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Physicochemical, Pasting, Thermal and Morphological Characteristics of Indian Water Chestnut (*Trapa natans*) Starch

Starch extracted from Indian water chestnut was investigated for its physicochemical characteristics. The results were compared with those obtained from two commercial starches (corn and potato). The pasting properties were tested in the Rapid Visco Analyser and thermal properties with a differential scanning calorimeter. Water chestnut starch possessed higher breakdown viscosity (BV) and setback viscosity (SV) than corn and potato starches. However, the pasting temperature of water chestnut starch was not significantly different from that of corn starch. Lower $\Delta H_{\rm gel}$ values were obtained for water chestnut starch than for the other two starches whereas the onset, peak and conclusion temperatures of gelatinization ($T_{\rm o}$, $T_{\rm p}$ and $T_{\rm c}$) for water chestnut starch were quite comparable with corn starch. Scanning electron micrographs showed similarity in starch granule shape between water chestnut and potato starch with corn starch showing surface wrinkles on starch granule surfaces.

Keywords: Water chestnut; Characterization; RVA; DSC; Retrogradation

1 Introduction

Non-conventional food resources and their value addition have attracted attention in the recent years for their potential use as functional ingredients in food formulations. Water chestnut (Trapa natans L. var. bispinosa Roxburgh), locally known as 'Singhara' is one of the important annual aquatic warm season crops. Water chestnut belongs to the family Trapaceae. It is found commonly floating on the water surface of lakes, tanks and pools throughout India and similar countries. It is a valued crop in Asia, of which it is native, as well as many other parts of the world. Water chestnut is an important commodity in food industry because of its unique taste [1]. The dark-brown corms are peeled before cooking or canning. The kernel is delicious and contains carbohydrates, proteins and essential minerals.

Lee and Hwang [2] reported the free sugars in Chinese water chestnut juice as sucrose (8.58%), glucose (1.64%) and fructose (1.58%) being major components and maltose as trace component. The potassium content was 408.57 mg/100 g, being the major mineral present. The bulk of the edible region consists of starch-rich, thin-walled storage parenchyma similar

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in appearance to potato, interspersed with vascular strands. Water chestnut starch possesses the peculiarity that it originates from the kernel of an aquatic plant.

Murty et al. [3] studied some physicochemical properties of water chestnut starch. Water chestnut starch granules swelled and solubilized less between 60 and 70°C, a little above 75°C. Hizukuri et al. [4] investigated the properties of starch extracted from water chestnut (bispinosa Makino) and reported that the starch showed Cc or Ca type X-ray diffraction pattern, the susceptibility of granules to amylases was reported to be intermediate between those of corn and potato starches at 40°C. Xu and Shoemaker [5] studied the gelatinization properties of water chestnut starch and found that the starch granules obtained from water chestnut were of various shapes: round, half cap, polyangular and cubic. The gelatinization temperature of water chestnut starch was found to be slightly higher than that of potato starch. They also studied the effect of varying concentrations of different sugars on gelatinization of lotus root starch and water chestnut starch and concluded that starch from both sources followed the same trend as have been found with other starches using the microscopic heating-stage and differential scanning calorimetry.

A great deal of work has been done on properties of starches from other non-conventional resources. The present study on physicochemical, pasting, thermal and morphological characteristics of water chestnut will sup-



port the development of modified non-cereal starches for various applications. Hence, the objective of the present investigation was to characterize the water chestnut starch and compare it with that of corn and potato starch to explore it for further use.

2 Materials and Methods

2.1 Materials

Water chestnuts of a variety having purple-red color shell, popularly known as *lal* (red) variety, were procured from a local market from three different outlets. Commercial corn and potato starches were also purchased from a local market.

