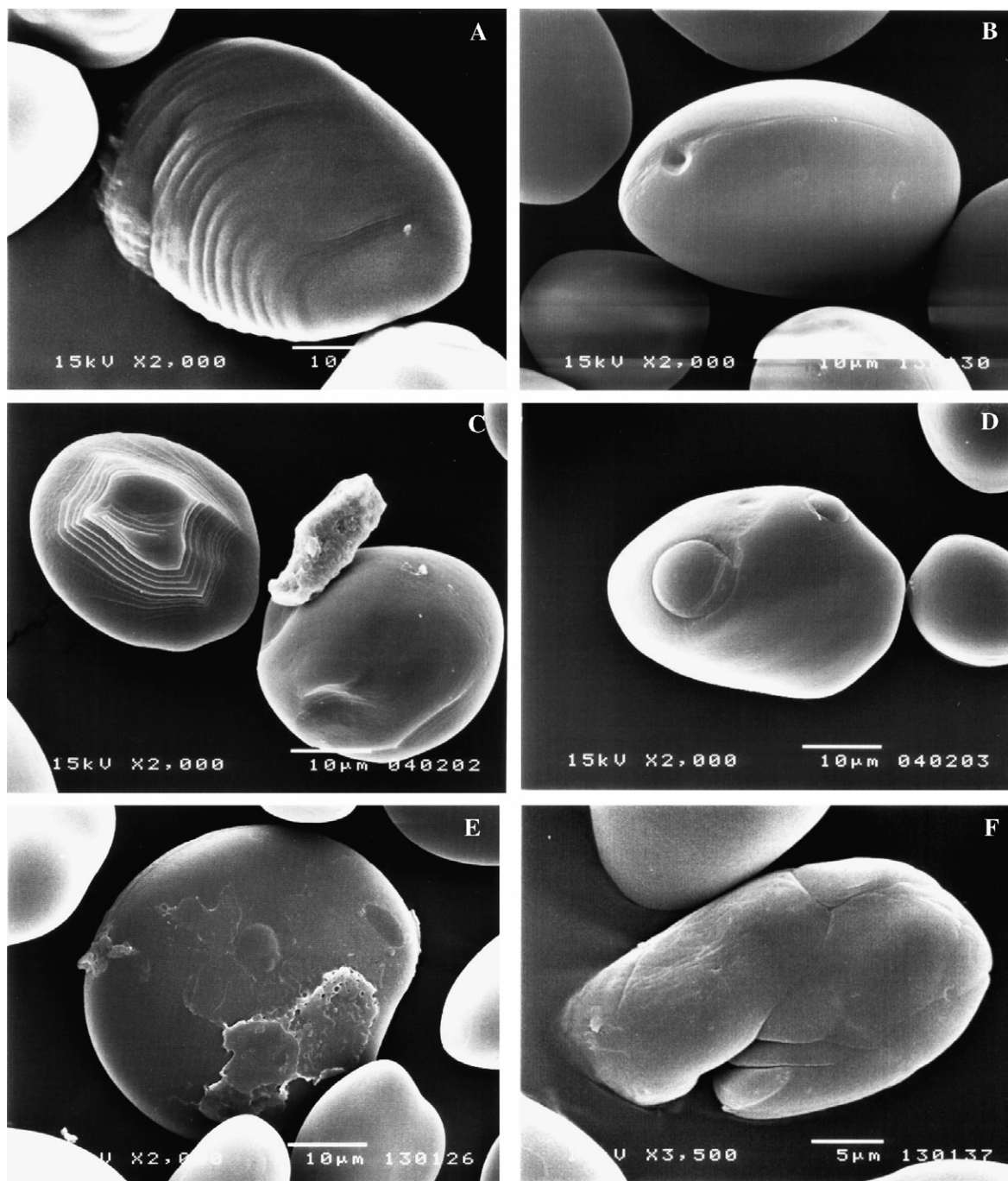


Fig. 4. Scanning electron micrographs of starch granules isolated from mother segment magnified by 2000 $\times$  (A–E, bar as 10  $\mu$ m) and 3500 $\times$  (F, bar as 5  $\mu$ m).

