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Carbohydrate Polymers 71 (2008) 310–315

Carbohydrate Polymers

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Physicochemical properties of water caltrop (*Trapa taiwanensis* Nakai) starch during growth period

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Received 14 April 2006; received in revised form 14 April 2007; accepted 1 June 2007
Available online 16 June 2007

Abstract

The physicochemical properties of the starch from Taiwan's water caltrop (*Trapa taiwanensis* Nakai) during growth were investigated. The results showed that the starch content of water caltrop increased from 39.7% to 74.7% on a dry weight basis as growth progressed (14th to 42nd day). The shape of the starch granules was oval and polyangular during growth period. The particle size of starch increased with the increase of from 15.31 to 24.16 μm with physiological aging. The X-ray diffraction patterns could be classified as a typical A-type crystalline structure. The gelatinization onset temperature (T_o), peak temperature (T_p), and gelatinization enthalpy (ΔH) increased with increasing growth time. The results of rapid viscosity analysis of water caltrop starch showed that the pasting temperature, peak viscosity, final viscosity and setback value increased during growth. The increase in starch granular size could be the major factor influencing starch physicochemical properties during maturity.

Keywords: Water caltrop starch; Morphology; X-ray diffraction; Thermal and pasting properties

1. Introduction

Water caltrop (*Trapa taiwanensis* Nakai) is the corm of sedge. It grows in shallow water fields, ponds or swampy lands. Water caltrop is one of the most popular vegetables in Asia countries, due to its taste and medical properties. It is used to treat inflammation, swelling, sore mouth and throats (Wu, 1987). The matured water caltrops are 3–5 cm wide and 5–6 cm long, with two short spines. The fresh, tender, starchy pulps are consumed after boiling water. They are sweet, delicious and thirst-quenching (Anonymous, 1976).

Water caltrop starch is used to thicken food such as soups and sauces. Water caltrop has a reasonably high content of starch which ranges from 65% to 80% of dry matter (Yang, Chang, Sung, & Lii, 1978). Several reports indicated that starch properties vary with cultivars and growth times. Christensen and Madsen (1996) reported that the starch granule size, peak viscosity and phosphorus content increased but amylose remained unchanged during potato growth. The starch content of yam tubers increased but the gelatinization temperature decreased as growth progressed (Huang, Lin, & Wang, 2006). Identification of native starch sources that have desired functionality and unique properties is needed (Duxbury, 1989). Among the most important functional properties of starches are their thermal and pasting properties. The thermal and pasting behaviors of starch are important for the evaluation and estimation of process design, unit operation and the quality of final starch-based products (Perez, Breene, & Bahnassey, 1998a; Perez, Breene, & Bahnassey, 1998b). Pasting behaviors are usually studied by observing changes in temperature of gelatinization, viscosity and retrogradation.

Little information is available on the morphological characteristics, thermal properties, pasting properties and

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doi:10.1016/j.carbpol.2007.06.009

swelling power of water caltrop starch during growth. The objective of this study was to establish the functional characteristics of the isolated starches from water caltrop during growth. Water caltrop starches were isolated and the physicochemical properties of the starches measured in terms of morphology, semi-crystalline structure, thermal and pasting properties.

2. Materials and methods

2.1. Materials

Water caltrops (*T. taiwanensis* Nakai) were grown in a local farm (Tainan, Taiwan). Water caltrops were planted in water field with area of $20 \times 40\ \mathrm{m}^2$ under 45 cm depth of water. The maturity of water caltrop is usually the indicator of quality in the farm markets. According to the experience of farmers, the maturity of water caltrops is approximately 40–45 days. The water caltrop was planted on the 3rd June, 2004 and was harvested at five dates after planting at 14, 21, 28, 35 and 42 days into the growing season. After harvesting, the water caltrops were stored at 4 °C for 2 h where starch was immediately isolated and shipped to a laboratory.

2.2. Starch preparation

Water caltrops starches were extracted and purified using the procedure of Xu and Shoemaker (1986). Water caltrops were washed, unshelled, diced and then blended in a domestic blender in distilled water (pulp:water = 1:3). After blending, the mixtures were passed through a 200 mesh sieve. The residues were centrifuged at 5000 rpm, and the supernatant discarded. The yellow gummy pellet was scraped to remove the top layer which was discarded. The remainder of the pellet was collected and washed several times with distilled water. The mixtures were centrifuged again. All precipitates were collected and dried in a conventional oven at 40 °C overnight. The starch was ground gently with a mortar and pestle to a size suitable to passing through a 100 mesh sieve. The powdered starch was collected and stored in a sealed glass jar in a freezer.

