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PHYSICO-CHEMICAL CHARACTERIZATION OF SWEET CHESTNUT (*CASTANEA SATIVA* L.) STARCH GROWN IN TEMPERATE CLIMATE OF KASHMIR, INDIA

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Studies were conducted to characterize the chestnut starch for physico-chemical properties. Chemical composition of chestnut starch showed low levels of protein and ash indicating purity of starch. The results revealed low water and oil absorption capacity of chestnut starch. Starch showed high swelling power and low solubility index. Swelling power and solubility index of chestnut starch increased with increase in temperature (50–90 °C). The results revealed high initial, peak, setback, breakdown, and final viscosity but low paste development temperature. Transmittance (%) of the starch gel was low and decreased with increasing storage period. The chestnut starch gel showed increase in % water release (syneresis) with increase in time of storage but was less susceptible to repeated cycles of freezing and thawing. Starch was also characterized for granule morphology. Starch granules were of round and oval shapes, some granules showed irregular shape.

Keywords: chestnut, amylose, syneresis, freeze-thaw, pasting

Chestnut (*Castanea sativa* L.) is species of family "Fagaceae" of flowering plants. The tree and its edible nuts are referred to by several common names, such as "sweet chestnut" or "Marron". Chestnut is a medium to large sized deciduous tree that requires mild climate and adequate moisture for growth and good harvest. They appear in late June–July in the northern hemisphere and by autumn, the female flowers develop into spiny capsules, containing 3–7 brownish nuts that are shed during October. It has been the staple food in Southern Europe, Turkey, South-Western Europe, and Asia Minor from the millennia, largely replacing cereals. Until the development of potato, the whole forest dwelling community relied on chestnuts as a source of carbohydrates. In 1879, it was referred to as "temporary but complete substitution for cereals". During the British colonial rule in the mid-1700s–1947, sweet chestnut was widely introduced in the temperate parts of the Indian subcontinent mainly in the lower and middle Himalayas (Miller, 1968).

Chestnuts are characterized by high level of starch and low levels of fat and protein, but are good sources of total amino acids containing aspartic acid, glutamic acid, leucine, alanine, and arginine. It also comprises of considerable levels of vitamins, fibres, essential fatty acids, and minerals (Borges et al., 2008). There is increasing evidence showing that the consumption of chestnuts has become more important in human nutrition due to the health benefits provided by the presence of bioactive components (Blomhoff et al., 2006).

Starch is the reserve carbohydrate in the plant kingdom generally deposited in the form of minute granules ranging from 1–100 μm in diameter**.** Chemical composition of the chestnut

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reveals starch as the major component. Starch is one of the most important biopolymers used in numerous food and non-food industrial applications (Othaman et al., 2010). The major physicochemical and functional properties of the starch are gelatinization, retro-gradation, solubility, water absorption capacity, syneresis, thermal and rheological behaviour in pastes and gels (Rondan-Sanabria & Finardi-Filho, 2009). These properties are influenced by shape, molecular structure, and botanical source of native starch. Efforts have been made to find the native starches with suitable characteristics for food industry, not requiring chemical or genetic modifications. Study on the new natural starches is essential for their best usage and also to increase the utilization of starchy flours. The aim of the present study was to isolate and characterize the starch from sweet chestnut in order to know its potential applications in food industry.

1. Materials and methods

1.1. Materials

The seeds of sweet chestnut were purchased from a local market in Srinagar, J and K, India. Seeds were dehulled and stored at 5 °C until further use within two days. All the reagents used in the study were of analytical grade.

1.2. Methods

1.2.1. Starch isolation. Starch was isolated according to the method of Wani and co-workers (2014).

1.2.2. Composition. Moisture (925.10), protein (960.10), fat (920.85), and ash (923.03) contents were determined according to the methods of (AOAC, 1990) procedures. Starch was calculated by difference (100–% protein–% fat–% ash).

1.2.3. Apparent amylose content. Apparent amylose contents of the starch samples were determined by the method of Williams and co-workers (1970).

1.2.4. Colour. Colour of the starch was determined using colour flex Spectro colorimeter (Hunter lab colorimeter D-25, Hunter Associates Laboratory, Ruston USA) after being standardized using Hunter lab colour standards and their Hunter *L* (lightness), *a* (redness to greenness) and *b* (yellowness to blueness) values were measured.

