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Starch properties of *Amaranthus pumilus* (seabeach amaranth): a threatened plant species with potential benefits for the breeding/amelioration of present *Amaranthus* cultivars

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Abstract

This study reports on the physicochemical properties of starch isolated from the seeds of *Amaranthus pumilus* (a threatened plant species) and compares it to that of the commonly cultivated/commercially produced *Amaranthus hypochondriacus* K343 (Plainsman). Seeds of both investigated species were found to possess comparable quantities/levels of total starch. Although no significant differences ($P \geqslant 0.05$) were found between the composition of the two starches with regard to their moisture, ash, protein and fat contents, the starch of *A. pumilus* was found to contain significantly higher ($P \geqslant 0.05$) amounts of amylose than that of *A. hypochondriacus*. The higher levels of amylose found in *A. pumilus* starch were believed to be responsible for the higher differential scanning calorimetric T*o*, T*p* and T*e* starch temperatures than those of *A. hypochondriacus* starch which would indicate higher levels of ordered crystalline structure in the former. Scanning electron microscopy and integrated light scattering techniques both revealed a uniform average starch granule diameter of approximately 1 μm for both investigated species. This study further supports and compliments a previous study which showed that much genetic diversity exists between *A. pumilus* and *A. hypochondriacus*, indicating that potential breeding possibilities for the improvement of commonly cultivated amaranth lines do exist, if *A. pumilus* is not first driven to extinction.

1. Introduction

Much scientific information concerning the nutritional attributes and benefits associated with the consumption of amaranth grain abound, especially when it is incorporated into thermally processed foods (Lehmann, 1996; Hozova, Buchtova, Dodok, & Semanovic, 1997). Other studies have demonstrated a variety of important and unique nutraceutical type applications for grain amaranth, including its use as an adjunct to lower blood glucose response in non-insulin-dependent diabetics and in other applications to lower blood serum cholesterol levels (Lehmann; Chaturvedi, Sarojini, Nirmalammay, & Satyanarayana, 1997; Dunz & Lupton, 1992). The development of grain amaranth with enhanced characteristics for the above purposes, has been aided by the fact that there is a great deal of genetic variation available within the species. Although several amaranth species, do exist, a few wild species, including *Amaranthus pumilus* (Seabeach Amaranth) are quickly being extirpated from their native habitats and this may reduce the overall biodiversity available to plant breeders for the amelioration of present amaranth cultivars. With fewer than 55 remaining populations left in the wild (or approximately 1000 individuals known), *A. pumilus* has received federal protection as a threatened plant species under the United States Endangered Species Act (Weakley, Bucher, & Murdock, 1996). If not driven to extinction, the seabeach amaranth may potentially serve as a useful plant resource in future breeding programs due to its favourable genetic traits, such as tolerance to high soil salinity and large seed size.

In a study by Marcone (1999), *A. pumilus* was found to possess a larger and more desirable seed size and weight (2–3-fold higher) than *Amaranthus hypochondriacus* K343 (most commonly cultivated commercial species), allowing for greater biomass production in addition to

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lower levels (one-half) of free carbohydrate. In addition to having higher edible-oil content than K343, its lower saturated and unsaturated (S/U) ratio make it potentially a better source of nutritional oil. Moreover, the 2 fold-higher quantity of vitamin E found in *A. pumilus* and the higher levels of squalene also found, may one day serve as a renewable crop source of this compound and, hopefully, diminish the world's dependence on marine animals.

Although several important compositional differences have been observed between the seeds of*A. pumilus* and *A. hypochondriacus* (Marcone, 1999), little else is known about any differences that may exist between their starch compositions, i.e. the main constituent within each seed.

