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PHYSICOCHEMICAL CHARACTERIZATION OF STARCH ISOLATED FROM ETHIOPIAN POTATO (*PLECTRANTHUS EDULIS*)

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ABSTRACT: Starch from the tubers of Ethiopian potato (*Plectranthus edulis*) (Fam. *Lamiaceae*) has been isolated and examined for its chemical composition, amylose content and physicochemical properties. The starch yield was about 80.4% on dry weight basis. The proximate composition of the starch on dry weight basis was found to be 0.14% ash, 0.21% lipid, 0.43% protein, and 99.2% starch. The amylose content was 30.6%. Its true density and moisture content values were 1.47 g/mL and 11.2%, respectively. Scanning electron microscopy (SEM) of the starch granules showed characteristic morphology that was by and large oblong (elliptical) with some oval-shaped granules. The starch has normal granule size distribution with a mean particle size of 36.2 µm. The DSC thermograms of the starch obtained from starch-water mixtures (1:1), exhibited gelatinization onset temp. (T_o) of 69.2 ^oC, peak temp. (T_p) of 74.3 ^oC and endset temp. (T_e) of 83.3 ^oC. X-ray diffraction pattern of the starch was typical B-type with a distinctive maximum peak at 17.5^o 2θ.The starch possesses higher swelling power and moisture sorption pattern but lower solubility values than those of potato starch at all temperatures studied. Considering the high yield and some similar physicochemical properties to those of potato starch, *P. edulis* (Ethiopian potato) can be explored as an alternative source of starch for various applications.

Key words/phrases: Differential scanning calorimetry, Ethiopian potato, *Plectranthus edulis*, Scanning electron microscopy, Starch, Swelling power

INTRODUCTION

Plectranthus edulis, Vatke [Fam. Lamiaceae], is large, erect, and aromatic herb with hirsute, decumbent and glandular stems and edible underground potato-like tubers on slender rhizomes, and with a height up to 150 cm (Hedberg *et al*., 2006). Figure 1 shows the image of *P. edulis*, which is one of the traditional root crops indigenous to Ethiopia. *P. edulis* is cultivated during the rainy season in mid and high altitudes (1880 to 2200 m above sea level) in the north, south and west Ethiopia primarily for its edible tubers). Tubers of *P. edulis* have long been used for food, as they are rich in carbohydrate (Zemede Asfaw and Zerihun Woldu, 1997; Mulugeta Taye *et al*., 2007).

Locally, *P. edulis* is known by various vernacular names such as *Wolaita donuwa*, *Dincha Oromo*, *Gurage dinch*, *Hadiya dinch or Agew dinch;* and generally, as Ethiopian potato (Mulugeta Taye *et al*., 2007; Yeshitila Mekibib, 2007). It is one of the four economically important tuber crops of the genus *Plectranthus*, together with *Plectranthus esculentus* (Livingstone potato), *Plectranthus parviflorus* (Sudan potato) and *Plectranthus rotundifolius* (Madagascar potato) (Mulugeta Taye, 2008; Rice *et al*., 2011). *P. edulis* is grown from tuber pieces, whole tubers, stem cuttings and sprout cuttings, but tuber pieces are the most commonly used. The tuber pieces are planted in a 5-10 cm deep hole at a spacing of ~ 60–100 cm (Mulugeta Taye *et al*., 2007). For the plant to become fully grown it requires about 7-8 months (Mulugeta Taye, 2008).

Starch is the primary component of most plant storage organs - tubers, cereal grains and legume seeds - and remains an essential food source of calories in the human and animal diet (Buléon *et al*., 1998). It is also one of the most widely used biomaterials in the food, textile, cosmetics, plastic, adhesives, paper and pharmaceutical industries (Tsige Gebre-Mariam and Schmidt, 1996; Buléon *et al*., 1998; Fortunak *et al*. 2015). New applications of starch are steadily emerging, including low-calorie fat mimetics, biodegradable packaging materials, thin films, carrier matrices for controlled release of agrochemicals, and thermoplastic materials (Biliaderis, 2009). These diverse industrial

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applications of starch are premised on its availability at low cost, high caloric value, inherent excellent physicochemical properties and the ease of its modification to other derivatives (Tsige Gebre-Mariam and Schmidt, 1996).

