# Extraction of Starch from Differently Treated Horse Chestnut Slices

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## ABSTRACT
Starch was isolated from dehydrated Horse Chestnut slices dried at temperature of 50, 60, 70, 80 and 90°C and rehydrated chips (dried at 50 and 60°C) at rehydration temperature of 25 and 40°C. Optimization was done on the basis of starch yield. The highest yield was found in the sample dried at 50°C. Physicochemical properties of optimized starch were determined. Color values indicate that the starch was light in color with L value of 96.2. The starch was having a neutral pH with zero carboxyl content. The bulk density value was 0.85g/ml and sediment value was 36ml. Light transmittance showed a decreased trend with increased storage period of 120hrs. Syneresis and freeze thaw values were increased from 0 to 3.24 % and 0 to 20.21 % with storage period.

## General Terms
Horse Chestnut starch, centrifuge, spectrophotometer, grinder

## Keywords
Horse Chestnut starch, Bulk density, Sediment value, Paste clarity, Color, Syneresis

## 1. INTRODUCTION
Indian Horse Chestnut or Himalayan Chestnut (*Aesculus indica*), locally known as *Han dun* is a large deciduous tree found in moist and shady ravines of Jammu and Kashmir, Himachal Pradesh and Uttar Pradesh [1, 2] yielding large number of seeds, which ripen in October [3]. The seeds are about 3.5 cm in diameter with a hard shining black rind from outside and lime white cotyledons inside [4]. The seeds possess edible uses. In Himachal Pradesh, seeds with reduced level of bitterness after being kept in running water are dried and ground into flour called Tatttwakhar, used for making *Halwa* (porridge) and also mixed with wheat flour to make *chapattis* [5]. During famine times, the seeds have been used as food by various tribes of North and North Eastern India [6]. The seeds constitute about 50.5% moisture, 5.85% sugar, 0.39% protein, 1.93% ash [4] and about 38.3% starch on dry weight basis [7]. Due to this satisfactory starch content, Horse Chestnut seeds can prove as an inexpensive non-conventional source of starch.

The utilization of starch as additive in various industrial applications has stimulated the development of various extraction methodologies with high purity and well defined physical and functional properties. Important advances have been made during the last decades in the development of methods for starch extraction. However, the successful characterization of starch mainly depends upon the purity of the isolated starch. A good representative starch sample must contain >96 % (w/w) pure starch and be devoid of other plant components, such as fibre (soluble and insoluble), protein, and lipids.

The objective of the present study was to standardize the extraction of starch from dried and rehydrated Horse Chestnut slices.

## 2. MATERIALS AND METHODS

### 2.1 Materials
Indian Horse Chestnut seeds were harvested from trees located in rural areas of Anantnag, J and K, India during the month of October, 2013. The seeds were washed with water, oven dried at 60°C to remove excess moisture and stored at 5° C until further use. All the reagents and chemicals used in the study were of analytical grade and were obtained from M/s. Loba Chemie Pvt. Ltd, Mumbai (India).

### 2.2 Sample preparation
Seeds were peeled manually and the kernels were cut into slices of 4 ± 0.4 mm. The slices were dipped into water containing 0.5% KMS and 1.0% citric acid for 30 min [8]. The slices were then dried at temperature of 50, 60, 70, 80, and 90°C and the sample dried at 50 and 60°C was rehydrated at 25 and 40°C. All the prepared slices were then taken for starch extraction.

### 2.3 Starch isolation
Starch was extracted from dried and rehydrated Horse Chestnut slices, using alkaline steeping method [9]. Dried and rehydrated Horse Chestnut slices were steeped in 0.25 % NaOH solution (w/v) with ratio of 1:3 and stored at 4°C for 6 hrs. The steeped chips were ground along with alkali with a laboratory grinder and filtered through 100 mesh sieve, kept for settlement followed by 2-3 washings with distilled water. The slurry was again filtered through 300 mesh sieve and then centrifuged at 3000 rpm for 15 min. The aqueous phase obtained on centrifugation was discarded and the upper non-white layer was scraped off. The white starch layer was re-suspended in distilled water and centrifuged 2-3 times. The starch was then collected and dried in hot air oven at 40°C.

### 2.4 Physicochemical properties of starch
*2.4.1 Yield and composition*

Optimization was done on the basis of yield and the sample with highest yield was taken for further analysis. Moisture content, crude protein, fat, and ash content of the purified starch was determined [10].