2.2 Isolation of starch

Isolation of starch was done by the following method. The white kernels obtained from fresh fruit by peeling were dipped in 0.1% aqueous potassium metabisulfite solution for 30 min. The kernels were then homogenized with cold water in a blender. The homogenate was filtered through a 100-mesh sieve (149 μ m) and the filtrate was kept undisturbed for several hours. Then the precipitated primary starch was recovered by decantation. The crude starch thus obtained was treated with 0.2% aqueous NaOH for 1-2 h. followed by several washings with cold water to remove traces of alkali until the supernatant no longer showed any pink color with phenolphthalein. The precipitated starch cake thus obtained was dried overnight in an oven at 40°C. The starch was ground gently with a mortar and pestles to pass it through a 100 mesh sieve (149 µm), packed in double plastic bags and stored in refrigerated conditions at 4°C till further use.

2.3 Physicochemical properties of starch

2.3.1 Moisture content

The moisture content of the starch samples was determined by oven-drying of two representative sample of about 2 to 3 g each and heated for 24 h at 105°C to constant weight.

2.3.2 Amylose content

Apparent amylose contents of the starch samples were determined by the method of *Williams* et al. [6]. A starch sample (20 mg) was taken, 10 mL of 0.5 M KOH was added and the suspension was mixed thoroughly. The

dispersed sample was transferred to a 100 mL volumetric flask and the volume was made upto the mark with distilled water. An aliquot of the test starch solution (10 mL) was pipetted into a 50- mL volumetric flask and 5 mL of 0.1 M aqueous HCl was added followed by 0.5 mL of iodine reagent. The volume was diluted to 50 mL and the absorbance was measured at 625 nm (UV Spectrophotometer, Electronics Corp. of India Limited, Hyderabad, India). The content of amylose was determined from a standard curve developed using standard amylose and amylopectin blends from potato starch (Central Drug House, Bombay, India).

2.3.3 Water binding capacity (WBC)

The water binding capacity of the different starches was determined using the method described by Yamazaki [7] as modified by Medcalf and Gilles [8]. A suspension of 5 g starch (dry weight basis) in 75 mL distilled water was agitated for 1 h and centrifuged $(3000 \times g)$ for 10 min. The free water was removed from the wet starch, drained for 10 min and the wet starch was weighed.

2.3.4 Bulk density (loose and packed)

For loose bulk density, an empty and dried 50-mL measuring flask was weighed. The starch sample was allowed to fall freely into the flask up to the mark, with a gentle tapping. The flask was weighed again along with the sample. For packed bulk density the starch sample was tapped inside the measuring flask with the help of a rubber pad and more sample added upto the mark before weighing. The results were reported as grams per milliliter.

2.3.5 Swelling power and solubility index

The swelling power and solubility index were determined by the method of *Leach* et al. [9]. The values for swelling power were reported in grams per gram and that of solubility index in percent.

2.4 Turbidity

The turbidity of suspensions of the starch samples was measured as described by *Perera* and *Hoover* [10]. An aqueous starch suspension (1%) was heated in a boiling water bath for 1 h with constant stirring and then cooled for 1 h at 30°C. The samples were stored for 5 days at 4°C in a refrigerator under covered conditions to prevent loss/gain of moisture and turbidity was determined every 24 h

by measuring absorbance at 640 nm (UV Spectrophotometer, Electronics Corp. of India Limited, Hyderabad, India) against water blank.

2.5 Syneresis

A starch suspension (2%, w/v) was heated at 85°C for 30 min in a temperature-controlled water bath, followed by rapid cooling in an ice water bath to room temperature. The starch sample was stored for 24, 48 and 120 h at 4°C. Syneresis was measured as percentage amount of water released after centrifugation at 3200 rpm for 15 min.

2.6 Morphological studies

Scanning electron micrographs (SEMs) were taken with a Jeol JSM-6100 scanning electron microscope (Jeol Ltd., Tokyo, Japan). Starch samples were suspended in ethanol to obtain a 1% suspension. One drop of the starchethanol suspension was applied on an aluminum stub using double-sided adhesive tape and the starch was coated with gold-palladium (60:40). An accelerating potential of 20 kV was used during micrography.