Figure 1. Image of *P. edulis* plant (A) and its tubers (B)

Starches from various plant species of Ethiopia including *Ensete ventricosum* (Tsige Gebre-Mariam and Schmidt, 1996), *Dioscorea abyssinica* (Tsige Gebre-Mariam and Schmidt, 1998), *Colocasia esculenta* (Million Adane *et al*., 2006), *Manihot esculenta* (Getahun Paulos *et al*., 2009) have been investigated for their physicochemical properties and applications in pharmaceutical and other industries. Investigation and characterization of starches from newer/ alternative sources can reduce the burden on the limited sources of starch like potato, cassava, maize, rice and other tubers and cereals. Starch generally occurs in the form of granules which vary in shape, size, physicochemical and functional characteristics depending on its plant origin (Tsige Gebre-Mariam and Schmidt, 1996, Pérez *et al.* 2009, Salar *et al.,* 2013). The current work reports on the granule compositions and some physicochemical properties of the starch isolated from the tubers of *P. edulis* for its potential pharmaceutical and related industrial applications.

MATERIALS AND METHODS

Materials

Fresh mature *P. edulis* tubers were collected from Shashigalle Kebele, Wolaita Zone, Ethiopia and authenticated at the Department of Biological Sciences, College of Natural Sciences, Addis Ababa University. Sodium hydroxide (Loba Chemie Pvt. Ltd., Mumbai, India), sodium metabisulphite and hydrochloric acid (BDH Chemicals Ltd, Poole, England), Lugol's solution (SIGMA Chemicals Co., USA), amylose and amylopectin (Merck, Germany), Potato starch BP (BDH LTD., Poole, UK), Sodium chloride (Oxford Laboratory, Mumbai, India) were used as received.

Methods

Extraction of P. edulis starch

Starch isolation was carried out following the method described by Tsige Gebre-Mariam and Schmidt (1998). Tubers of *P. edulis* were thoroughly washed and all foreign materials removed. The washed tubers were peeled and pulverized using a blender and suspended in large quantities of distilled water containing 0.075 % (w/v) of sodium metabisulphite. The material was then passed through fine muslin cloth to remove cell debris and the translucent suspension collected, re-filtered through the muslin cloth and allowed to settle. The sedimented starch was washed several times with sodium metabisulphite solution until the suspension was clear and free of suspended impurities. The resulting starch was air-dried at room temperature. Finally, the dried starch was ground and sieved through a 224 µm mesh sieve and kept in a tight sealing container.

Chemical composition

Amylose content of the starch was determined by a colorimetric assay method (Tsige Gebre-Mariam and Schmidt, 1996). The fat content was estimated by acid hydrolysis; ash content by measurement of the residue left after combustion in a furnace at 550 °C; and moisture content by oven drying according to the method outlined by the Association of Official Analytical Chemists (AOAC) (2000). Protein was estimated by determination of nitrogen via elemental analysis,

using a conversion factor of 6.25 (AOAC) (2000). Each analytical determination was performed in triplicate.

Physicochemical characterization of the starch

Morphological characterization

Electron micrographs of the starch sample were obtained using an environmental scanning electron microscope (ESEM XL30 FEG, Hillsboro, USA) in a wet-mode at an acceleration voltage of 12KV as described elsewhere (Ochalek *et al*., 2012).