of the granule surface. More damaged granules, greater areas of eroded surface, and large numbers of small particles deposited on the surface were observed with longer periods of hydrolysis. The small particles were believed to be pieces of starch granules which were the products of hydrolysis. A rough surface due to superficial corrosion was clearly visible after hydrolysis for 72 h. Fragments of granules were also observed. Most of the granules were degraded by this mode of action (exocorrosion). Another mode of enzyme attack was observed with the granules

having big holes or circular indentations. In this case, starch granules could be eroded by endocorrosion, revealing an internal layered structure. Mode of attack by  $\alpha$ -amylase on canna starch (principally digested through surface corrosion) was similar to that reported for other B- or C-type crystalline starches such as potato, ginkgo, yam and banana starches (Demirkan, Mikami, Adachi, Higasa, & Utsumi, 2005; Jayakody, Hoover, Liu, & Donner, 2007; Spence & Jane, 1999), but different from many A-type crystalline starches such as maize, barley, sorghum and wheat

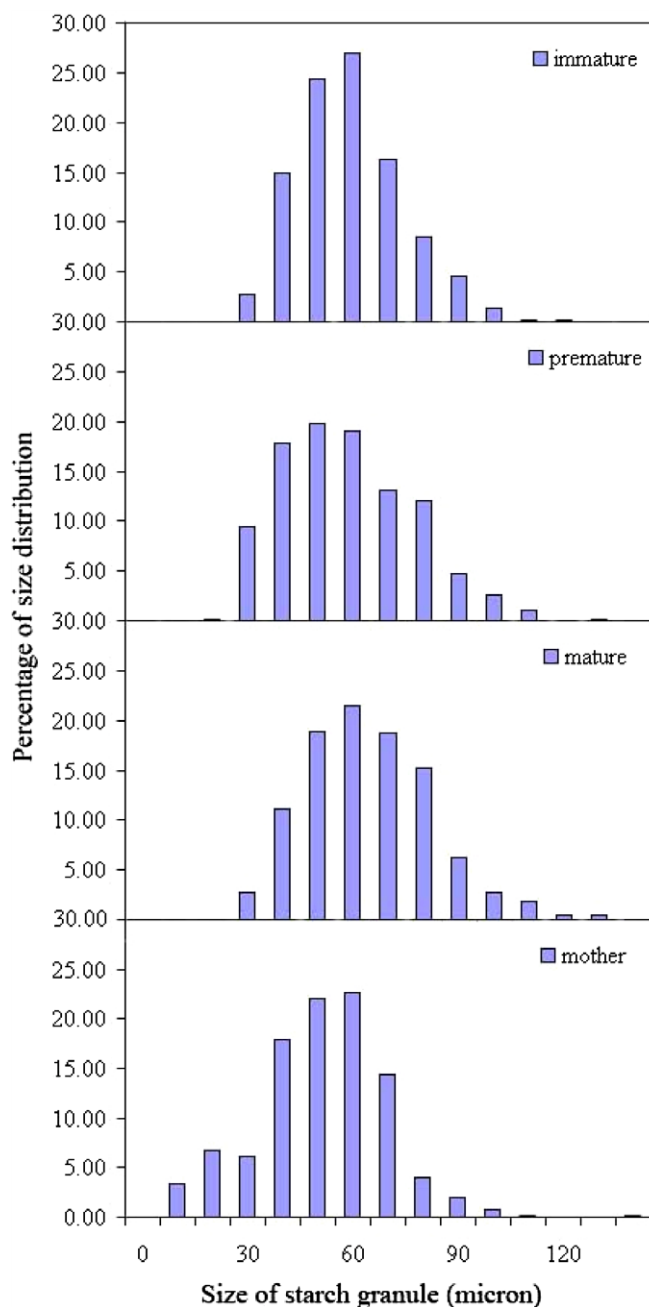


Fig. 5. Granule size distribution profiles of canna starches (number-based) isolated from immature, premature, mature and mother segments.

starches, where erosion of specific areas resulted in circular pits/pinholes and digestion occurring in the radial direction (Demirkan et al., 2005; Kunamneni & Singh, 2005; Stevnebø, Sahlström, & Svihus, 2006; Svihus, Uhlen, & Harsstad, 2005).