2.7 Pasting properties

The pasting properties of the starches were evaluated with the Rapid Visco Analyser (RAV-4, Newport Scientific, Warriewood, Australia). Viscosity profiles of different starches were recorded using starch suspensions (6%, w/w; 28 g total weight). A programmed heating and cooling cycle was used, where the samples were held at 50°C for 1 min, heated to 95°C at 12°C/min, held at 95°C for 2.5 min, before cooling from 95 to 50°C at 12°C/min and holding at 50°C for 2 min. Parameters recorded were pasting temperature, peak viscosity, trough viscosity (minimum viscosity at 95°C), final viscosity (viscosity at 50°C), breakdown viscosity (peak-trough viscosity) and setback viscosity (final-trough viscosity).

2.8 Thermal properties

Thermal characteristics of isolated starches were studied by using a DSC (DSC-204 F1, Phoenix, Netzsch, Seib, Germany) equipped with a thermal analysis data station. Starch (3.5 mg, dry weight) was loaded into a 40-µL capacity aluminum pan (ME-27331, Mettler Toledo, Greifensee, Switzerland) and distilled water was added with the help of a Hamilton microsyringe to achieve a starchwater suspension containing 70% water. Samples were hermetically sealed and allowed to stand for 1 h at room temperature before heating in the DSC. The DSC analyzer was calibrated using indium and an empty aluminium pan was used as reference. Sample pans were heated at a rate of 10°C/min from 20 to 100°C. Thermal transitions of starch samples were defined as T_o (onset), T_p (peak of gelatinization) and T_c (conclusion), and ΔH_{gel} was referred to as enthalpy of gelatinization.

2.9 Statistical analysis

The data reported in all the tables were subjected to oneway analysis of variance (ANOVA) using Minitab Statistical Software version 13 (Minitab, Inc., State College, USA).

3 Results and Discussions

3.1 Physicochemical characteristics of starches

Water chestnut starch contained 0.2% of protein, 0.2% ash and 0.1% lipids, thus giving 99.5% pure starch. The moisture content was higher in native potato starch than in native water chestnut and native corn starches (Tab. 1).

The apparent amylose content of the starches was 23.5, 22.3 and 28.4%, respectively, for native corn, water chestnut and potato starches. *Hizukuri* [4] reported almost similar content of apparent amylose in starch of

Tab. 1. Physicochemical characteristics of different starch sources.

Source	Moisture [%]	Amylose	Water binding capacity [%]	Bulk density [g/mL]		Swelling	Solubility
		[%]		Loose	Packed	power [g/g]	[%]
Water chestnut	7.85b	22.3a	88.5a	0.66b	0.96b	9.72a	12.5b
Corn	7.78b	23.5b	98.2b	0.49a	0.68a	11.21b	13.9c
Potato	6.72a	28.4c	106.0c	0.81c	0.98b	21.58c	0.02a

Average of triplicate measurements, n = 3.

Mean values in the same column with different letters are significantly different (P < 0.05).

water chestnut. *Kim* et al. [11] and *Wiesenborn* et al. [12] observed similar content of amylose in starches from American potato cultivars whereas 35 corn landraces showed amylose content in the range of 16.1–23.3% [13].

Water chestnut starch exhibited the lowest water binding capacity of all three starches with potato starch having the highest value. The formation of hydrogen bonds between the hydroxyl groups of different starch chains lowers the water binding capacity [14]. The differences in water binding capacity are probably more likely due to differences in average amylopectin branch chain-length among the starches. Loose association of amylose and amylopectin molecules in the native starch granules has been reported to be responsible for high WBC. The difference in the degree of availability of water binding sites among the starches may also have contributed to the variation in water binding capacity among the different starches [15]. Loose and packed bulk densities of the three starches also differed with corn having the lowest packed bulk density of 0.68, water chestnut having 0.96 and potato the highest at 0.98 g/mL.