Particle size analysis

Particle size distribution was analyzed using a Malvern Mastersizer 2000 laser diffractometer (Malvern Instruments Ltd, Worcestershire, WR14 1XZ, UK). The mean volume particle size and specific surface area as well as the uniformity of particle size distributions were determined using Mastersizer S, PSS0003-01 software (2002). Particle sizes (D10%, D50% and D90%) were determined as mean of three determinations and D90/D10 ratios were calculated as measure of size distribution width.

Crystallinity

X-ray powder diffraction of the starch sample was taken with a Bruker AXS diffractometer (Bruker-AXS, Advanced D8, Germany) operating in the 2θ mode using computer software (Operational Soft Diffract+ 2000). A Cu target tube operated at a power setting of 40 kV (30 mA) with single crystal graphite monochromator equipped with a microprocessor to analyze peak position was employed. The scanning regions of the diffraction angle, 2θ were 4-35^{o}, which cover most of the significant diffraction peaks of starch crystallites (Buléon *et al*., 1998).

Gelatinization

Differential scanning calorimetry (DSC) measurements of the starch-water mixture was carried out using DSC 200 thermal analyzer (Netzsch, Selb, Germany) as described by Tsige Gebre-Mariam and Schmidt (1996). Starch sample of known moisture content was thoroughly mixed with distilled water (starch/water ratio of 1: 2 (w/w), on dry basis) and allowed to equilibrate in closed glass jars for 24 h at room temperature. Portion of well mixed starch slurry was transferred into aluminum DSC sample pans and sealed hermatically. The sealed pan was placed in the DSC cell and heated from 20 ^{o}C to 110 ^{o}C at a rate of 10 ^{o}C/min. An empty sealed pan was used as a reference in a nitrogen environment. From the DSC trace: onset (T_o), peak (T_p) and endset (T_e) temperatures of the gelatinization (^{o}C), and enthalpy of gelatinization (ΔH_{gel}, J/g) were obtained.

Density and related properties

Bulk density

Bulk density of *P. edulis* starch was determined by carefully pouring 100 g powder (m) into a 250 mL measuring cylinder. The volume (V_b) was then read directly from the cylinder and used to calculate the bulk density according to Eq. 1.

$$Bulk density\ (\rho b) = m/V_b \qquad \text{Eq. (1)}$$

For comparison, the bulk density of potato starch was also determined similarly.

Tapped density

For tapped density, 100 g of starch powder (m) was tapped 500 times in a 250 mL graduated measuring cylinder using mechanical densitometer (ERWEKA, Germany) to a constant volume (V_t), and the tapped density was calculated using Eq. 2.

$$Tapped density\ (\rho t) = m/V_t \qquad \text{Eq. (2)}$$

Carr's index

Carr's index (% compressibility) was calculated from the difference between the tapped (ρt) and bulk (ρb) densities using the following relationship:

$$Carr's Index\ (CI) = ([\rho t - \rho b]/\rho t)x100 \qquad \text{Eq. (3)}$$

Hausner ratio

Hausner ratio was obtained from the ratio of tapped and bulk densities of the starch as follows.

$$Hausner\ ratio\ (HR) = \rho t/\rho b \qquad \text{Eq. (4)}$$

True density

True density was determined by liquid displacement method using xylene as immersion fluid (Odeku *et al.,* 2008). All results are the mean of three determinations.

Moisture sorption, swelling and solubility

Pyrex desiccators containing distilled water, saturated solution of NaCl or appropriate concentrations of NaOH were prepared to provide different relative humidity (RH) chambers (Tsige Gebre-Mariam and Schmidt, 1998) and stored at room temperature. Starch samples were pre-dried in an oven (Kottermann® 2711, Germany) for 4 h at 120 ^{o}C. Five samples of dried starch, each weighing 2 g, were placed in Petri dish (dried and weighed) and transferred to particular RH chamber. Samples were equilibrated for four weeks at room temperature. The weights after four weeks were recorded and the moisture uptake of each sample was calculated as the weight difference of the starches before and after equilibration in a given RH chamber. Water sorption capacities of the starches were expressed as percent moisture uptake. Solubility and swelling power (SP) of the starch at various temperatures were determined using the method described elsewhere (Bello-Pérez *et al.,* 2000).

