Physicochemical properties of starches from seed and rhizome of *Enhalus acoroides*

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ABSTRACT

Starch, especially from terrestrial plants, is widely used in food and non-food industries. Marine macrophytes such as *Enhalus acoroides* also provide a considerable amount of starch in their storage parts such as rhizomes and seeds, but these have yet to be explored to fully understand their functionality. This study focused on the morphology, chemical composition and functional properties of starch from *Enhalus acoroides* collected from Tanjung Adang Laut shoal, Johor, Malaysia.. Starch granules from the seeds were oval, 4.6-28.0 μm in length along the major axis, and possessed a centric Maltese cross, whereas starch granules from the rhizome were rod-shaped, 28.9-53.8 μm long, and with both centric and eccentric Maltese crosses. Seeds yielded 48.9% total starch, much higher than 14.4% in rhizomes. In contrast, resistant starch and amylose accumulated in the rhizomes were 75.3% and 18.8% compared to 1.7% and 10.8% in the seeds, respectively. As for proximate composition, the seeds contained 7.5% protein and 1.4% lipid, both higher than the 3.3% protein and 0.3% lipid in the rhizomes. The phosphorus content of rhizome starch was higher (429.6 mg/kg) than in the seed starch (117.9 mg/kg). X-ray diffractograms showed that the seed starch possessed A-type crystallinity, while rhizome starch was categorized under C-type crystallinity. For starch gelatinization, the seed starch required higher energy (7.7 Jg^{-1}) than the rhizome starch (4.8 Jg^{-1}) while the viscosity value of both seed and rhizome starches were similar. The findings obtained may serve as baseline data and as a guideline on the usage of *E. acoroides* starches in food and non-food formulations.

KEYWORDS

Enhalus acoroides, starch, amylose, crystallinity, resistant starch

INTRODUCTION

Starch is the most common carbohydrate in human diets and is present in many staple foods. They occur mainly in storage organs of plants such as seeds, fruits, rhizomes and tubers. Aside from being consumed as the principal energy source for the body, starch is also widely utilized in food and non-food industries. As a storage polysaccharide, it is available in storage organs not only of terrestrial plants but also of marine plants, e.g., rhizomes (Drew 1983; Ralph et al. 1992) and seeds (Phang et al. 2002) of seagrasses.

Enhalus acoroides is a large seagrass which can be found throughout Southeast Asian coastal waters (Short et al. 2001) and is the dominant species in seagrass meadows of Sungai Pulai estuary, Johor, Malaysia. The above ground plant parts consist of long strap-like leaves and flowers whereas the ground parts comprised cord-like roots and thick rhizomes with black bristles (Fig. 1a). Rhizomes of *E. acoroides* have been utilized by local people, especially for medicinal purposes. The long stalk of female flowers bears edible fruit and in the coastal areas of Southeast Asia the seeds have been traditionally consumed raw or boiled due to their starchy and sweet taste (Ridley 1924; Burkill 1935; Montano et al. 1999, Japar Sidik et al. 2006). Montano et al. (1999) also suggested that *E. acoroides* seeds can be one of the staple foods, mainly in coastal areas, as this plant is very productive and a single fruit contains 8-11 seeds weighing approximately 6.4 g (Fig. 1b). The high fertility of *E. acoroides* in Tanjung Adang Laut, which produce fruits throughout the year, is advantageous for coastal communities in providing them with food sources. Furthermore, proximate composition of flour and starch derived from *E. acoroides* seeds are comparable to wheat, cassava and arrowroot. The study on seagrass starches, especially those on the functionality of their seeds (such as those of *Enhalus*

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acoroides and *Thalassia hemprichii*), are very limited, although their morphology and nutrient composition have been studied in some details (Montano et al. 1999; Phang et al. 2002; Japar Sidik et al. 2003). Therefore the present study aimed to evaluate the physicochemical properties of starches from seeds and rhizomes as sources for food formulation (e.g., biscuits and porridge) and non-food applications (e.g., paper and board manufacturing, pharmaceuticals and cosmetics).