1.2.5. Bulk density. Bulk density was measured as a ratio of mass to volume following the method of Wani and co-workers (2014).

1.2.6. Water and oil absorption capacity. Two and a half grams of starch on dry weight basis (db) was mixed with 20 ml distilled water or mustard oil and then stirred for 30 min at 25 ºC. The slurry was then centrifuged at 3000 × *g* for 10 min (5810R, Eppendorf, Hamburg, Germany) and the supernatant was decanted. Gain in weight was expressed as percentage of water/oil absorption capacity.

1.2.7. Swelling and solubility index. Swelling power and solubility index of the starches were determined using 2% db (w/v) aqueous suspension of starch at 50, 60, 70, 80, and 90 °C by the method of Wani and co-workers (2014).

1.2.8. Syneresis. Starch suspensions (6%, w/w db) were heated at 90 °C for 30 min in a water bath (SWB-10L-1-Taiwan) with constant stirring at 75 r.p.m. The starch sample was stored for 0, 24, 48, 72, 90, and 120 h at 4 °C in separate tubes for each day. Syneresis was measured as % amount of water released after centrifugation at 3000×*g* (5810R, Eppendorf, Hamburg, Germany) for 10 min (Wani et al., 2014)**.**

1.2.9. Freeze thaw stability. Freeze thaw stability was determined by the method of Hoover and Ratnayake (2002).

1.2.10. Light transmittance (%). Light transmittance was measured according to the method of Wani and co-workers (2010).

1.2.11. Pasting properties. The pasting properties of the starches were measured using a Rapid Visco analyser (Tech Master, Perten Instruments Pvt Ltd, Australia). An aqueous dispersion of starch – 14% moisture basis (10.7%, w/w; 28 g total weight) was equilibrated at 50 °C for 1 min, heated at the rate of 12.2 °C min^{-1} to 95 °C, held for 2.5 min, cooled to 50 °C at the rate of 11.8 °C min^{-1} and again held at 50 °C for 2 min. A constant paddle rotational speed (160 r.p.m.) was used throughout the entire analysis, except for rapid stirring at 960 r.p.m. for the first 10 s to disperse the sample.

1.2.12. Scanning electron microscopy. The starch granules were placed on an adhesive tape attached to a circular aluminium specimen stub. After coating vertically with gold–palladium, the samples were photographed at an accelerator potential of 5 kV using a scanning electron microscope (Hitachi S-300H-Tokyo, Japan).

2. Results and discussion

2.1. Chemical composition

The moisture content of chestnut starch was 10.62% (Table 1). The low moisture content usually reflects the high stability during storage. The protein (0.32%), lipid (0.00%), and ash contents (0.45%) were less than 1%. The values of ash, lipid, and protein are lower than a previous study on sweet chestnut starch (Demiate et al., 2001). However, the results are comparable with previous studies on other starches (Wani et al., 2010, 2014). Correia and Beirao-da-Costa (2012) reported that the chemical composition of the starch is affected by method of isolation.

Table 1. Chemical composition of chestnut starch (n=3)

Parameter	Value
Moisture (%)	10.62±0.17
Protein (%)	0.32±0.01
Fat (%)	0.00±0.00
Ash (%)	0.45±0.01
Amylose (%)	20.73±0.23

The values in the table are mean ± standard deviations

2.2. Apparent amylose content

The apparent amylose content of the chestnut starch was found to be 20.73% (Table 1). The results are comparable to amylose content of sweet chestnut starch (Demiate et al., 2001) and rice starches (Wani et al., 2013). The amylose content of starch is related to some factors, such as species, climatic conditions, and harvesting period (Copeland et al., 2009). The amylose content of the starch plays an important role in the physico-chemical (syneresis, freeze thaw stability, pasting properties) and functional (swelling power, solubility, water absorption capacity) properties of starch.

2.3. Colour

Isolated chestnut starch appeared to the naked eye as a white powder, presented high values of the lightness *"L"* (96.54), low value of *"a"* (0.89) and *"b"* (6.26) (Table 2). A low value of chroma and high value of lightness has been desired for starch to meet the consumer preferences (Ikegwu et al., 2010).