2.3. Starch content

The starch content of the water caltrop was determined by an AACC methods (1995), using the Megazyme total starch content assay kit (Meagzyme International Ireland Ltd., Wicklow, Ireland). The starch content of water caltrop was calculated and expressed on a dry weight basis.

2.4. Morphological properties

Scanning electron micrographs (SEM) were obtained using scanning electron microscope (Model ABT-150S, Topon Corp., Japan). Isolated starch was mounted on an aluminum stub use double-sided adhesive tape. The sample was coated with gold–palladium (Model JBS-ES 150, Ion sputter coater, Topon Corp., Japan). An accelerating potential of 15 kV was used during electron micrography in this study.

2.5. Particle size

The particle size of starches was measured by the method of Tecante and Doublier (1999) using a Laser Particle Sizer (Analysette 22 compact Laser Particle Sizor, Fritsch, Germany). The isolated starches were dispersed in distilled water at 4%. The instrument output had a volume distribution as the fundamental measurement, with medians of $D[V,0.1]$, $D[V,0.5]$, and $D[V,0.9]$ diameters. The output data $D[V,0.1]$, $D[V,0.5]$, $D[V,0.9]$ means that the diameter of the granules for which 10%, 50%, and 90% of the starch volume was made up of granules that were smaller.

2.6. X-ray diffraction

X-ray diffraction of the starches were measured using a Siemens X-ray diffractometer (Model D5000, Siemens Co., Madison, USA) with the operating conditions set as follows: target voltage 40 kV, current 30 mA, scanning range (2θ) 4–30°, scan speed 0.02°/s, receiving slit width 0.2 nm.

2.7. Swelling power and solubility

Swelling power and solubility of starches from water caltrop were determined by the method of Waliszewski, Aparicio, Bello, and Monroy (2003). Starch suspensions (1% w/w) were prepared in flasks and were heated to 60, 70, 80 and 90 °C, respectively, for 30 min. All flasks were shaken every 5 min and left to cool to room temperature, then centrifuged at 4000*g* for 15 min. The precipitates were weighed and calculated for swelling power. The supernatant was decanted into beaker and dried in an oven for 2 h at 105 °C. The dried solid was weighed and solubility calculated.

2.8. Thermal properties

The thermal properties of the isolated starches were measured using a differential scanning calorimeter (Model DSC822, Mettler–Toledo Co., Switzerland). Starch (3 mg) and distilled water (9 mg) were loaded into an aluminum pan and hermetically sealed. The pan was allowed to stand for 2 h at room temperature in order to attain an even distribution of water before heating the calorimeter. An empty aluminum pan was used as reference. The scanning temperature range was 30–100 °C at a heating rate of 5 °C/min. The onset (T_o), peak (T_p) and conclusion (T_c) temperatures and enthalpy (ΔH) of gelatinization of the dry starches were calculated automatically.

2.9. Pasting properties (Ross, Walker, Booth, Orth, & Wrigley, 1987)

The pasting properties of isolated starches were investigated with a Rapid Visco-Analyzer (Model RVA-3D, Newport Scientific, Ltd., Sydney, Australia). An 8% starch–water suspension (28 g total weight) was prepared and subjected to a heating–cooling cycle. The starch suspensions were equilibrated at 50 °C for 1 min, heated to 95 °C at 6 °C/min, held at 95 °C for 5 min, cooled to 50 °C at 6 °C/min, and held at 50 °C for 2 min. The paddle speed was set at 960 rpm for the first 10 s, then 160 rpm for the remainder of this experiment. The viscoamylograph described the various characteristics of the starch including peak temperature, peak viscosity (P), viscosity at 95 °C after 5 min holding (H), and final viscosity at end of 50 °C holding period (F) which were obtained from a typical diagram of Rapid Visco-Analyzer pasting curves (Zhou, Robards, Glennie-Holmes, & Helliwell, 1998). The unit of pasting properties of water caltrops starch is expressed as RVU, where RVU is the unit of viscosity from the Rapid Visco-Analyzer. Three replicates of each sample were carried out.

2.10. Statistical analysis

The mean values and standard deviations of each analysis are reported. All data were analyzed by using single factor of ANOVA. When F-value was significant ($p < 0.05$) in ANOVA, then Duncan's new multiple range test were calculated to compare treatment means.