Recently, starches with novel properties have attracted the interest of several researchers for expanded applications in foods and other non-food formulations. Amaranth starch has received particular attention because of its very small uniform granules with diameters of 1–2 μm (Uriyapongson & Rayas-Duarte, 1994; Radosavljevic, Jane, & Johnson, 1998; Baker & Rayas-Duarte, 1998) compared to 3–8 μm of rice starch, the smallest commercially produced starch. The extremely small starch granules of amaranth provide unique functional properties in food and non-food applications, e.g. food thickeners, paper coatings, laundry starch, dusting powders, cosmetics, fat replacers, thickeners in the printing of textiles and biodegradable plastics (Ahamed, Singhal, Kulkarni, Kale, & Pal, 1996; Lehmann, 1992; Breene, 1991). Although it has become exceedingly clear that *A. pumilus* has many characteristics superior to K343, little information is known about its starch, which constitutes the major component within the seed and would dominate the overall characteristics of any manufactured products. It was, therefore, the objective of this study to examine the starch properties of the threatened *A. pumilus* species and compare it to that of the more common and commercially cultivated *A. hypochondriacus*, grown under identical and controlled environmental conditions.

2. Materials and methods

2.1. Materials

Seeds of the threatened plant species *Amaranthus pumilus* (seabeach amaranth) were obtained from the USDA (Ames, Iowa) PI553083 under provision 50CFR17.7 (a) of the US Endangered Species Act, whereas *A. hypochondriacus* K343 (Plainsman) was obtained from the Department's germplasm collection. Both species were cultivated under identical greenhouse environmental conditions and seed was collected at maturity and stored at −20°C.

2.2. Starch isolation and purification

Starch from both *Amaranthus* species was isolated and purified using a modified wet-milling procedure based on the method of Baker and Rayas-Duarte (1998).

2.3. Chemical analyses

Ash, moisture, fat and protein contents of starch were determined according to AACC approved methods 08-01, 44-15A, 30-25 and 46-13 (AACC, 1983). Starch content in amaranth seed was determined according to the starch-gluco-amylase method 76-11 (AACC, 1995). Amylose content of starch samples was estimated using the method of Williams, Kuzina, and Hlynka (1970).

2.4. Scanning electron microscopy

Starch samples were mounted on aluminium stubs and coated with gold/palladium (60/40) to a thickness of 25–30 nm, using an Anatech hummer VII Sputter Coater (Alexandra, VA). Following coating, samples were visualized and photographed under a Hitachi S-570 Scanning Electron Microscope. The size of starch granules was estimated by measuring the diameter of >50 randomly selected granules from various photomicrographs.

2.5. Determination of starch granule size by integrated laser light scattering

The diameters of starch granules and their specific surface area were determined by integrated light scattering using a Mastersizer X (Malvern Instruments Inc., Southboro, MA). The samples were diluted circa. 1:500 in filtered Milli-Q water and measured at ambient temperature (22°C). The presentation factor used was 0303, representing a relative refractive index between the continuous and the dispersed phase (refractive index of 1.41 and absorption of 0.001). The scattered light was detected at 31 angles and analyzed by an inverse fourier transformation by the instrument.

2.6. Thermal properties

Thermal properties of starch were determined in three independently isolated starch samples (in triplicate) using a DuPont Modulated Differential Scanning Calorimeter Model 2910 equipped with a TA Instruments DSC Cell (New Castle, Delaware, USA) and a liquid nitrogen cooling accessory (TA Instruments) and data evaluated using General V4 IC DuPont 2000 analysis software. Thermograms were obtained employing a heating rate of 5°C/min, from 25–100°C. High purity (99.9%) indium and gallium were used to calibrate the system. Starch samples (3.0 mg) were weighed into DSC aluminium pans and 10 μl of deionized water added with a microsyringe. After sealing the pans, they were allowed to equilibrate for 3 h. An empty pan was used as a reference. Thermal transitions of starch were defined in terms of onset (*To*), peak ((*Tp*), and end of gelatinization (*Te*) temperatures (°C), and enthalpy of gelatinization (Δ*H*, J/g).

2.7. Calorimetric determination

The colour of starch (L^*, a^* and b^* values) samples were determined using a Minolta Chroma Meter CR-2006 (Minolta Camera Co. Ltd. Osaka, Japan).

2.8. Statistical analysis

Statistical analysis was performed using SAS Statistical Analysis System package (SAS Institute, 1990). Significant differences among treatments were determined by Duncan's multiple range test ($P \leqslant 0.05$).