Swelling power and solubility can be used to assess the extent of interaction between the starch chains, within the amorphous and crystalline domains of the starch granule [16]. The swelling power in water chestnut was lowest at 9.7 g/g whereas its value for potato starch was found to be highest at 21.6 g/g. A low swelling behavior of water chestnut starch has also been reported earlier [4]. Water chestnut starch exhibited a slightly lower water solubility than corn starch. The swelling power of starch has been reported to depend upon the water holding capacity of starch molecules by hydrogen bonding [17]. Hydrogen bonds stabilizing the structure of the double helices in

crystallites are broken during gelatinization and are replaced by the hydrogen bonds with water, and swelling is regulated by the crystallinity of the starch [18]. Sandhya Rani and Bhattacharaya [19] indicated that starch granules with low amylose content being less rigid, swell freely when heated. However, the starch granules with higher amylose content are better reinforced and thus more rigid.

3.2 Turbidity

Turbidity values for all three starch suspensions increased progressively during storage time (Fig. 1). The increase in turbidity during storage has been attributed to the interaction between leached amylose and amylopectin chains that led to development of junction zones, which reflect or scatter a significant amount of light [10]. Turbidity development in starch pastes during storage have been reported to be affected by factors such as granule swelling, granule remnants, leached amylose and amylopectin, amylose and amylopectin chain lengths [20]. Native potato starch showed lowest turbidity followed by water chestnut, whereas highest turbidity values were recorded for corn starch.

3.3 Syneresis

The syneresis in starch gels from the three different sources is presented in Tab. 2. The syneresis of starches progressively increased with the increase in storage duration of the starch gels from the three sources. Starch gels are metastable and non-equilibrium systems and therefore undergo structural changes during storage [21]. The values obtained after five days showed lower syneresis in

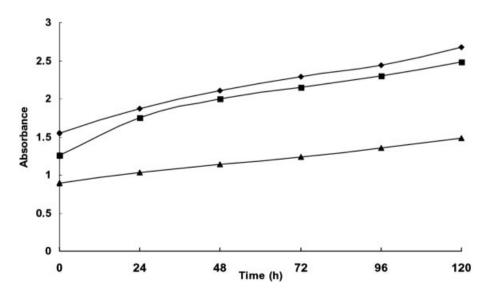


Fig. 1. Effect of storage on turbidity of different starch pastes. Water Chestnut Starch -■-■-, Corn Starch -◆-◆-, Potato Starch -▲-▲-.

Tab. 2. Effect of storage on syneresis of gels prepared from different starch sources.

Source		Syneresis [%]	
	24 h	48 h	120 h
Water chestnut Corn Potato	73.2b 76.5c 41.6a	75.8b 78.5c 53.3a	78.6b 82.3c 61.7a

Average of triplicate measurements, n = 3.

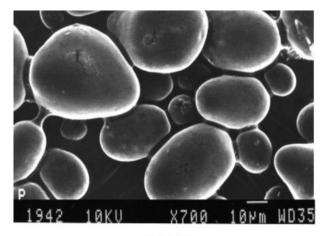
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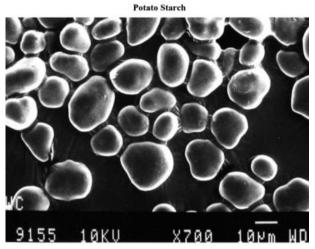
water chestnut starch than in corn starch and potato starch exhibited the lowest syneresis values of all the starch sources. This is because potato starch has phosphomonoester groups with negative charges that repel each other, limiting reorganization of amylopectin molecules that allow for strong gel formation. The increase in syneresis (%) during storage has been attributed to the interaction between leached out amylose and amylopectin chains which led to development of junction zones, which would reflect or scatter a significant amount of light [10]. Amylose aggregation and crystallization has been reported to be complete within the first few hours of storage while amylopectin aggregation and crystallization occurred during later stages [22].