Table 2. Physico-chemical properties of chestnut starch (n=3)

Physicochemical property	Values
Colour	
L	96.54±0.25
a	0.89±0.15
b	6.26±0.15
Loose bulk density (g ml^{-1})	0.40±0.02
Tapped bulk density (g ml^{-1})	0.73±0.01
Water absorption capacity (g g^{-1})	0.91±0.03
Oil absorption capacity (g g^{-1})	0.66±0.01

Values reported are mean ± standard deviation.

2.4. Bulk density and true density

Bulk density (loose and tapped) of the chestnut starch is given in Table 2. The chestnut starch showed the loose bulk density of the order of 0.40 g ml^{-1} and tapped bulk density of the order of 0.73 g ml^{-1}. Tapped bulk density of native Indian Horse Chestnut starch was 0.86 g ml^{-1} (Wani et al., 2014).

2.5. Water and oil absorption capacity

Chestnut starch presented the water and oil absorption capacities of 0.91 g g^{-1} (91%) and 0.66 g g^{-1} (66%) at 25 °C (Table 2). Shubeena and co-workers (2015) reported water and oil absorption capacity of 2.09 and 2.54 g g^{-1}, respectively, for Indian horse chestnut starch. Low water absorption capacities of the starches may be attributed to the involvement of the large proportion of the hydroxyl groups in the formation of the hydrogen bonds between the starch chains not with the water (Hoover & Sosulski, 1986). Imbibition of water is an important functional trait in the foods such as dough, custards, and sausages (Ikegwu et al., 2010).

Moreover, oil absorption capacity is useful in structure interaction in the food, especially in flavour retention, improvement of palatability, and extension of shelf life, particularly in bakery or meat products (Adebowale & Lawal, 2004). The ability of the food to absorb water and oil may help to enhance the sensory properties such as flavour retention and mouth feel.

2.6. Swelling power and solubility index

Swelling power and solubility index of the chestnut starch was studied over a temperature range of 50 °C to 90 °C. The swelling power of the chestnut starch increased steeply over the studied range of temperature. The swelling power of chestnut starch showed a rapid increase from 50 °C (2.41 g g^{-1}) to 90 °C (19.35 g g^{-1}) (Table 3). These results are higher than previous studies on sweet chestnut starch (Correia et al., 2012a). The lower swelling power found in the previous study than in the present study may be due to higher amylose content of samples in the previous study. Bello-Perez and co-workers (2002) reported that the swelling is due to the breaking of the intermolecular hydrogen bonds in the amorphous region of the granule that allows irreversible and progressive water absorption. Swelling power has been found to be positively correlated with gelatinization temperature, amylopectin chain length, and amylose content (Singh et al., 2003)

The solubility of the chestnut starch was found to increase in the range of 0.05–8.4% with the increase in temperature from 50–90 °C (Table 3). The results are comparable to previous study on sweet chestnut starch (Correia et al., 2012a). However, it was lower than rice starch (Wani et al., 2013). Solubility has been reported to be mainly due to leaching of the amylose from the amorphous part of the swollen granules, and the solubility mainly depends on lipid content of the starch and ability of the starch to form the amylose lipid complex, because amylose lipid complexes are insoluble in water and require higher temperature to dissociate (Morrison, 1988). The swelling power and solubility index provides the evidence of the magnitude of interaction between the starch chains within the amorphous and crystalline domains (Singh et al., 2003). The extent of this interaction is influenced by amylose to amylopectin ratio in terms of molecular weight/distribution, degree and length of branching and conformation.

Table 3. Swelling power and solubility index of chestnut starch (n=3)

Temperature (°C)	Swelling power (g g^{-1})	Solubility index (%)
50	2.41±0.30	0.05±0.01
60	3.26±0.19	0.55±0.1
70	9.29±0.28	1.60±0.16
80	13.21±0.47	5.05±0.19
90	19.35±0.22	8.40±0.28

Values reported are mean ± standard deviation

2.7. Syneresis

The results obtained indicate the increase in % syneresis of chestnut starch gels with increase of storage period. Syneresis of starch gels was 26.34% after 120th h of refrigeration (Table 4). The increase in the syneresis has been attributed to the interaction between leached amylose

and amylopectin chains leading to the development of junction zones (Perera & Hoover, 1999). Syneresis characterizes the stability of starch during storage and is also an index of starch retrogradation degree at low temperature (Wang et al., 2010). Starch gels are meta-stable and non-equilibrium systems and tend to show structural changes during storage (Ferrero et al., 1994). The phenomenon of the syneresis is undesired for use of starch in food applications and especially in foods that are refrigerated.