Statistical analysis

Analysis of Variance (ANOVA) was performed using statistical software Origin 8 (Origin Lab™ Corporation, USA). Tukey multiple comparison test was used to compare the individual difference in the physicochemical properties of the starches. At 95% confidence interval, p values less than or equal to 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Chemical composition

Starch is the major constituent of *P. edulis* tuber, accounting for about 80.4 ± 0.45% on dry weight basis. The proximate composition and selected properties of *P. edulis* and potato starch granules are shown in Table 1.

As shown in the Table, *P. edulis* starch has comparable starch content, 99.2% to that of potato starch, 99.4%. The lipid and protein contents of *P. edulis* starch were significantly higher than potato starch ($p \leq 0.05$). *P. edulis* starch has relatively lower ash and moisture content than potato starch. The relative amounts of the two homopolymers of starch, amylose (linear) and amylopectin (branched), vary depending on its botanical source. Amylose, in aqueous solution, strongly interacts with iodine (I_3^-) to form a blue colored helical complex (λ_{max} 600–610 nm). Amylopectin, on the other hand, weakly interacts with iodine (I_3^-) to form a violet colored complex (λ_{max} 530–540 nm). Hence, this difference in the "iodine binding capacity" was used for the estimation of amylose content of the starch samples (Tsige Gebre-Mariam and Schmidt, 1996). Accordingly, the amylose content of *P. edulis* starch was found to be 30.6%. The corresponding value of potato starch was 28.8%.

Table 1. Proximate composition and some physicochemical properties of *P. edulis* and potato starch granules

Content (%)	*P. edulis* starch	Potato starch
Amylose	30.6 ± 0.62	28.8 ± 0.04
Ash	0.14 ± 0.01	0.30 ± 0.03
Protein	0.43 ± 0.03	0.15 ± 0.05
Lipid	0.21 ± 0.03	0.10 ± 0.02
Starch	99.2 ± 0.02	99.4 ± 0.02
Moisture	11.2 ± 0.17	12.3 ± 0.21

Physicochemical characteristics

Morphology

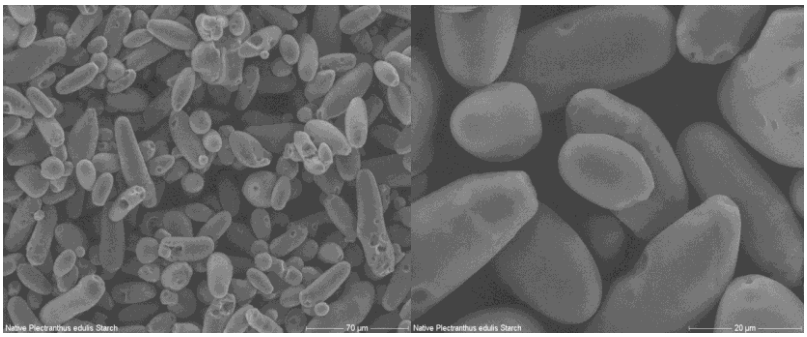
Particle morphology is an important property in the characterization and identification of powdered pharmaceutical excipients. It can also be used to predict certain functional properties that relate to flowability and compactibility of the powder (Builders *et al*., 2013).

Figure 2 shows the scanning electron micrographs of *P. edulis* starch. As can be seen from the figure, the starch granules have characteristic - oblong (elliptical) morphology and oval in shape, existing as single entities. By and large, the surface of the granules is smooth, but some exhibit fissure (cracks) when viewed at larger magnifications. These cracks could be due to aging of the plant when the starch was isolated (Vasanthan *et al*., 1999), which exposes the starch granules to enzyme catalytic digestion (Leach and Schoch, 1961; Fannon *et al*., 1992).