3. Results and discussion

3.1. Starch content

Dry matter and starch content of plant origin acts as important indicators for quality evaluation of starchy food products (Lisinska & Leszczynski, 1989; Treche & Agbor-Egbe, 1996). During the initial growth period of seven days, the internal part of water caltrop is mostly liquid, containing very little dry matter and starch. Although dry matter and starch content increased remarkably over the earlier stages of the growth (8–14 days), there were significant differences between the plants of the water caltrop. In order to obtain enough starch content from each pulp of water caltrop for analysis, the growth period after the 14th day was considered acceptable for quality analysis. The results showed the dry matter and starch content of water caltrop significantly increased during the growth period (14th to 42nd day) ($p < 0.05$) (Table 1). Over the 42 days growth period, dry matter and starch content of water caltrop increased slightly at the earlier stages (14–28 days) and after that increased significantly at the stages of maturity (28–42 days). The highest dry matter and starch content, up to 74.7%, of water caltrop were observed at the final harvest time (42nd day), this time is judged to be the harvesting time based on starch content.

Table 1
Dry matter and starch content of water caltrop during growth

Growth time (day)	Dry matter (%, w/w)	Starch (%, dry matter)[e]	Starch (%, fresh pulp)[e]
14	11.3[d]	39.7[d]	6.6[d]
21	13.2[c]	56.5[c]	9.4[c]
28	14.2[b]	65.8[b]	11.2[b]
35	15.5[b]	68.9[b]	12.3[b]
42	20.7[a]	74.7[a]	14.1[a]

[a–d] Means with different letters within the same column differed significantly ($p < 0.05$) ($n = 3$).

[e] Starch content of water caltrops were expressed on the basis of dry matter and fresh pulp, respectively.

3.2. Morphological characteristics and particle size

The results of SEM indicated the shape of the water caltrop starch granules appeared to be round, oval and polyangular during growth period (Fig. 1). The granule size of water caltrop starches significantly increased as growth progressed (14–42 days). On the 42nd day, the granule size is the largest, ranging in 15–25 μm. At final harvest time, the size of starch granule appeared to be larger than the earlier stages of growth. The granule size measurement of the water caltrop starches were carried out using a laser diffraction analyzer. The results of particle size of water caltrop starch from the laser diffraction (Table 2) agreed with the results from the scanning electron micrographs. The size of water caltrop starches, expressed as the average diameter $D(4.3)$, increased with increasing of growth time from 15.31 μm (14th day) to 24.16 μm (42nd day). Several pieces of research have shown a similar dependence of size present in sweet potato starch (Noda, Takahata, Sato, Hisamatsu, & Yamada, 1995), potato starch (Liu, Weber, Currie, & Yada, 2003) and yam starch (Huang et al., 2006) during growth.

3.3. X-ray pattern

Starch granules present different crystalline structures naturally. An X-ray diffractometry has been widely used to reveal the characteristics of the crystalline structure of starch granules (Zobel, Young, & Rocca, 1988). The X-ray diffraction patterns of water caltrop starches at different physiological ages all show similar characteristics with four main reflection intensities (Fig. 2). The four distinctive peaks appeared at 15.2°, 17.1°, 18.2° and 23.5° (2θ) supporting a typical A-type X-ray pattern. Comparing the five dates of the growth period, the similar X-ray diffraction patterns indicated that the semi-crystalline structure of water caltrop starch was not affected by the growth time. Previous studies indicated that the semi-crystalline structure of most root and tuber starches were not effected by different growth periods (Huang et al., 2006; Liu et al., 2003; Noda et al., 1995) and this agrees with our study.