Table 1
Comparison of the chemical, physical and thermal properties of the isolated starch and flour from the seeds of *Amaranthus hypochondriacus* K343 (plainsman) and *Amaranthus pumilus* (seabeach amaranth; a threatened plant species)[a]

	Amaranthus hypochondriacus (plainsman)	*Amaranthus pumilus* (seabeach amaranth)
Chemical analysis of starch (%)		
Total starch (in seed)	61.0a	61.4a
Amylose	4.17a	8.20a
Moisture	11.1a	10.3b
Ash	1.3a	1.0a
Protein	0.05a	0.07b
Fat	0.87a	1.1a
Morphological properties of starch granules		
Starch granule size by S.E.M. (μm)	1.1a	1.1a
Starch granule size by light scattering (μm)	1.09a	1.07a
Specific surface area (m^2/cm^3)	5.5282a	5.5977a
Starch colour		
L^*	93.20a	93.85a
a^*	0.85a	0.83a
b^*	3.07a	2.97a
Thermal properties of starch/flour		
*T*o (°C) (Starch/Flour)	(62.0/64.2)a	(63.4/66.2)b
*T*p (°C) (Starch/Flour)	(70.6/73.2)a	(71.6/74.3)b
*T*e (°C) (Starch/Flour)	(78.9/77.5)a	(80.0/82.8)b
Δ*H* (J/g) (Starch/Flour)	(13.0/6.6)a	(12.2/6.3)a

[a] *To*, *Tp* and *Te*, onset, peak and end temperatures, respectively. Δ*H*, enthalpy change. Values within each row are not significantly different ($P \geqslant 0.05$). Values represent the means of three replications.

3. Results and discussion

3.1. Chemical composition of starches

No significant difference ($P \geqslant 0.05$) was observed between the total seed starch contents of *A. pumilus* and the more common and commercially cultivated *A. hypochondriacus* K343 (i.e. ~60%) which fell within the range reported in the literature for other studied *Amaranthus* species (Uriyapongson & Rayas-Duarte, 1994). The proximate composition and amylose contents of both isolated starches are shown in Table 1. The air-dried starches showed no significant differences