Table 4. Syneresis and freeze-thaw stability of chestnut starch pastes (n=3)

Time	Syneresis (% water release)	Freeze-thaw stability (% water release)	
0 h	0.00±0.00	0 thaw	0.00±0.00
24 h	6.27±0.22	1 thaw	7.13±0.13
48 h	9.73±0.70	2 thaw	9.95±0.77
72 h	18.89±0.36	3 thaw	11.98±0.60
96 h	22.47±0.47	4 thaw	20.92±0.79
120 h	26.34±0.34	5 thaw	23.93±0.05

Values reported are mean ± standard deviation

2.8. Freeze-thaw stability

Freeze-thaw stability of the chestnut starch was studied and results are given in Table 4. Results indicate increase in the % water release with increase of the storage time. Chestnut starch gels showed less separation of water after the first cycle of freezing and thawing. Chestnut starch gels released 23.93% water after the 5th cycle of freezing and thawing. The results are lower than reported in earlier studies on sweet chestnut starch (Demiate et al., 2001) but are comparable with studies on Indian horse chestnut starch (Wani et al., 2013). When the starch gels are frozen, phase separation occurs due to formation of ice crystals. Syneresis in the freeze thawed gel is due to the increase of the molecular association between the starch chains, in particular retrogradation of amylose, expelling water from gel structure.

2.9. Transmittance

Chestnut starch gel had low transmittance value of 2.3% at 0 h and it decreased progressively to 0.78 after 120 h of storage at 4 °C (Fig. 1). The decreases in percent transmittance of the gel with an increase of storage may be due to the retrogradation of starch. In chestnut starch the decrease in transmittance was sharp up to second day, after which the decrease was small. Perera and Hoover (1999) reported that increase in the turbidity was mainly due to rapid formation of double helical junction zones upon cooling, resulting from continued interaction between the leached amylose-amylopectin chains through hydrogen bonding.

2.10. Pasting properties

The chestnut starch had a peak viscosity of 3670.0 cP (Table 5). Peak viscosity is the ability of the starch granules to swell freely before their physical breakdown (Ikegwu et al., 2010). High peak viscosity may be attributed to unrestricted swelling of starch granules. Peak

viscosity has been reported to be influenced by the extent of amylose leaching, amylose lipid complex formation, friction between swollen granules, granule swelling, and competition between leached amylose and remaining un-gelatinized granules for free water (Liu et al., 1997). Higher peak viscosity in comparison with commercial corn and potato starches may be advantageous for their applications as a thickening agent in food systems (Wani et al., 2014).

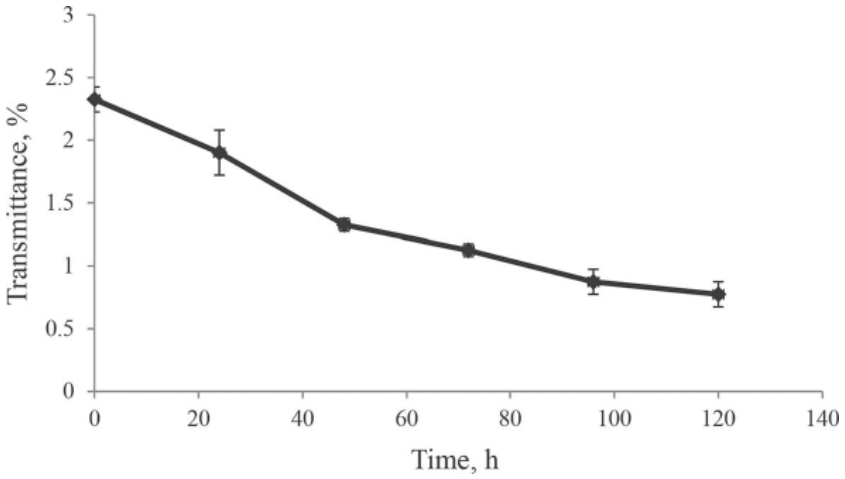

Fig. 1. Transmittance (%) of chestnut starch (n=3)