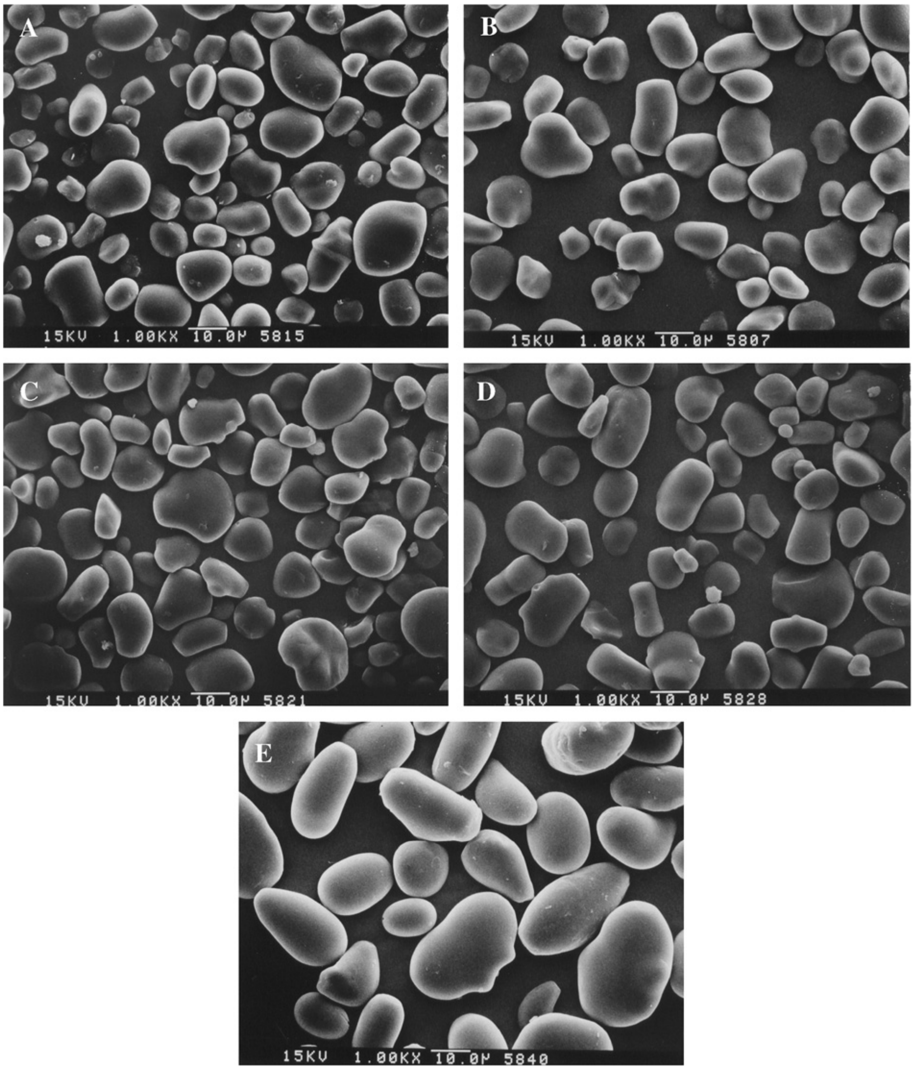

Fig. 1. Scanning electron microscopy of water caltrop starch during growth (A, B, C, D and E are representative of 14, 21, 28, 35 and 42 days, respectively), (bar in each picture is 10 μm).

Table 2
Granular size value of water caltrop starch during growth

Growth time (days)	Granular size of water caltrop starch (μm)				
	$D(4,3)$[e]	$D(3,2)$[f]	$D(V,0.1)$[g]	$D(V,0.5)$[h]	$D(V,0.9)$[i]
14	15.31[c]	7.97[c]	5.29[d]	14.48[d]	25.96[d]
21	17.42[b]	9.32[b]	7.45[c]	16.07[c]	28.34[c]
28	17.78[b]	9.46[b]	7.71[c]	16.52[c]	29.62[b]
35	18.12[b]	10.00[b]	8.58[b]	17.24[b]	30.90[b]
42	24.16[a]	13.76[a]	13.71[a]	23.09[a]	38.31[a]

[a–d] Means with different letters within the same column differed significantly ($p < 0.05$) ($n = 3$).

[e] $D(4,3)$ granule diameter derived from the volume distribution.

[f] $D(3,2)$ ratio of total volume of particles to the total surface area.

[g] $D(V,0.1)$ median of 10% granule diameter.

[h] $D(V,0.5)$ median of 50% granule diameter.

[i] $D(V,0.9)$ median of 90% granule diameter.

3.4. Swelling power and solubility

Swelling power of starch indicated the degree of water absorption of starch granules, while solubility reflected the degree of solubility during the starch swelling procedure (Carcea & Acquistucci, 1997). The swelling power of water caltrop starches at different physiological stages are summarized in Table 3. Several studies indicated that the swelling power of starch was strongly affected by heating temperature and this agrees with our study. The highest swelling power of water caltrop starch from five different development stages were obtained when the starch suspension was heated to 90 °C (Table 3). The swelling power of water caltrop starch increased with the growth period from the 14th to 42nd day at all heating temperature (60–90 °C). However, there was no significant differences in swelling power between the growth period of 14–21 and 35–42 days, respectively. Wang, Wu, Lai, and Huang (2001) indicated that the swelling power of taro starch increased as growth progressed. The solubility of water caltrop starches during growth period is shown in Table 4. As the heating temperature increased, the solubility of water caltrop starch increased which agreed with the other studies on starches from different sources (Wang et al., 2001). As with the