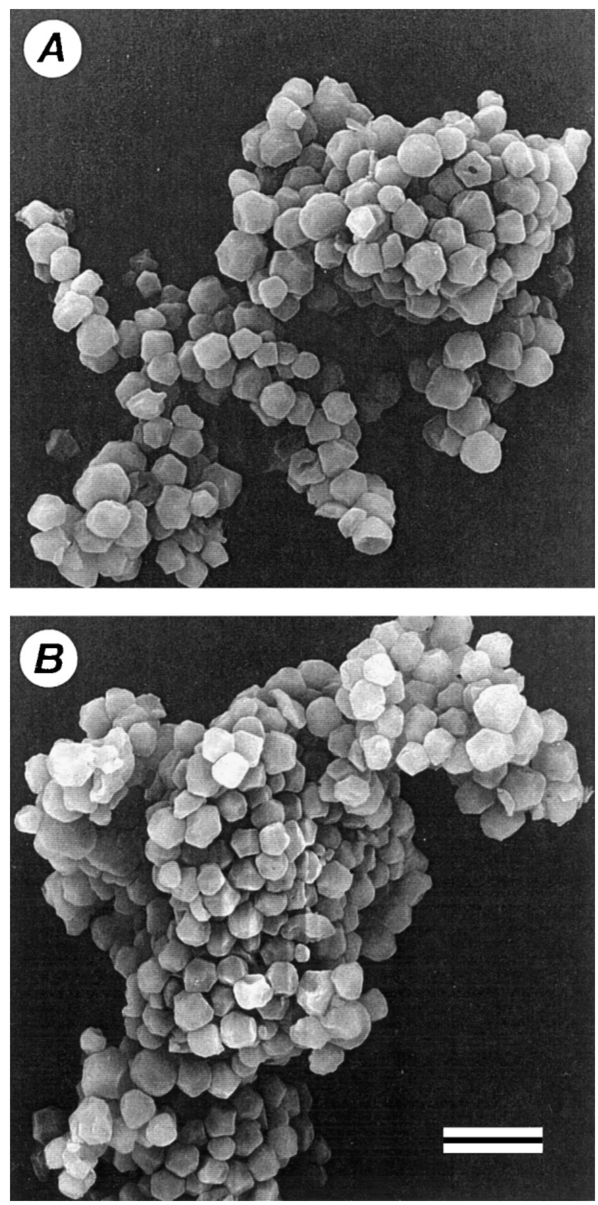

Fig. 1. Scanning electron micrographs of starch isolates from (A) *Amaranthus pumilus* and (B) *Amaranthus hypochondriacus* magnified by 2500. Bar represents 3.0 μm.

($P \geqslant 0.05$) in moisture, ash, protein, fat or colour and were comparable to those of other studied *Amaranthus* species (Uriyapongson & Rayas-Duarte). On the other hand, *A. pumilus* starch had significantly higher ($P \leqslant 0.05$) amylose content than *A. hypochondriacus* starch (Table 1). Since low levels of amylose, typically found in amaranth starch (as in this study), cause amaranth starch to perform poorly in bread and cake formulations (Stone & Lorenz, 1984), the higher levels of amylose presently found in *A. pumilus* may potentially improve its overall performance in the above products and thus awaits further verification.

3.2. Starch granule morphology

Examination of the morphological properties of *A. pumilus* starch granules by scanning electron microscopy revealed a uniform average diameter of 1 μm, similar to those of starch granules isolated from *A. hypochondriacus* (Fig. 1 and Table 1). This finding was further substantiated by the results of integrated laser light scattering analyses which also show a comparable average diameter falling within a very narrow size distribution range (Fig. 2 and Table 1). Scanning electron microscopy also revealed that starch granules from both