Enlarged views of surface morphology of granules hydrolyzed for 72 h are shown in Fig. 8. Cracked and pitted surfaces resulting from exocorrosion are demonstrated in Fig. 8A and B, respectively. Small protrusions of approximately a few hundred nanometers in size were observed at the surface of hydrolyzed granules. According to the blocklet structure described by Gallant, Bouchet,

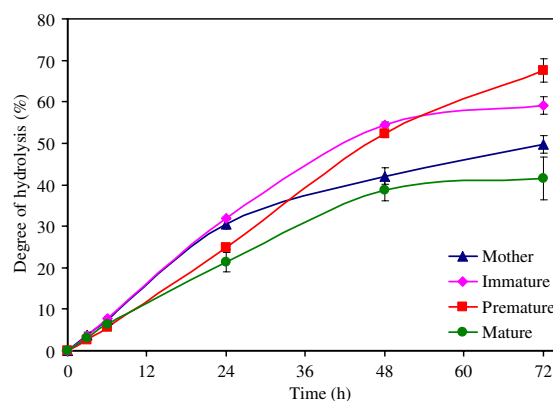


Fig. 6. Enzymatic hydrolysis of canna starches isolated from immature, premature, mature and mother segments.

and Baldwin (1997) and Tang, Mitsunaga, and Kawamura (2006), these protrusions were probably “blocklet units” of canna starch granules. Protrusions having a size of 10–50 nm have been observed at the surface of wheat starch granules, whereas larger, more or less spherical protrusions (200–500 nm) were evident at the surface of potato starch granules (Gallant et al., 1997).

### 3.2.4. Chemical composition

The proximal analyses, amylose content and inorganic components of the starch samples are presented in Table 2. The data revealed that the starches from all segments contained very low amounts of protein (0.06%) and lipid (non-detectable). The low content of lipid and protein is typical for root and tuber starch. Ash content of starches from immature, premature and mature segments was similar (0.18–0.33%), whereas a significantly higher ash content (1.69%) was found in starch from the mother segment. Except for sodium, a similar trend was observed for phosphorus, calcium and iron content. Phosphorus, calcium and iron of starches from immature, premature and mature segments ranged between 251–264, 195–243 and 9–11 ppm, whereas those of starch from mother segment were 312, 421 and 33 ppm, respectively. Sodium content of all starch samples was in a range of 82–95 ppm. Phosphorus in starch is found in three major forms: phosphate monoester, phospholipids and inorganic phosphate (Lin & Czuchajowska, 1998). The phosphorus in canna starch was reported to be phosphate monoester in which a major part (about 70%) was linked to C-6 of amylopectin, and the phosphate groups are located mostly in long B-chain of amylopectin (Takeda & Hizukuri, 1982; Thitipraphunkul et al., 2003b). Therefore, it is possible that the starch from mother segments may have a higher proportion of long B-chain of amylopectin than the starches from other segments. The phosphorus, although existing at a low concentration, plays an important role in starch functional properties (Jane, Kasemsuwan, Chen, & Juliano, 1996). On the other hand, the function of calcium or other cations was still not clearly known, but it is