Granule size and specific surface area

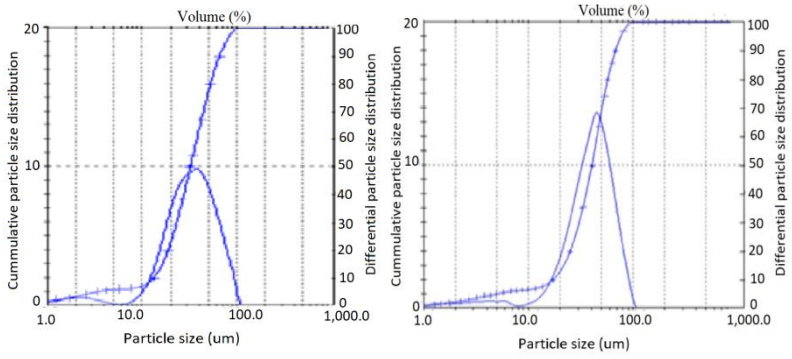
The volumetric particle size distributions of *P. edulis* and potato starches are shown in Figure 3. The mean volumetric particle sizes of *P. edulis* and potato starches were found to be 36.2 μm

and 41.5 µm, respectively (Table 2). Due to its relatively smaller particle size, *P. edulis* starch has a higher specific surface area than potato starch.



(A) **(B)**

Figure 2. Scanning electron micrographs of *P. edulis* starch granules at: (A) 500X and (B) 2000X.



(A) **(B)**

Figure 3. Volumeteric granule size distribution of *P. edulis* starch (A) and Potato starch (B).

Table 2. Volumetric granule size and size distribution parameters of *P. edulis* and potato starches

Parameters	***P. edulis***	**Potato**
Average particle size (µm)	36.2 ± 20.9	41.5 ± 20.1
50% of the particles (D50%) (µm)	< 32.6 ± 1.2	< 40.5 ± 2.0
10% of the particles (D10%) (µm)	< 13.3 ± 0.8	< 17.1 ± 1.3
90% of the particles (D90%) (µm)	< 65.9 ± 1.1	< 68.2 ± 2.5
Distribution width/span	4.94 ± 0.22	3.99 ± 0.15
Specific surface area (m^2/g)	0.30± 0.02	0.24± 0.03

Crystallinity

Starch has a definite crystalline nature. Its crystallinity has been attributed to the well-ordered structure of the amylopectin molecules inside the granules (Buléon *et al.*, 1998). The branches of amylopectin form double helices which are arranged in crystalline domains (Manek *et al.,* 2005). On the contrary, amylose largely makes up the amorphous regions which are randomly distributed between the amylopectin clusters (Tester *et al.*, 2004). The principal crystalline patterns of native starches from various botanical sources can be classified as either A, B or C type (Hizukuri, 1985; Manek *et al.,* 2005).

The X-ray powder diffractograms of *P. edulis* and potato starch are presented in Figure 4. As depicted in the figure, the X-ray diffraction pattern of *P. edulis* starch is similar to that of potato starch. The characteristic peak of B-type crystallinity at 4.5° 2θ of most tuber starches is observed for both starches. Moreover, both starches exhibited maximum peak at 17.5° 2θ. The other significant peaks appear at around 14.5°, 19.5°, 22.2°, and 24.6° 2θ confirming typical B-type patterns. Thus, *P. edulis* starch has B-type crystallinity like other tuber starches.