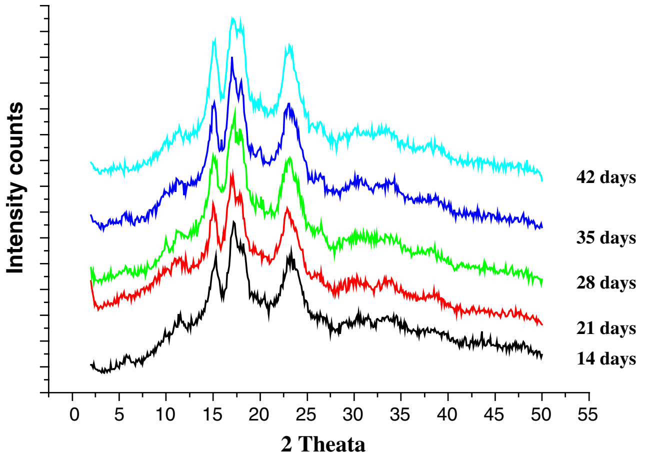

Fig. 2. A typical X-ray diffraction pattern of water caltrop starch during growth.

Table 3
Swelling power of water caltrop starch during growth

Growth time (days)	Swelling power (g/g starch)			
	60 °C	70 °C	80 °C	90 °C
14	3.57^{c}	3.68^{b}	12.77^{d}	23.74^{b}
21	3.47^{c}	3.67^{b}	14.28^{c}	23.18^{b}
28	3.98^{b}	3.98^{b}	22.20^{b}	27.88^{a}
35	4.72^{a}	4.25^{a}	23.35^{a}	25.46^{a}
42	4.88^{a}	4.55^{a}	24.09^{a}	27.85^{a}

$^{a–d}$ Means with different letters within the same column differed significantly ($p < 0.05$) ($n = 3$).

Table 4
Solubility of water caltrop starch during growth

Growth time (days)	Solubility (%)			
	60 °C	70 °C	80 °C	90 °C
14	0.34^{c}	0.68^{b}	11.24^{d}	24.54^{b}
21	0.38^{c}	1.12^{a}	13.56^{c}	24.05^{b}
28	0.59^{b}	1.23^{a}	19.17^{b}	31.14^{a}
35	0.66^{a,b}	1.35^{a}	19.42^{b}	30.82^{a}
42	0.76^{a}	1.43^{a}	21.05^{a}	31.93^{a}

$^{a–d}$ Means with different letters within the same column differed significantly ($p < 0.05$) ($n = 3$).

swelling power, the solubility of water caltrop starch significantly increased as the growth progressed ($p < 0.05$).

3.5. Thermal properties

Thermal properties of water caltrop starches at different growth time are summarized in Table 5. All starches showed a single symmetrical endotherm during the gelatinization process (Fig. 3). All the parameters of gelatinization temperature (T_o, T_p, T_c) and enthalpy (ΔH) increased with increasing the growth time ($p < 0.05$) (Table 5). Of the five stages of development, the gelatinization enthalpy (ΔH) of water caltrop starch showed an increasing tendency where the 42 days of growth times exhibited the highest ΔH. The highest T_o and ΔH of gelatinization and the narrowest gelatinization range was seen at a harvest time of 42 days. This indicates that the degree of in the starch structure was highest at the final harvest stages compared with the earlier stages. Barichello, Yada, Coffin, and Stanley (1990) observed that a more thermo-stable granular structure and a more highly ordered crystalline structure due to the higher proportion of longer chains in the amylopectin, resulting in more resistance to gelatinization. Consequently, the more resistance granules required a higher energy to disorganize their structure at the harvest stages of water caltrop starches. Previous studies from Wang et al. (2001) showed an increase in the thermal properties of taro starch during the growth period that agrees with this study.