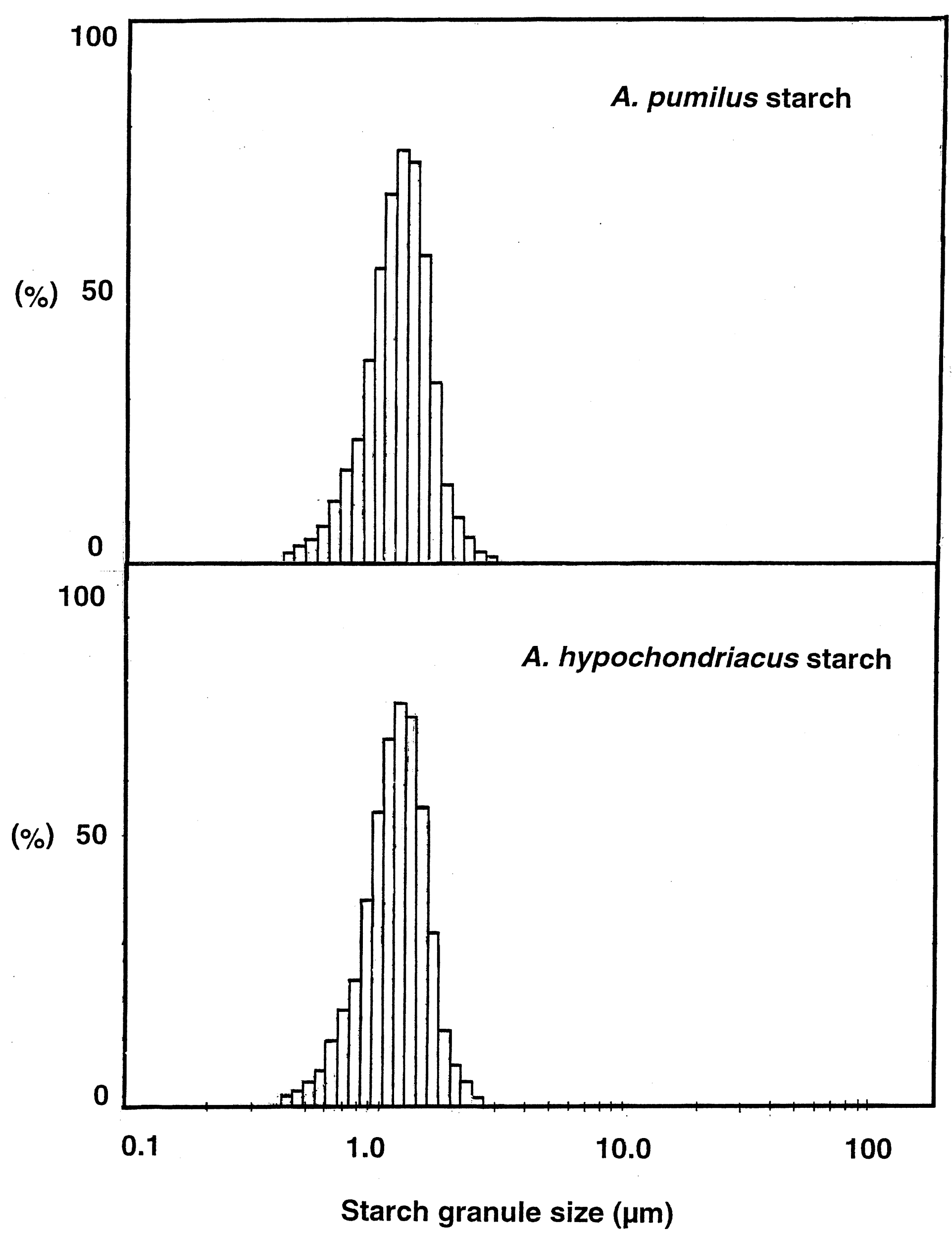

Fig. 2. Integrated light scattering size distribution of starch granules of *Amaranthus pumilus* and *Amaranthus hypochondriacus*.

species were polygonal in shape with very smooth surfaces and with no evidence of any fissures. The smooth granule surfaces of both starch isolates would indicate a relatively low level of amylase activity within the seed in addition to resistance to mechanical shear during isolation (Quian and Kuhn, 1999). These morphological characteristics are in agreement with the findings of other investigators (Hoover, Sinnott, & Perera, 1998; Radosavljevic et al., 1998).

3.3. Thermal properties

Gelatinization and melting transition properties of starches were also studied using differential scanning calorimetry and typical DSC curves shown in Fig. 3. Both the starch and flour of *A. pumilus* seed revealed a small but significantly higher ($P \leqslant 0.05$) onset temperature (*To*) as compared to their respective counterparts in *A. hypochondriacus,* which may suggest that *A. pumilus* starch has amorphous regions which are thermally and structurally more stable than the former (Leszkowait, 1988) (Table 1). The corresponding peak (*Tp*) temperature values were slightly, but also significantly higher in both *A. pumilus* starch and flour than the *A. hypochondriacus* samples, suggesting that starch from the former has a greater resistance to gelatinization (Hoover & Sosulski, 1985). The significantly higher *Tp* temperatures of *A. pumilus* starch would suggest either a more highly ordered crystalline structure (Hoover & Sosulski) or, possibly, a fewer number of amorphous regions (Table 1). The end temperature (*Te*) is thought to be indicative of the true melting of crystallites undisturbed by amorphous regions (Maurice, Slade, Sinnett, & Page, 1985; Table 1). The significantly higher (*Te)* temperatures of *A. pumilus* would suggest a more stable and/or more highly ordered crystalline region. The higher content of amylose found in *A. pumilus* starch correlates well with DSC data since the (lower amylose) higher amylopectin content of *A. hypochondriacus* and, therefore, higher degree of chain branching would disrupt the