MATERIALS AND METHODS

Isolation of native starch

Rhizomes and mature fruits of *E. acoroides* were collected randomly within the shoal of Tanjung Adang Laut, Johor (1°19'49.91"N 103°33'58.81"E) at low tide during its fruiting season (January until May 2016). A total of 163 mature fruits were collected in January (12 fruits), February (15 fruits), March (40 fruits), April (26 fruits), and May (70 fruits). The fruits were cut open to obtain the seeds and the white cottony coat covering the seeds was removed. As for the rhizome, six mature plants were harvested monthly (January until May 2016) from the same area where the fruits were collected. Approximately 1 kg of 1,533 seeds and 2 kg of 30 rhizomes were obtained, cleaned, labeled and brought to the laboratory for further isolation process. These samples of seeds and rhizomes from different months were pooled into a single composite sample of "seeds" and "rhizomes", hence no comparison was made among months to examine possible seasonal variation in starch content and composition. Native starches (or non-modified starch) of seeds and rhizomes were isolated following the method of Kurzawska et al. (2014). The outer skin of the rhizome was peeled and then chopped into 1 cm fragments. The plant materials (seeds and rhizomes) were added to water (in a ratio of 1:10) in a blender (Panasonic, Osaka, Japan) and blended for 10 minutes until a smooth paste was formed. The slurry was sieved through 100-mesh linen cloth into the beaker. The resultant slurry was transferred into a 50 ml centrifuge tube and then centrifuged at 8000 rpm at 20°C for 20 minutes. The supernatant was discarded and the pellet formed was then oven-dried at 40°C for 24 hours. The dried starch was ground using mortar and pestle, sieved (250 μm), labeled and stored in a tightly closed container and kept in a desiccator.

Morphological properties

Approximately 0.2 mg starch powder was stained with 0.25% Lugol's solution and observed under a compound light microscope (DM 750, Leica Microsystem, Wetzlar, Germany) equipped with polarized filter and analyzer. Images of starch granules with birefringence were captured. Starch granules were also examined in detail with a scanning electron microscope Jeol JSM-6400 (Jeol Ltd., Tokyo, Japan) with an energy dispersive X-ray analyzer (EDS) PGT Spirit, at an acceleration of 20 keV. Samples of starch were mounted on aluminum specimen stubs with double-sided adhesive tape and sputtered with a 20-30 nm gold layer using a sputter coater before observation.

Chemical compositions

Macro components (total starch and amylose) and resistant starch were determined using the Megazyme assay kit (Megazyme International Ireland Ltd., Bray, Ireland). Microcomponents, i.e., protein, lipid and phosphorus were determined for content by following the official method of AOAC International (2005).

Functional properties

The degree of crystallinity of starches was quantitatively estimated and analyzed through 2θ of 5°-45° using X-ray diffractograms (Xpert Pro MPD, Philips, Netherlands). Thermal properties of starches were obtained using differential scanning calorimeter, DSC (Model-823e, Mettler-Toledo, Switzerland) from 25°C to 120°C at the rate of 10°C/min. Rheological properties of starches suspended in distilled water were determined using Rotational rheometer (C-DG26.7/QC, RheolabQC, Anton Par Ltd, Germany), following the method of Chrungoo and Devi (2015).

Statistical analysis

Mean, standard deviation and range were computed for triplicated determination. Data for morphological, physicochemical and thermal properties were statistically analyzed using SPSS 16.0 Statistical Software Program. Means were compared using independent sample T-Test and Levene's Test was performed for equality of variances.

RESULTS

Seed starch granules are small with a mean size of 14.7±6.9 μm (mean±SD, *n*=30, range 4.6-28.0 μm) and oval with distinct centric Maltese cross. Starch granules from rhizomes are large with a mean size of 40.6±6.9 μm (mean ± SD, *n*=30, range 28.9-53.8 μm) (Fig. 1 c-f) and rod-shaped with Maltese cross displayed either centric or eccentric. The total starch content of the seeds and the rhizomes was 48.9%