### *2.4.2 Color*

Color measurements of HCN starch was carried out in triplicates, using Color Flex Spectrocolorimeter (Hunter Lab Colorimeter D-25, Hunter Associates Laboratory, Ruston, USA), and results were expressed in terms of $L^*$, $a^*$ and $b^*$ values. The $L^*$ value indicates the lightness/ darkness of the sample, 0–100 representing dark to light. The $a^*$ value gives the degree of redness/greenness of the sample, with a higher positive $a^*$ value indicating more red. The $b^*$ value indicates the degree of yellowness/blueness of the sample, with a higher positive $b^*$ value indicating more yellow. The functions chroma [$C^* = (a^{*2} + b^{*2})^{1/2}$] and hue angle [$h^* = \tan^{-1}(b^*/ a^*)$] were also calculated.

### *2.4.3 pH of starch suspension*

The pH of each starch suspension was determined using a digital pH meter (Hanna, USA). Starch samples for pH measurements were prepared by suspending 1 g of starch in 25 mL of water at 25°C and agitating for 5-10 min [11].

### *2.4.4 Carboxyl content*

The carboxyl content of HCN starch was determined by the standard method [12].

### *2.4.5 Bulk density*

Bulk density of HCN starch was measured by the method of Wani, et al. (2013). Starch sample was gently transferred into 10ml previously weighed graduated cylinder. The sample was then packed by gently tapping the cylinder on a laboratory bench several times until no further diminution of the sample level was observed after it was filled up to the mark. The weight of the filled cylinder was taken, and bulk density was calculated as weight of the sample per unit volume (g/mL). The measurements were made in triplicates [13].

### *2.4.6 Paste clarity*

The clarity (% transmittance at 650 nm) of starch paste was determined with slight modification to the method described by Sandhu and Singh (2007). A 1% aqueous suspension of starch adjusted to pH 7.0 was heated in boiling water bath for 30 min with intermittent shaking. After that the suspension was cooled down to 25°C. The light transmittance was read at 650 nm against water blank [14].

### *2.4.7 Sediment volume*

Sediment volume of HCN starch was determined with slight modification to the method of Tessler (1978). Starch (1g, dry basis) was weighed into beaker and 95 ml of distilled water was added. The pH of the starch slurry was then adjusted to 7.0 using 5% NaOH or 5% HCl following which the slurry was cooked in a boiling water bath for 15 min. The mixture was then stirred thoroughly and transferred to a 100 ml graduated cylinder, the volume was made up to 100ml with distilled water. The cylinder was then sealed and kept at room temperature for 24 hrs for settlement of starch granules. The volume of the sediment consisting of starch granules was then measured for sediment volume [15].

### *2.4.8 Light transmittance*

Transmittance of HCN starch was measured according to the method followed by Craig, et al. (1989). A 1% aqueous suspension of starch sample was heated in a boiling water bath for 1 h with constant stirring. The suspension was then cooled for 1 h at 30°C. The samples were stored at 4°C in a refrigerator and transmittance was determined after storage period of 0, 24, 48, 72, 96 and 120h at 640 nm against water blank with a UV spectrophotometer (Shimadzu UV-1601, Japan) [16].

### *2.4.9 Syneresis*

Syneresis of HCN starch was determined by the modified method of Wani, et al. (2010). 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 rpm. The starch sample was stored for 0, 24, 48, 72, 96, and 120 h at 4°C in separate tubes for each day. Syneresis was measured as % amount of water released after centrifugation at 3000x g for 10 min (5810R, Eppendorf, Hamburg, Germany) [17].

### *2.4.10 Freeze thaw stability*

Freeze thaw stability of HCN starch was determined by the method of Hoover and Ratnayake (2002). Aqueous starch slurry (6%, w/v db) was heated in a water bath (SWB-10L-1-Taiwan) at a temperature of 90°C, for 30 min. The gels were subject to cold storage at 4°C for 16hr and then frozen at -16 °C. To measure freeze thaw stability, the gels frozen at -16 °C for 24 h, were thawed at 25°C for 6 h and then refrozen at -16°C. Five cycles of freeze thaw were performed. The tubes were centrifuged at 1000x g for 20 min at 10°C and the released water was measured as freeze thaw stability [18].