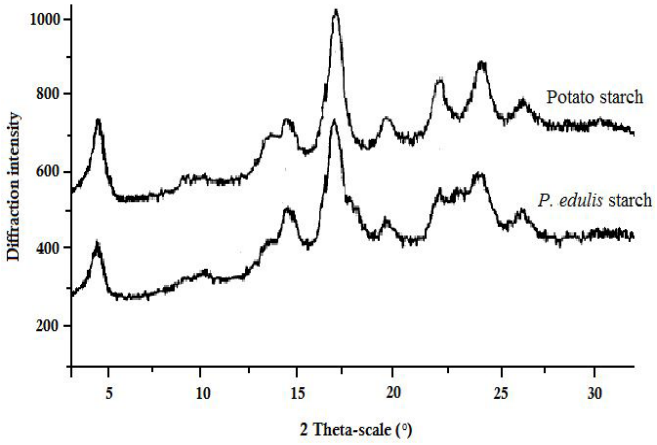


Figure 4. X-ray powder diffractograms of *P. edulis* and potato starches.

Gelatinization

DSC provides an accurate, easy and reproducible method for the determination of the onset (T_o), peak (T_p), endset temperatures (T_e) and the enthalpy (ΔH_{gel}) of gelatinization (Tsige Gebre-Mariam and Schmidt, 1996; Tsige Gebre-Mariam and Schmidt, 1998; Moorthy, 2002).

Figure 5 shows the DSC thermograms of *P. edulis* and potato starches analyzed at a starch-water ratio of 1:2. As shown in Figure 5, the T_o, T_p and T_e of *P. edulis* starch are higher (69.2, 74.3 and 83.3 ^oC, respectively) than potato starch (62.5, 68.3, and 79.4 ^oC, respectively). This suggests that *P. edulis* starch is more resistant to heat than potato starch. This is probably due to the high fat, and/or associative forces in *P. edulis* starch. Studies have shown that fat forms insoluble complexes with amylose, leading to high gelatinization temperatures (Kunle *et al.*, 2003; Manek. *et al.*, 2005). Starch composition (amylose/amylopectin ratio, lipid complexed amylose chain, and phosphorus) and granule architecture (crystalline/amorphous ratio), size and shape of the starch granules, genetic and environmental factors, etc., have been known to influence the gelatinization process (Tester and Morrison, 1990; Ratnayake *et al.*, 2002; Singh *et al.*, 2003).

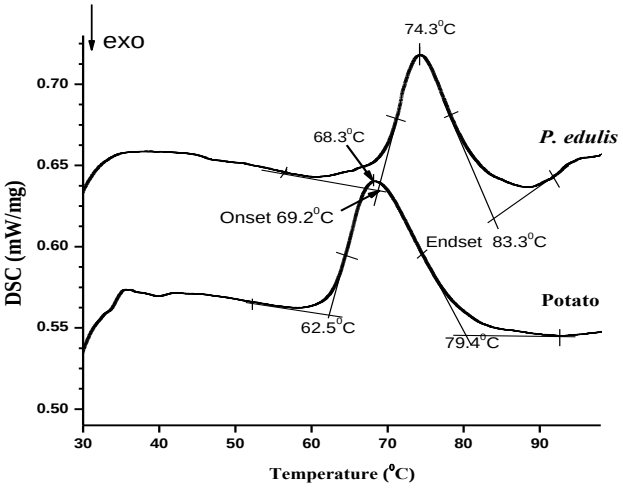


Figure 5. DSC thermograms of *P. edulis* and potato starches.

For *P. edulis* starch, the gelatinization enthalpy was about 3.81 J/g, whereas for potato starch it was 5.00J/g; while ΔT (T_e-T_o) was 14.1 for *P. edulis* starch and 16.9 for potato starch. Since amylopectin has more crystalline nature, slightly higher amylopectin content of potato starch could be responsible for its higher enthalpy of gelatinization, which is in agreement with the findings of Ihegwuagu *et al.* (2009) that semi-crystalline starch had higher ΔH_{gel} value than an amorphous starch.