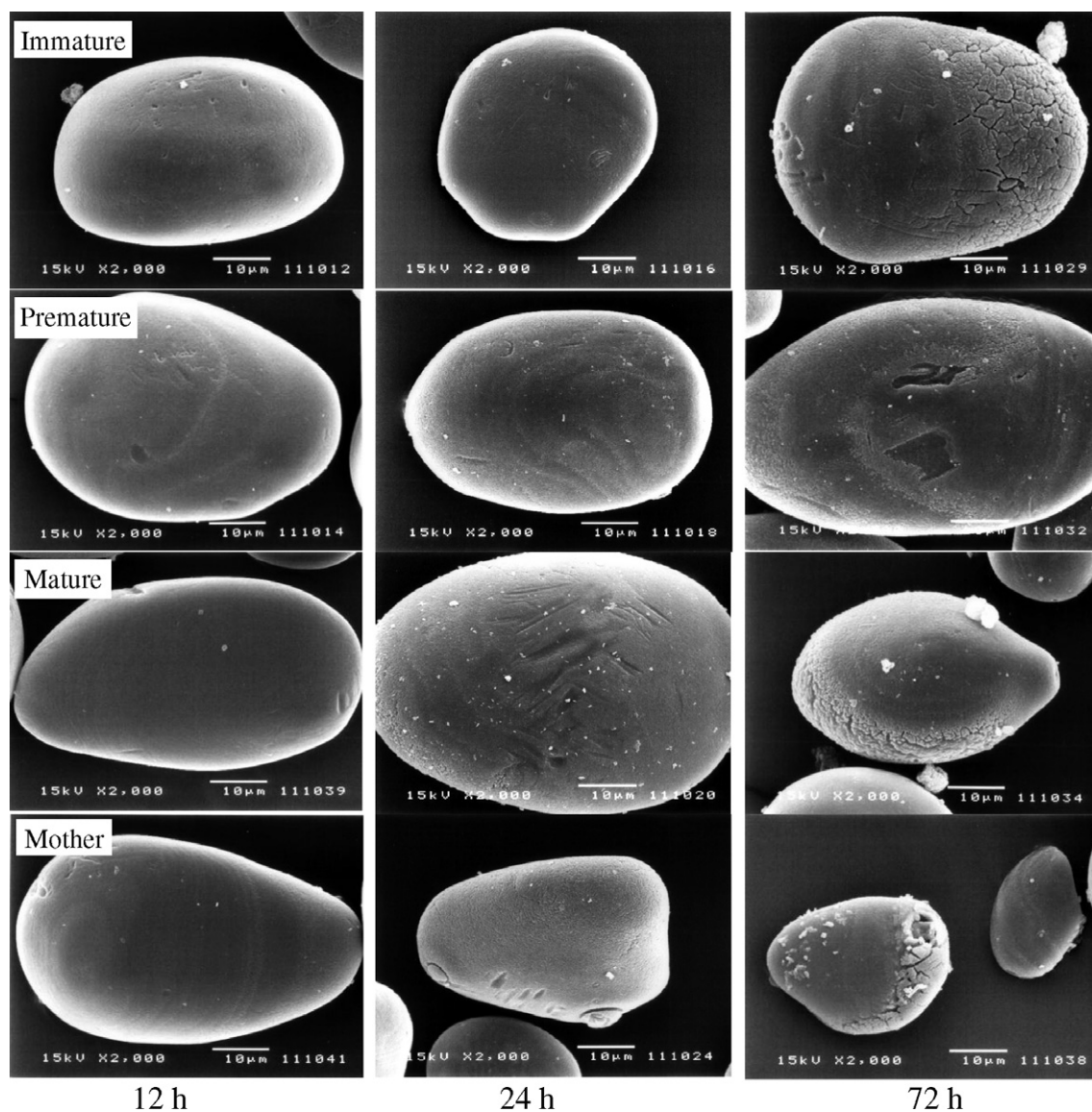


Fig. 7. Scanning electron micrographs of starch granules from immature, premature, mature and mother segments hydrolyzed for 12, 24 and 72 h (bar as 10 µm).

believed that calcium may function as a cross-linking agent in canna starch. Amylose content of the starch samples, except for starch from mature segment, was at approximately 31%. Starch from mature segment had significantly higher amylose content (36%) than others. Broad variation of amylose content (19–37%) in canna starch, depending on the method of measurement and/or source of starch, has been reported by many researchers (Hung & Morita, 2005; Inatsu et al., 1983; Santacruz et al., 2002; Thitiprakhunkul et al., 2003a).