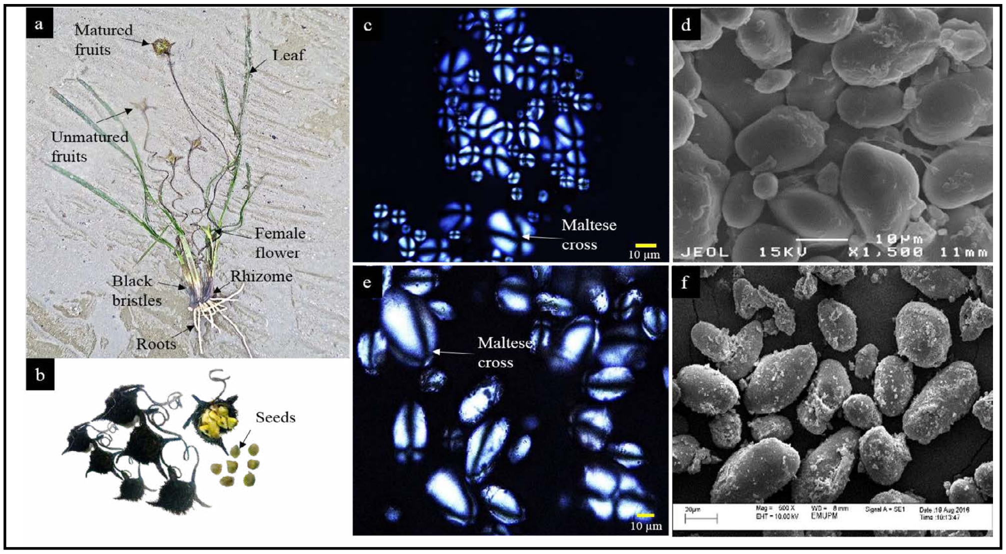

Figure 1. Morphology and starch characteristics of Enhalus acoroides, (a) plant parts, (b) fruits and seeds, (c) starch of seeds under a polarized microscope and (d) under SEM, (e) starch of rhizome under a polarized microscope and (f) under SEM.

and 14.4%, respectively, while the amylose content of the seeds and the rhizomes was 10.8% and 18.8%, respectively (Table 1). Resistant starch in the rhizomes was 75.3% while it was 1.65% in the seeds. The contents of protein, lipid, and phosphorous in the seeds were 7.5%, 1.4% and 117.9 mg/kg, respectively, while those in the rhizomes were 3.3%, 0.2% and 429.6 mg/kg, respectively. Ash values in the rhizomes and the seeds were 5.0% and 5.6%, respectively (Table 1).

Functional properties of starches were investigated when the starch underwent gelatinization and retrogradation during the heating process. Starch granules possess two important structures, amylose (amorphous) and amylopectin (semi-crystalline) molecules, arranged in alternate layers. *E. acoroides* seed starch had A-type crystallinity with sharp peaks at 15.8° 2θ and 23° 2θ, and unresolved doublets at 17° 2θ and 18° 2θ, while rhizome starch has a C-type crystallinity with strong peaks at 14.8° 2θ, 24.1° 2θ and a small peak at 20.6° 2θ (Fig. 2). Rhizomes comparatively had a higher relative crystallinity of 21% compared to 18% in seed starch. Rhizome starch also displayed a higher gelatinization peak at 96.5°C compared to 87.2°C for seed starch. In contrast rhizome starch has lower gelatinization enthalpy of 4.8 Jg^{-1} compared to 7.7 Jg^{-1} for seed

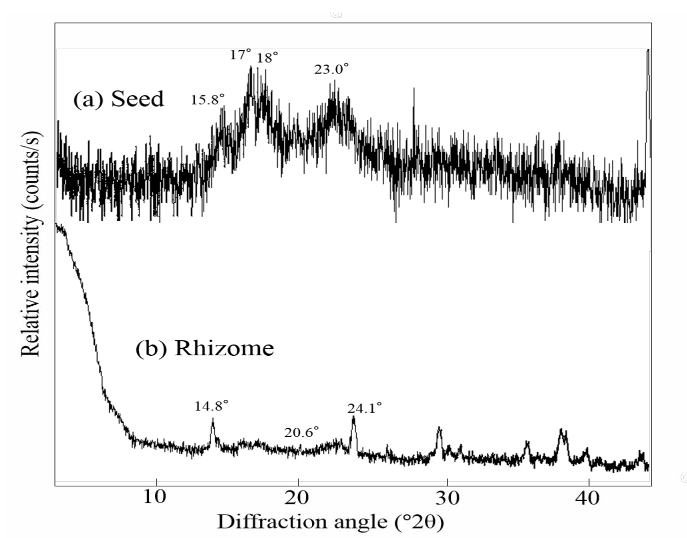

Figure 2. X-ray diffractograms showing characteristic peaks of (a) A-type crystallinity in seed starch, (b) C-type crystallinity in rhizome starches of *E. acoroides*.