3.4 Morphological properties

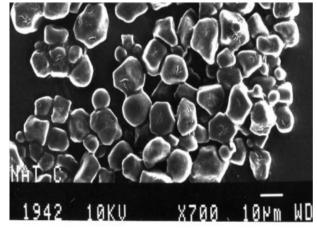
The granular shape, size and morphology of the different starch granules are shown in Fig. 2. The SEMs of the native water chestnut and native potato starches showed similarity in shape whereas a significant difference was observed when compared to corn starch. Results obtained in this study are comparable with earlier morphological studies on water chestnut starch [4]. Water chestnut and potato starch granules were observed to be smooth, oval to irregular or cuboidal, however, native corn starch granules were less smooth, angular and oval to polyhedral in shape. The sizes of the granules present in water chestnut starch ranged from small to medium and that of potato starch from small to large. The diameter of starch granules varied between 5 to 30, 12 to 45 and 3 to 15 μm , respectively, for water chestnut, potato and corn starches.

When viewed under a scanning electron microscope, the surface of the granules in native corn starch showed the presence of slight surface wrinkles or scratches whereas most of water chestnut starch granules were found to be smooth but some had slight wrinkles. In comparison potato starch granules showed relatively smooth surfaces. The difference in granule morphology may be attributed to





Water Chestnut Starch



Corn Starch

Fig. 2. Scanning electron micrographs of native potato, water chestnut and corn starch granules.

the biological origin, biochemistry of the amyloplast and physiology of the plant [23, 24]. *Fannon* and *BeMiller* [25] also observed the presence of wrinkles on the surface of

corn, sorghum and millet starch granules. *Leach* and *Schoch* [26] assumed that these wrinkles or scratches are related to the botanical source of the starch. This also may be due to difference in methods of extraction of starch from raw sources and also storage conditions of starch.

3.5 Pasting properties

The pasting properties of the three different starches are summarized in Tab. 3. The pasting properties of starches have been reported to be influenced by size, rigidity, amylose to amylopectin ratio and swelling power (degree of swelling as well as shear stability) of the granules [27]. A rapid increase in the viscosity of the starches was noted with the increase in the temperature as shown in Fig. 3. Potato starch exhibited the highest peak viscosity (PV) of

8112 mPa s (Tab. 2) followed by water chestnut with 5554 mPa s and corn with 2907 mPa s. The increase in viscosity with temperature may be attributed to the removal of water from the amylose exuded by the granules as they swell [28].

Trough viscosity also followed the same trend. Breakdown viscosity (BV) was observed to be lowest in native corn starch but highest in native water chestnut starch. Final viscosity (FV) was highest in potato starch at 7277 mPa s followed by water chestnut and corn starch at 5624 and 2956 mPa s, respectively. The increase in final viscosity might be due to the aggregation of the amylose molecules [22]. The setback (final viscosity minus trough viscosity) is the viscosity increase resulting from the rearrangement of amylose molecules that have leached from swollen starch granules during cooling, and is generally used as a measure of the gelling ability or retrogradation

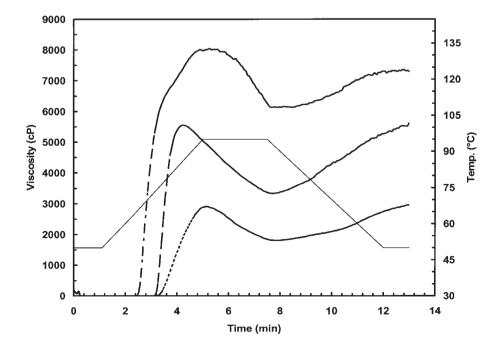
Tab. 3. Pasting properties of different starch sources.

Source	PV [mPa s]	TV [mPa s]	BV [mPa s]	FV [mPa s]	SV [mPa s]	P _{Temp} [°C]
Water chestnut	5554b	3338b	2216c	5624b	2286b	75.05b
Corn	2907a	1807a	1100a	2956a	1149a	76.75b
Potato	8112c	6128c	1984b	7277c	1149a	66.95a

PV, peak viscosity; TV, trough viscosity; BV, breakdown viscosity; FV, final viscosity; SV, setback viscosity; P_{Temp} , pasting temperature.

Average of triplicate measurements, n = 3.

Values in the same column with different letters are significantly different (P < 0.05).



tendency of starch [29]. Water chestnut starch showed the highest setback in viscosity whereas, it was found to be same in corn and potato starches.