### 3.2.5. Crystalline structure

The data obtained by using X-ray diffractometer revealed that the crystalline pattern of canna starch was not affected by the stage of segmental development. In other words, the pattern of double helical packing in crystalline lamella of starches from immature, premature,

mature and mother segments was not different. Starches extracted from all different stages displayed a B-type crystalline pattern which was characterized by small peak at 5.6°, only one peak at 17° and a doublet at 22° and 24°.

### 3.2.6. Thermal properties

DSC-results of phase transitions associated with gelatinization and retrogradation of the starch samples are shown in Table 3. There was no significant difference in gelatinization temperature ( $T_0$ ,  $T_p$  and  $T_c$ ) as well as the enthalpy of gelatinization among the starches from immature, premature, mature and mother segments. The peak temperatures (often referred to as the gelatinization temperature) and enthalpy of canna starches were 73.6–74.2 °C and 16.6–18.0 J/g, respectively. The peak temperature of canna starch in this study was 3–4 °C higher than that reported by Hung and Morita (2005), Srichuwong,

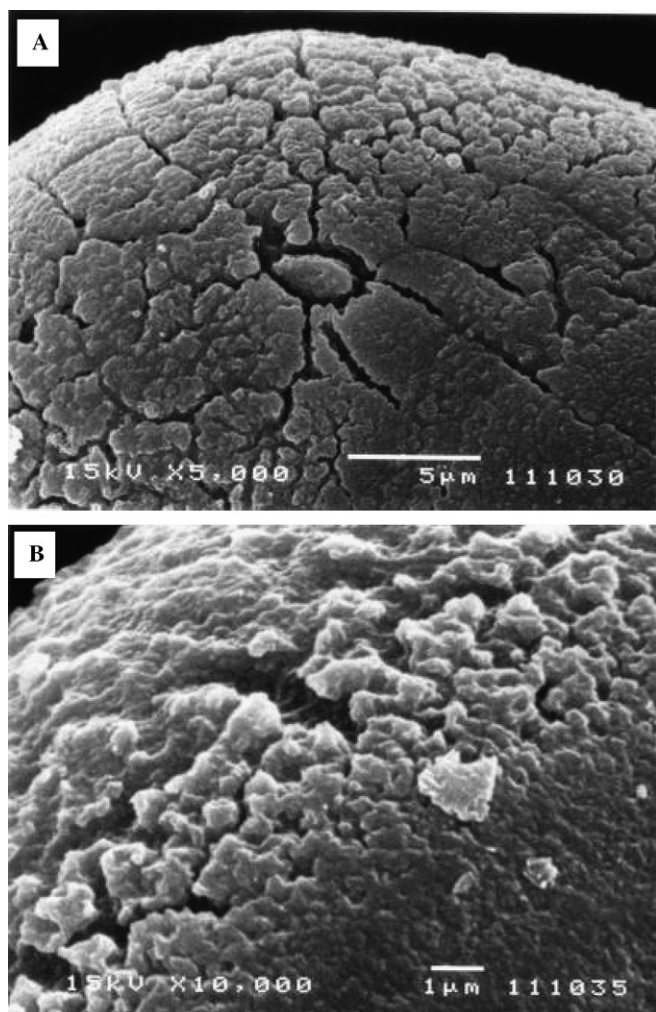


Fig. 8. Scanning electron micrographs of hydrolyzed starch granules magnified by 5000 $\times$  (A, bar as 5  $\mu$ m) or 10000 $\times$  (B, bar as 1  $\mu$ m).

Table 2  
Chemical composition (dwb) of starches isolated from immature, premature, mature and mother segments

Component	Immature segment	Premature segment	Mature segment	Mother segment
Protein (%)	0.06	0.06	0.06	0.06
Lipid (%)	nd <sup>a</sup>	nd	nd	nd
Ash (%)	0.18	0.19	0.33	1.69
P (ppm)	257.9	250.8	264.2	311.9
Ca (ppm)	195.1	242.5	233.1	421.2
Na (ppm)	81.8	95.2	82.3	84.2
Fe (ppm)	9.3	8.6	10.5	33.0
Amylose (%)	31.1	31.0	36.2	30.9

<sup>a</sup> Not detected.