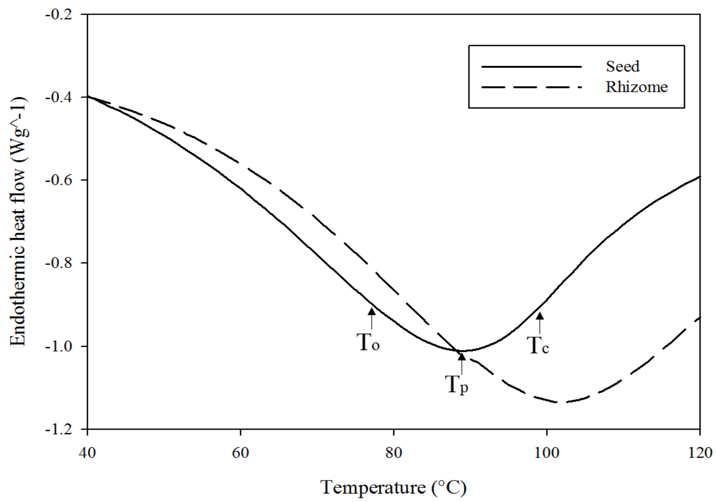

Figure 3. Plots of endothermic heat flow versus temperature obtained from DSC (differential scanning calorimeter) of *E. acoroides* seed and rhizome starches. T_o: temperature onset, T_p: temperature peak, T_c: temperature conclusion.

(Fig. 3). Rheological behavior of *E. acoroides* native starches in rhizomes and seeds showed comparable viscosity of 15.8-17.5 mPas and 14.2-16.7 mPas, respectively.

DISCUSSION

The sizes of starch granules of *E. acoroides* rhizomes in the present study (mean=40.6 µm) are comparable to those from a previous study (38.1 µm) by Phang et al. (2002) while there is no published data reported on starch granules from *E. acoroides* seeds. Pomeranz (1991) categorized the starch granule sizes based on commercial starch: large (15-100 µm, e.g., potato starch), medium (10-25 µm, e.g., corn starch) and small (3-8 µm, e.g., rice starch). Based on this classification, starch from seeds of *E. acoroides* are medium-sized (14.7 µm) while those of the rhizomes are large (40.6 µm). Large starch granules are more preferable in food formulation as they give unique swelling capabilities and also form highly viscous pastes. In contrast, small starch granules have an advantage as a fat substitute as they are digested four times faster by glucoamylase than large granules (Kang et al. 1985).

The yield of starch from the seed obtained in the present study (48.9%) is comparable to the 50% reported by Montano et al. (1999), but lower than those of the commercial crops (ca. 90%: Table 1). According to Tester et al. (2004), regular starches comprise approximately 20-30% amylose and 70-80% amylopectin; waxy starches contain <10% amylose while >40% for high-amylose starches. In the present study, *E. acoroides* seed starch is categorized as waxy starch while rhizome starch as regular starch, like those in corn, tapioca and potato (Table 1). Total starch in rhizomes of *E. acoroides* (14.4%) is lower than that in rhizomes of *Thalassia hemprichii* from Merambong shoal (34.3%) (Phang et al. 2002).

Waxy starch such as in rice gives a stickier and softer texture in undergoing starch gelatinization, while more amylose in starch will result in firmer structure and increase the gel strength (Cruz et al. 2013). Nowadays, people become more aware of quality foods containing resistant starch as the latter plays a significant role in maintaining intestinal health. Resistant starch is also considered as a functional dietary fiber defined as the sum of starch that passes through the small intestine without getting digested and is then fermented in the colon of a healthy individual (Baixauli et al. 2008). The rhizome of *E. acoroides* has a higher resistant starch content which also surpassed the other commercial starches from tapioca, corn and potato. Thus, it can be an alternative source of low-cost indigestible carbohydrate especially for people living in coastal areas.

As for minor components, both rhizome and seed starches of *E. acoroides* have a comparable amount of lipid, and higher amount of other macro-components such as moisture, protein and ash compared to the commercial starches (Table 1). Higher moisture content and minor nutrient composition were recorded for presently tested seed starch compared to previous data recorded by Montano et al. (1999). To obtain the purest starch form, the isolation method needs to be improved because excess protein and lipid in starch can cause foam and browning as well as produce undesirable off flavors. Higher phosphate content in

Table 1. The chemical composition of different starches from the present and previous studies. Data are expressed as the mean values ± standard deviation (*n*=3) for the present study.