### *2.4.11 Statistical analysis*

he reported data in tables are an average of triplicate observations and were subjected to statistical analysis using Statistica-log software package version 7 (StatSoft Inc., OK, USA).

# 3. RESULTS AND DISCUSSION

## 3.1 Physicochemical properties

### *3.1.1 Proximate composition*

Highest yield starch was extracted from chips dried at 50°C as shown in Table 1. The proximate analysis of HCN starch revealed 10.97 ± 0.23% moisture, 0.31 ± 0.14% protein, 0% fat and 0.29 ± 0.9% ash. The physicochemical properties of native starch are shown in Table 2.

**Table 1. Yield of starch extracted from various treated HCN slices**

| Starch | Yield (%) |
|---|---|
| Sample dehydrated at 50°C (D1) | 28±1.4 |
| Sample dehydrated at 60°C (D2) | 20±1.32 |
| Sample dehydrated at 70°C (D3) | 15±1.54 |
| Sample dehydrated at 80°C (D4) | 10±1.37 |
| Sample dehydrated at 90°C (D5) | 5±0.64 |
| D1sample rehydrated at 25°C (25D1) | 9.24±1.2 |
| D1 sample rehydrated at 40°C (40 D1) | 8.09±1.03 |
| D2 sample rehydrated at 25°C (25 D2) | 8.49±0.95 |
| D2 sample rehydrated at 40°C (40 D2) | 6.25±0.87 |

The values are means ± standard deviation of three replicates.

### *3.1.2 Color measurement*

As shown in Table 2, a high degree of whiteness was observed for HCN starch with $L^*$ value of 96.2. Boundries et al., (2009) have concluded that $L^*$ values greater than 90 give a satisfactory whiteness for starch purity [19]. The chroma ($c^\circ$) and hue ($h^\circ$) values of starch sample was 96.23 and 84.30, respectively.

**Tab. 2. Physicochemical properties of HCN starch**

| Parameters | HCN starch |
|---|---|
| *Color values* | |
| L | 96.2±0.02 |
| A | -2.43±0.02 |
| B | 2.77±0.04 |
| $c^*$ | 96.23 ± 0.03 |
| $h^\circ$ | 84.30 |
| Yield (%) | 28±1.02 |
| Amylose (%) | 26.10±0.51 |
| pH | 7±0.11 |
| Carboxyl content (%) | 0 |
| Bulk density (g/mL) | 0.85±.025 |
| Sediment value (ml) | 36±0.34 |
| Paste clarity (A) | 0.832±0.14 |

The values are means ± standard deviation of three replicates

### *3.1.3 pH of starch suspension*

The pH of HCN starch suspension is shown in Table 2. The starch suspension is having a neutral pH and any modification done to the starch results in increase or decrease in pH value. Results have reported decreased pH value due to the breakdown of starch molecules [11], which presumably induce COOH formation that could be responsible for increase in acidity of the modified starch.

### *3.1.4 Carboxyl content*

The carboxyl content of native starch is listed in Table 2. The carboxyl content value is zero due to the neutral pH of the starch. Any modification involving use of chemical treatment results in increase in carboxyl content. Reports on the increase in carboxyl content after the oxidative treatment has been discussed by different researchers [20, 21]. Acids like formic, pyruvic, acetic, glucouronic etc., are said to be responsible for the elevation in carboxyl content [22].

### *3.1.5 Bulk density*

Bulk density (BD) is a reflection of the load a sample can carry if allowed to rest directly on another. The bulk density of native starch as presented in Table. 2, was 0.85 g/mL. Bulk density is generally affected by the particle size and density of powder. However, smaller the particle size, the more is the resistance for flow of powders. The high bulk density of material suggests their suitability for use in food preparations. In contrast, low BD would be an advantage in the formulation of complementary foods [23].

### *3.1.6 Paste clarity*

Paste clarity of starch suspension is presented in Table 2. The paste clarity of the suspension is because of variation in water penetration and absorption of the starch granules, which ultimately led to difference in swelling of starch and resulting in more or less transmittance of light [16]. Bhandari and Singhal (2002) observed in amaranth starch that the starch paste clarity is directly affected by degree of swelling of starch [24].