Sunarti, Mishima, Isono, and Hisamatsu (2005a) and Thitipraphunkul et al. (2003a). The variation in gelatinization temperature of starch samples from different regions might be due to differences in cultivar and/or environmental conditions of planting. In addition, phase transition temperature and enthalpy of the retrograded canna starch

Table 3

Thermal properties of canna starches isolated from immature, premature, mature and mother segments

Source of starch	Gelatinization ( $^{\circ}$ C)				Retrogradation ( $^{\circ}$ C)			
	$T_o$	$T_p$	$T_c$	$\Delta H$ (J/g)	$T_o$	$T_p$	$T_c$	$\Delta H$ (J/g)
Immature segment	71.0	73.8	77.4	16.6	40.1	63.9	79.9	8.2
Premature segment	71.5	74.2	77.6	17.7	39.8	63.9	79.6	7.8
Mature segment	70.9	73.6	76.9	18.0	38.5	65.2	78.6	6.5
Mother segment	70.8	73.6	76.8	17.7	37.3	66.0	79.4	6.5

samples from the different segments were similar. Melting temperature and enthalpy of retrograded starches ranged between 63.9–66.0  $^{\circ}$ C and 6.5–8.2 J/g, respectively. The high value of enthalpy indicated that canna starch has a high degree of retrogradation. This result agreed well with previous reports by Hung and Morita (2005), Srichuwong et al. (2005a) and Thitipraphunkul et al. (2003a).

### 3.2.7. Pasting properties

The RVA pasting profiles of starches isolated from immature, premature, mature and mother segments are shown in Fig. 9. All the starch samples displayed a similar pattern: high viscosity, stability during holding at 92.5  $^{\circ}$ C and high setback, although slight differences in the values of pasting parameters were observed. Pasting temperature of all starch samples was at 74–76  $^{\circ}$ C, about 2–4  $^{\circ}$ C higher than that reported by Srichuwong, Sunarti, Mishima, Isono, and Hisamatsu (2005b) and Thitipraphunkul et al. (2003a, 2003b). Narrow ranges of peak viscosity (120–123 RVU), breakdown (17–21 RVU), final viscosity (216–233 RVU) and setback (114–126 RVU) were recognized among the starches from immature, premature and mature segments. Lowering in the paste viscosity of starch from mother segment throughout the measurement period could be observed, though it was not obvious. The peak viscosity, breakdown, final viscosity and setback of the starch from mother segment were 112, 16, 202 and 106 RVU, respectively.

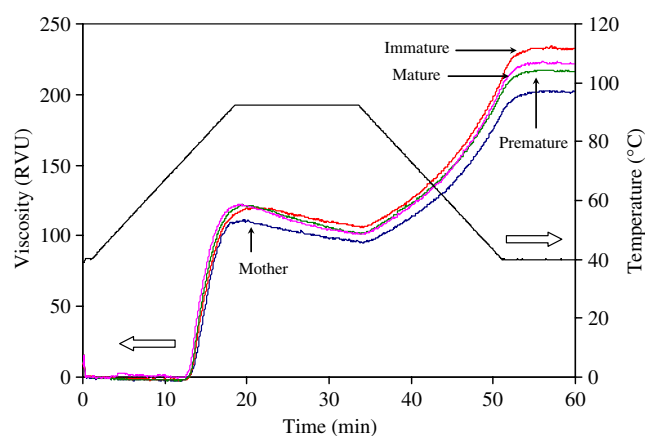


Fig. 9. Pasting profiles of starches (6%, w/w) from immature, premature, mature and mother segments measured by RVA.

#### 4. Conclusion

There were no significant changes in pasting and thermal properties, crystalline structure or chemical composition of canna starches during rhizomal development from immature to premature and mature stages. However, changes in size and morphology of starch granules were observed. Granule size tended to increase with progressive development of segments from immature to mature stages, and the full size granules seemed to have a rounder shape. Starch from mother segments had typical characteristics in both granule size and surface features. Canna starch granules were predominantly superficially hydrolyzed by  $\alpha$ -amylase, even though some endocorrosion may have also occurred.

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