The pasting temperature of native potato starch was the lowest (66.9°C), whereas water chestnut and corn starches had higher pasting temperatures (75 and 76.7°C, respectively). Pasting temperature of water chestnut starch has been reported to be 71°C [4]. *Shi-Yung Xu* and *Shoemaker* [5] reported higher gelatinization temperature for water chestnut starch than for potato starch. Three potato cultivars were reported having pasting temperature in the range of 68.2 to 74.4°C [30]. Pasting temperatures in the range of 74.9–84.7°C has been reported for Argentinian corn landraces [13].

3.6 Thermal properties

The gelatinization temperatures (onset, $T_{\rm o}$; peak, $T_{\rm p}$; and conclusion, $T_{\rm c}$) and enthalpy of gelatinization ($\Delta H_{\rm gel}$), for the three starches as measured by DSC are presented in Tab. 4. Water chestnut starch had the highest gelatinization temperatures which suggest that more energy is required to initiate gelatinization. The lowest values for $T_{\rm o}$, $T_{\rm p}$ (onset temperature, peak temperature) were recorded for potato starch whereas corn starch exhibited intermittent values between the other two sources (Fig. 4). Water chestnut starch exhibited the highest $T_{\rm c}$ of 75.1°C, followed by corn and potato starch with 74.4 and 73.6°C, respectively. These values are in agreement with earlier studies on potato starch from American cultivars [11] and with values observed for normal corn starches [31, 32].

Tab. 4. Thermal properties of different starch sources.

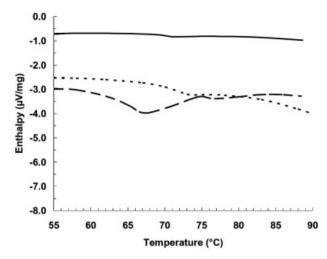
Source	T _o [°C]	T _p [°C]	T _c [°C]	$\Delta H_{\rm gel}$ [J/g]
Water chestnut	69.6±0.6ab	73.3±0.9c	75.2±0.8b	7.3±0.3
Corn Potato	68.7±1.2b 59.9±0.9a	71.6±0.7b 67.7±1.1a	$74.4 \pm 0.5 ab$ $73.7 \pm 0.6 a$	9.4±0.5b 12.5±0.1c

 $T_{\rm o}$ = onset temperature, $T_{\rm p}$ = peak temperature, $T_{\rm c}$ = conclusion temperature, $\Delta H_{\rm gel}$ = enthalpy of gelatinization [dwb, based on starch weight].

Average of triplicate measurements, $n=3,\pm$ means standard deviation.

Values in the same column with different letters are significantly different (P < 0.05).

The gelatinization enthalpy values of starches has been reported to be affected by factors such as granule shape, percentage of large and small granules, and the presence of phosphate esters [33]. $\Delta H_{\rm gel}$ observed for different



starches were 12.5, 9.4 and 7.3 J/g for native potato, corn and water chestnut starches, respectively. The variations in $\Delta H_{\rm gel}$ could represent differences in bonding forces between the double helices that form the amylopectin crystallites, which resulted in different alignment of hydrogen bonds within starch molecules [34]. X-ray diffraction analysis could have helped in assessing gelatinization of water chestnut starch. Earlier studies have suggested a semi-crystalline structure of water chestnut starch, but more detailed study about crystallinity of starch from this particular variety is necessary for the next step.

4 Conclusion

The physicochemical properties of water chestnut starch with corn and potato starch as reference were investigated. Water chestnut starch showed a lower swelling power than the two reference sources which suggests that the amylose is involved in strong mutual association or association with amylopectin. Water chestnut starch showed similarities with corn starch in its differential scanning calorimetry characteristics but had a lower enthalpy of gelatinization than the reference sources. Pasting properties studies by Rapid Visco Analyser presented highest values for breakdown and setback viscosities for water chestnut starch than the starches from other two sources. Having lower syneresis than that corn starch, the water chestnut starch can easily replace the former in preparation of frozen products and other industrial applications. This can make this native starch from non-conventional source an excellent alternative to corn and other conventional starches.

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