### *3.1.7 Sediment volume*

The sediment volume of HCN starch is 36ml as presented in Table 2. HCN starch is having highest sediment volume and upon modification of any starch sample there is decrease in sediment volume. The decreased values in modified starch are due to the disruption of granules resulting in decreased swelling and low volume makeup. Studies were done for acetylated and cross-linked rice starch which reported reduced sedimentation volume, due to decreased interaction between starch molecules by acetyl group, and inhibit swelling by cross-linking [25]. The decreased sediment volume may also be due to large starch granules which caused decrease in bond strength upon heating [26].

### *3.2.1 Light transmittance*

Light transmittance which provides the insight of starch paste when light passes through it can be used to indicate the clarity of starch paste and depends on the level of swollen and non-swollen granule remnants. Transmittance is the fraction of incident light that passes through a sample at a specified wavelength. The percent transmittance (%T) was measured as a function of wavelength for various starch pastes and it was observed that more opaque pastes gave a lower %T [27]. The effect of storage period on percent transmittance of investigated starches is presented in Table 3. The percent transmittance was reduced with increased storage period of the paste. Similar time-dependent reduction in transmittance has been reported for banana starch [28].

### *3.2.2 Syneresis*

As presented in Table 3, the syneresis of pastes increased with storage period and HCN starch paste displayed highest syneresis (0.00-3.24%) during 120 hr of storage. Perera and Hoover (1999) reported that the increase in percentage syneresis during storage is due to the interaction between leached amylose and amylopectin chains, which lead to the development of junction zones and release of water [29]. Amylose aggregation and crystallization have been reported to be completed within the first few hours of storage while amylopectin aggregation and crystallization occurs during later stages [30, 31].

### *3.2.3 Freeze-thaw stability*

Freeze-thaw stability of gelatinized starch paste is presented in Table 3. The lowest freeze-thaw stability (20.21% syneresis) was observed at 5thaw for HCN starch after five freeze thaw cycles. Freeze thaw stability of native starch was in the range of 0 - 20.21%. This may be attributed to degradation of molecular structure of starch and formation of smaller fragments which retain more water. The amount of water separated from the gels increased with storage time. Baker and Duarte (1998) have also reported low freeze–thaw gel stability for corn and amaranth starches [32]. Likewise, Bello-Perez et al., (1999) have found low gel stability in the same process for banana starch [33]. Low gel stability under these conditions suggests it is not convenient for use in food systems involving refrigeration or freezing processes.

**Tab. 3. Light transmittance, syneresis and freeze thaw stability of HCN starch**

| Parameter | HCN starch |
|---|---|
| *Light transmittance* | |
| 0h | 5.8±1.7 |
| 24h | 3.2±1.2 |
| 48h | 2.1±0.9 |
| 72h | 1.5±1.3 |
| 96h | 1.2±1.0 |
| 120h | 0.9±1.5 |
| *Syneresis* | |
| 0h | 0 |
| 24h | 0.24±0.5 |
| 48h | 0.67±1.2 |
| 72h | 2.1±0.6 |
| 96h | 2.43±0.9 |
| 120h | 3.24±1.4 |
| *Freeze thaw stability* | |
| 0thaw | 0 |
| 1thaw | 2.04±1.3 |
| 2thaw | 10.28±0.9 |
| 3thaw | 15.66±0.7 |
| 4thaw | 18.61±1.1 |
| 5thaw | 20.21±0.8 |

The values are means ± standard deviation of three replicates.

# 4. CONCLUSION

Horse Chestnuts having high content of starch, which presently go waste can have a great potential to be used in food industry either for the purpose of formulating newer products or can be used for replacement of conventional starches in food products. The Physicochemical, pasting, thermal and rheological properties of Horse Chestnut starch showed significant difference when compared with sweet potato, water chestnut, corn and mango kernel starches. The isolated starch showed ash, protein, lipid as 0.29%, 0.31%, and 0%, respectively with a yield of 28%. This indicates the process for starch extraction has been well carried out with minimum impurities and high yield. The characteristics of native HCN starch displayed high light transmittance and low syneresis. Thus, the starch can be used in food products which need refrigeration. However, the starch can be modified either physically by heat moisture or chemically by acid/alkali and the modified starch having varied properties can be utilized for processing of various industrial products both in food and non-food areas. The analysis of physicochemical properties of starch provides valuable information associated with the functional properties of starch. There is thus, a need for detailed investigation of Horse Chestnut to gather more information related to various properties of starch.

# 5. ACKNOWLEDGMENTS

The authors are grateful to Maulana Azad National Fellowship (MANF) for providing financial assistance in the form of Junior Research Fellowship.

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