Density and related properties

The bulk and tapped densities are indirect methods for predicting the flow and compactibility properties of pharmaceutical powders (Well, 2003). The density and its derived properties such as percent compressibility and Hausner ratio of *P. edulis* and potato starches are shown in Table 3. The two starches have comparable true densities. However, the bulk and tapped densities of *P. edulis* starch are relatively smaller than potato starch. This difference in their densities may be due to the difference in particle size and shape which affect the packing arrangements of the powder particles (Schüssele and Bauer-Brandl, 2003). Flow property is rated based on compressibility index (CI) and Hausner ratio (Carr, 1965, Hausner, 1967). Lower CI or lower Hausner ratio of a material indicate better flow properties than higher ones (Shah *et al*., 2008). *P. edulis* starch has higher Carr's index and Hausner ratio values which indicate that *P. edulis* starch has relatively lower flowability as compared to its counterpart. Generally, these values show that the starches have fair to passable flow characteristics as per Carr's flowability classification of powders (Carr, 1965; Staniforth, 2002; Schüssele and Bauer-Brandl, 2003).

Table 2. Density and related properties of *P. edulis* and potato starches.

Parameters	*P. edulis* starch	Potato starch
True density (g/mL)	1.47 ± 0.02	1.48 ± 0.01
Bulk density (g/mL)	0.58 ± 0.01	0.64 ± 0.09
Tapped density (g/mL)	0.73 ± 0.01	0.76 ± 0.02
Carr's index (%)	20.6 ± 1.77	16.2 ± 0.94
Hausner ratio	1.26 ± 0.02	1.18± 0.02

Swelling power and solubility of starch

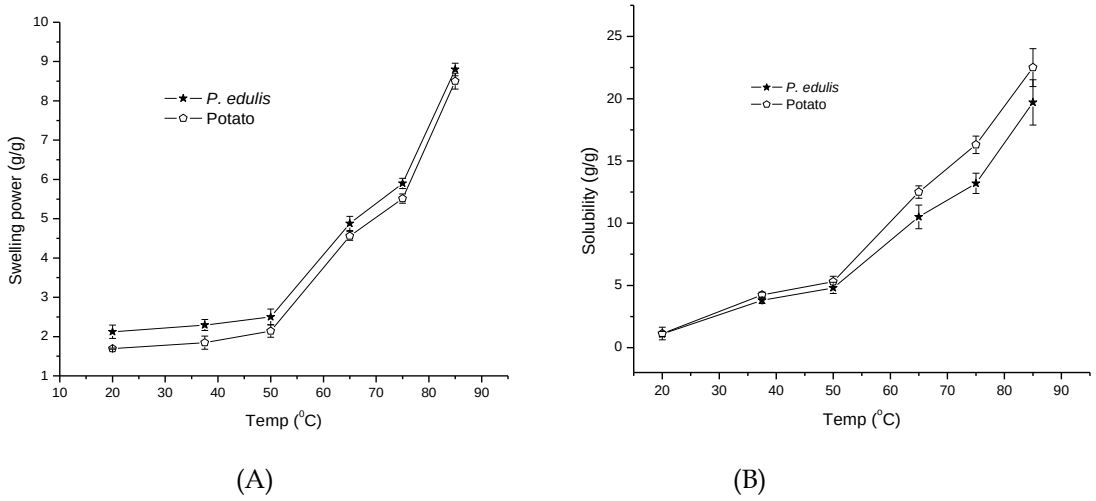
Swelling power indicates the water holding capacity of starch and has generally been used to demonstrate various types of starches. Factors like amylose-amylopectin ratio, chain length and molecular weight distribution, degree/length of branching and conformation, their non-carbohydrate (i.e., lipid and protein) contents and bonding forces within starch granules determine the degree of swelling and solubility (Schoch, 1994).