Table 5
Gelatinization properties of water caltrop starch during growth

Growth time (days)	Parameters of endothermic properties				
	T_o (°C)^{e}	T_p (°C)^{e}	T_c (°C)^{e}	Gelatinization range (°C)^{e}	ΔH (J/g)^{e}
14	69.6^{d}	76.1^{b}	82.7^{c}	13.1^{a}	13.0^{d}
21	70.6^{c}	76.3^{b}	83.9^{b}	13.3^{a}	14.0^{b,c}
28	72.3^{b}	76.1^{b}	83.4^{b}	11.1^{b}	15.5^{b}
35	73.8^{b}	76.4^{b}	83.1^{b}	9.3^{c}	15.6^{b}
42	78.4^{a}	80.9^{a}	84.7^{a}	6.3^{d}	17.4^{a}

$^{a–d}$ Means with different letters within the same column differed significantly ($p < 0.05$) ($n = 3$).

^{e} T_o = onset temperature; T_p = peak temperature; T_c = closed temperature; ΔH = enthalpy (J/g starch); Gelatinization range (°C) = ($T_c - T_o$).

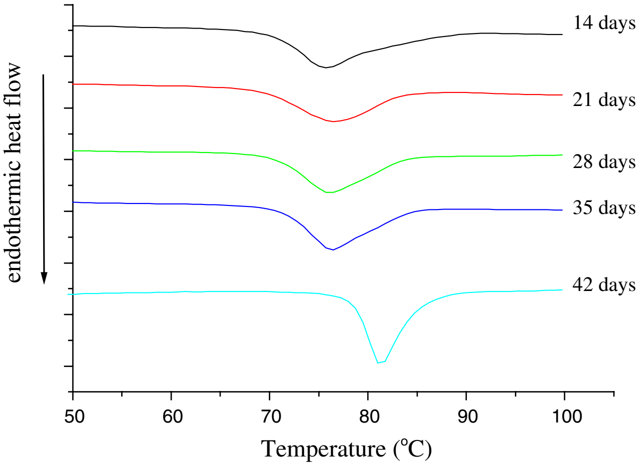

Fig. 3. The DSC thermal curve of water caltrop starch during growth.

3.6. Pasting properties

Starches from different plant sources exhibited their pasting behaviors which are important for the evaluation and estimation of process design, unit operation and quality of final starch products (Perez et al., 1998a, 1998b). Pasting behavior of starch is usually studied by observing the changes in the viscosity of a starch system based on rheological principles. The pasting properties of water caltrop starches were greatly affected by growth times (Table 6). As the growth times progressed, there was an increasing trend

Table 6
Pasting properties of water caltrop starch during growth

Growth time (days)	Parameters of pasting behaviors[e]					
	Pasting temperature (°C)	Peak viscosity (*P*)	Holding viscosity (*H*)	Final viscosity (*F*)	Breakdown ($P - H$)	Setback ($F - H$)
14	80.3[c]	313.7[d]	266.3[c]	493.7[b]	47.3[c]	227.3[b]
21	81.5[b,c]	321.3[c]	259.7[d]	432.7[d]	61.3[b,c]	183.0[d]
28	82.3[b]	336.2[b]	280.3[a]	495.3[b]	61.0[b,c]	215.0[c]
35	82.8[b]	336.9[b]	274.3[b]	481.0[c]	63.3[b]	207.7[c]
42	85.0[a]	361.0[a]	200.7[e]	543.7[a]	90.3[a]	343.0[a]

[a–d] Means with different letters within the same column differ significantly ($p < 0.05$).

[e] The unit of pasting properties of water caltrop starch is expressed as RVU, where RVU is the unit of viscosity from the Rapid Visco-Analyzer.

in pasting temperature for water caltrop starch. The results of pasting properties also indicated the peak viscosity and breakdown of water caltrop starch significantly increased with increasing growth time ($p < 0.05$), where the highest peak viscosity and breakdown was seen at the latest harvest time (42 days). Liu et al. (2003) indicated that potato starch with a shorter growth time resulted has smaller starch granules, which contributed to a low peak viscosity.

4. Conclusion

Dry matter and starch content increases with increasing growth time. The particle size of the water caltrop starches increased as growth progressed which agrees with swelling power results. The X-ray diffraction pattern of water caltrop starches showed a typical A-type crystalline structure and was not affected by growth time. The RVA results showed the pasting temperature, peak viscosity, breakdown increased with maturity of the water caltrops. The results suggested that water caltrop starches paste appeared to show a high setback after cooling, which resulted in a high tendency for retrogradation. This study provides useful information in term of changes in the granule size and thermograms as well as pasting behavior of water caltrop starch that will affect the choice of harvest time and help in the evaluation of the potential uses of water caltrop starch in value added products.

Acknowledgement

The authors express sincere thanks to National Science Council of Taiwan, ROC for the financial support (NSC 94-2214-E-005-006).

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