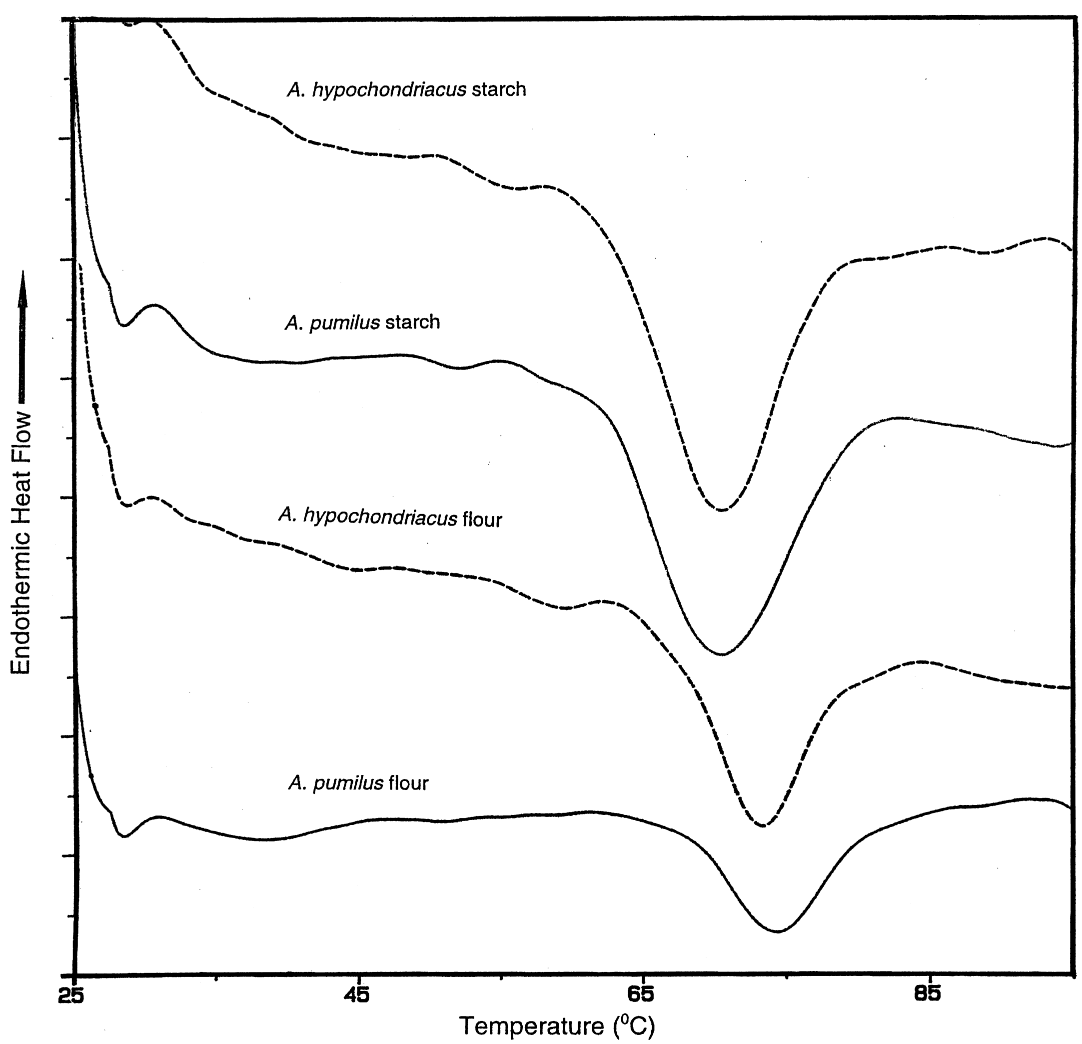

Fig. 3. Differential scanning calorimetric thermograms of *Amaranthus pumilus* and *Amaranthus hypochondriacus*.

organization of crystalline regions and decrease the amount of intra-and intermolecular hydrogen bonds required for stability (Biliaderis, Page, Slade, & Sirett, 1981). Despite this, it is important to note that no significant difference ($P \geqslant 0.05$) between the enthalpy of gelatination for *A. pumilus* flour and that of *A. hypochondriacus* flour was noted, indicating that the starch interacts with other seed components when used as part of a whole grain formulation.

Once a sustainable cultivated population of the threatened *A. pumilus* species is achieved, (permitting for the isolation of greater quantities of starch for analysis), apparent intrinsic viscosities as well as pasting properties will be examined and compared to those of *A. hypochondriacus* in order to complete/complement this study. Although further in-depth studies of starch properties will be needed, it is now clear that *A. pumilus* does show potential benefit for future amaranth amelioration breeding programs.

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