The swelling power and solubility profiles of *P. edulis* and potato starches are shown in Figure 6 A. Comparative studies have shown strong positive correlations between starch swelling and amylose content as well as amylose leaching (Zuluaga *et al*., 2007). The higher swelling power of *P. edulis* starch positively correlates with its slightly higher amylose content (Table 1). Granule size and specific surface area of the starches have also been found to affect their swelling patterns. The higher swelling power of *P. edulis* starch is also associated with its smaller granule size (36.2 *vs.* 41.5 µm) and higher specific surface area (0.30 *vs.* 0.24 m^2/g) than potato starch (Table 2). Small granules are associated with a higher rate of water absorption, earlier hydration and higher swelling than are larger granules (Chiotelli and Meste, 2002).

The swelling power and solubility of starches are affected by temperature (Loss *et al.,* 1981). The swelling power of *P. edulis* starch was found to be significantly ($p < 0.05$) higher than that of potato starch at lower temperature (20-50 ^oC). For instance, the swelling power of *P. edulis* starch was 2.30 g/g as compared to 1.70 g/g for potato starch, at 37.5 ^oC. At higher temperature, there is a large increase in the extent of swelling and solubility of both starches. This is due to the disruption of intermolecular hydrogen bonds, leading to more substantial loss of amylose and amylopectin from the granules. Water molecules solvate the liberated hydroxyl groups and the granules continue to swell. As a consequence of severe disruption of hydrogen bonds, the granules will be fully hydrated and finally the micellar network separates and diffuses into the aqueous medium (Hashim *et al.,* 1992).

The solubilities of *P. edulis* and potato starches increase with temperature. At lower temperature (20-50 ^oC), both starches show almost similar solubility profiles ($p>0.05$). Comparatively, potato starch has shown higher solubility than *P.*

edulis at all temperatures studied (Figure 6 B). A complex set of factors contribute to this difference in the solubilities of the starches: the source, inter-associative forces within starch granules, the difference in swelling power and chemical composition (lipid and protein), etc. of the starches.



(A) (B)

Figure 6. Swelling power (A) and solubility (B) of *P. edulis* and potato starches at different temperatures.

Moisture sorption properties

The Moisture is known to modify the flow and mechanical properties of many powders including starches (Tsige Gebre-Mariam and Schmidt, 1996; Staniforth, 2002). Figure 7 shows the moisture sorption profiles of *P. edulis* and potato starches equilibrated at different relative humidities (RHs) for four weeks. As shown in the figure, *P. edulis* starch shows slightly higher moisture sorption profile than potato starch at all RH values studied. However, this difference is not statistically significant ($p > 0.05$).

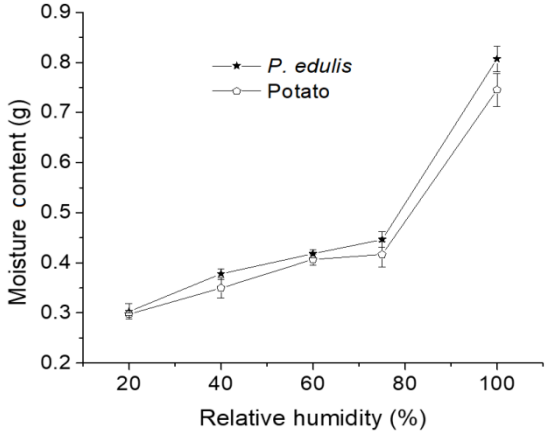


Figure 7. Moisture sorption patterns of *P. edulis* and potato starches.

The moisture sorption profile as well as the swelling power indicates that *P. edulis* starch might have superior disintegrant or at least a comparable property to potato starch.

CONCLUSION

The starch yield of *P. edulis* tubers is high, 80.4% on dry weight basis. The study on physicochemical properties revealed that *P. edulis* starch has comparable starch content to potato starch, but slightly higher amounts of protein and lipid. *P. edulis* starch also has slightly higher amylose content than potato starch, and hence higher swelling power. *P. edulis* starch exhibited higher gelatinization temperatures than potato starch indicating that it is more resistant to heat than potato starch. From the foregoing, it can be concluded that *P. edulis* starch could be an alternative to potato starch for various applications.

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