Species	Total starch (%)	Amylose (%)	Resistant starch (%)	Moisture content (%)	Protein (%)	Lipid (%)	Ash (%)	Phosphorus (mg/kg)
Seagrass starch								
E. acoroides seed[a]	48.9±0.4	10.8±1.1	1.7±0.1	15.9±0.2	7.5±0.1	1.4±0.2	5.0±0.7	117.9±40.3
E. acoroides rhizome[a]	14.4±0.8	18.8±0.2	75.3±0.7	14.4±0.3	3.3±0.10	0.3±0.01	5.6±1.3	429.6±40.2
E. acoroides seeds flour[b]	nd	nd	nd	9.80	8.8	0.2	6.4	2392
E. acoroides seeds starch[b]	50.0	nd	nd	11.00	0.8	0.1	0.5	210
Thalassia hemprichii seed[c]	70.4	nd	nd	nd	nd	nd	nd	nd
T. hemprichii rhizome[c]	34.3	nd	nd	nd	nd	nd	nd	nd
Commercial starch								
Zea mays (Corn)[d, e]	89.3	25.6	1.2	7.7	0.4	0.7	0.4	0.0
Manihot esculenta (Tapioca)[d]	91.0	16.3	9.7	7.5	0.1	0.1	0.2	0.0
Solanum tuberosum (Potato)[d, f]	89.4	25.0	66.5	9.4	0.1	0.1	0.2	0.1

[a]Present study, [b]Montano et al. (1999), [c]Phang et al. (2002), [d]Mishra & Rai (2006), [e]Moongngarm (2013) and [f]Englyst et al. (1992). nd: no data available.

starch caused the chains to come apart inside starch granules which lead to better access of water during starch gelatinization. This situation resulted in lower pasting temperature which increases the viscosity and paste clarity (Mishra and Rai 2006).

Investigation of functional properties including starch gelatinization, crystallinity and rheological behavior are the new findings in this seagrass study. This information, as well as their nutrient composition and starch morphology, are important factors in food formulation. The Maltese cross present in starches indicates an orderly arrangement of crystalline areas within a starch granule called polymorph and can be distinguished into A-, B- or C-type starches based on their peaks (Wang et al. 1998). A-type shows strong diffraction peaks at around 15° 2θ and 23° 2θ, and unresolved doublets at around 17° 2θ and 18° 2θ, B-type crystallinity shows strongest diffraction peak around 17° 2θ and small peaks around 15 2θ, 20° 2θ, 22° 2θ and 24° 2θ, and a characteristic peak at about 5.6° 2θ, while C-type crystallinity possesses a mixture of both (Cai et al. 2014). Generally, cereal starches have A-type crystallinity while rhizomes and tubers are mostly B-type starch (Zobel 1988). In the present study, the seed of *E. acoroides* had A-type starch crystallinity characterized by its loose structure and longer amylopectin branches. C-type starch normally can be found in high amylose starch (Zobel 1988), as in the *E. acoroides* rhizome starch in the present study. The high content of amylose (18.8%) influences its gelatinization enthalpy, i.e., high energy is needed to disrupt the highly ordered structure of the starch granules. Simultaneously, larger particle sizes of *E. acoroides* rhizome starch formed agglomerated particles during heating which caused its higher gelatinization peak (Maniglia and Tapia-Blacido 2016). In contrast, *E. acoroides* seed starch has a lower content of amylose (10.8%) and produced a higher viscosity slimy paste during beating.

Both the seeds and rhizome starches give promising potential to be used in food formulation. The seeds of *E. acoroides* yielded a considerable amount of total starch with high protein content and can be an alternative staple food source for coastal communities. On the other hand, the rhizome starch contained a good amount of dietary fiber that may benefit the human diet by lowering blood glucose level and improving insulin sensitivity as well as digestion. Starch gelatinization is a basic standard of processing starch granules or native starches to modify their functional characteristics for further utilization in food formulation. More studies need to be carried out on their nutrients and anti-nutrients so that consumers can acknowledge their nutritional information.

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