The trough viscosity, which is the minimum viscosity value in the constant temperature phase of RVA profile and measures the ability of the paste to withstand breakdown during cooling, is 1937.5 cP for the chestnut starch. The breakdown viscosity of chestnut starch is 1838 cP, and is regarded as measure of degree of disintegration of granules, and also shows the paste stability. The final viscosity of the chestnut starch is 4024.5 cP. Final viscosity is used to define the particular quality of starch and indicates the stability of the cooked paste in the actual use; it also indicates the ability to form various pastes or gels after cooling and less stability of the starch paste commonly accompanied with high value of breakdown (Ikegwu et al., 2010). The setback viscosity of the chestnut starch is 2207.5 cP. Setback viscosity is the measure of degree of re-association during cooling among starch molecules involving amylose (Charles, 2004) leached from the swollen starch granules, and is generally used as a measure of gelling ability or retrogradation tendency of starch (Singh et al., 2009). The setback value of the chestnut starch was found to be higher than the normal corn starch (2156.7 cP) but less than the *Brachystegia eurycoma* starch (2335.8 cP) (Ikegwu et al., 2010).

Pasting temperature of the chestnut starch was reported to be 70.73 °C. The result of the pasting temperature is in accordance to the encountered swelling power behaviour of this starch. Generally, high gelatinization temperatures indicate a high resistance to swelling.

Table 5. Pasting and morphological properties of sweet chestnut starch (n=3 for pasting and 25 for morphological properties)

Parameter	Values
Pasting properties	
Peak viscosity (cP)	3670.00±8.48
Trough viscosity (cP)	1937.50±21.9
Breakdown viscosity (cP)	1838.00±162.63
Final viscosity (cP)	4024.50±40.30
Setback viscosity (cP)	2207.50±87.5
Pasting temperature (˚C)	70.67±0.45
Morphological properties	
Average length (μm)	13.26±4.97
Length range (μm)	4.88-21.96
Average width (μm)	8.91±3.28
Width range (μm)	2.44-14.64

The values in the table are mean ± standard deviations

2.11. Granular structure

The SEM micrographs of the starch granules showed round and oval shapes with smooth surfaces (Fig. 2). Mean granule length, length range, mean granule width and width range of chestnut starch was 13.257±4.974 μm, 4.88–21.96 μm, 8.91±3.28 μm, and 2.44–14.64 μm, respectively (Table 5). Furthermore, the chestnut starch consisted of the small to medium size granules. Correia and co-workers (2012b) reported round and oval shaped starch granules with mean size range of 9.0–13.0 μm.

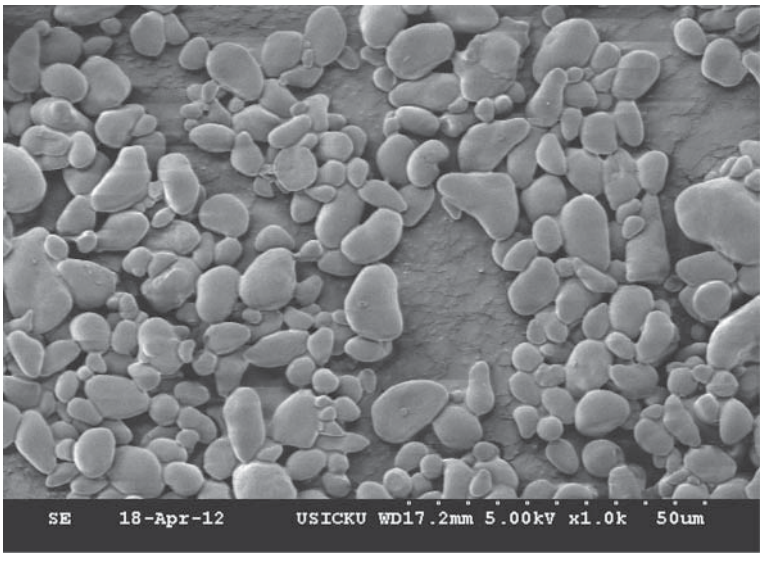

Fig. 2. Scanning electron micrograph of sweet chestnut starch

3. Conclusions

It can be concluded that chestnut starch showed similar characteristics to that of cereal starches with high swelling power and lesser tendency to retrogradate during freezing and refrigeration conditions. So it can be used as an ingredient in various food formulations, where low temperature is required. Further, its pasting properties reveal its use as a stabilizer and thickening agent in cooked paste. However, its high cost and low availability in the market may be limiting factor for its commercialization.

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