# DETERMINATION OF STRUCTURAL CHARACTERISTICS OF STARCH EXCTRACTED FROM SEEDS OF HERITIERA LITTORALIS DRYAND

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***Abstract:*** Starch was isolated from the seeds of *Heritiera littoralis* and further used to study the various properties. In addition, influence of structural features on thermal properties of starch observed. Starch characteristics were examined using polarized light microscopy (Photomicrography), Scanning electron microscopy (SEM), Fourier transform infrared (FT-IR) spectroscopy, X-ray diffraction (XRD) pattern, and Differential scanning calorimetry (DSC). The isolated starch was found to be creamy, crystalline, non-hygroscopic powder with yield of about 31.5%. Starch granules were found to be an oval to polygonal in shape with variable size (1-5µm). The isolated starch showed indicative of an intact granule structure. Isolated starch had the C-type XRD pattern. The enthalpy of gelatinization (ΔHgel) and percentage of retrogradation (%R) for this starch were 11.053 J/g and 83.51% respectively, which may related to XRD pattern and small granule size. Therefore, the present attempt was made to explore this starch is an important biomaterial. Knowledge of molecular structure can suggest an application of the starch in products to improve their functional characteristics, so structural features of starch were studied.

*Keywords: Heritiera littoralis, starch, thermal, XRD, DSC*

## I. INTRODUCTION:

Starch is one of the most widely present natural polymers. It has major economic importance. Starch is found mainly in the storage organs of plants in the form of granules. It is synthesized in semi-crystalline granular structure in grains, roots, tubers, leaves and fruits. The morphology and the structure of the starch granules depend on the botanical sources (Jane *et al.*, 1994), the organs of the plants (Vrinten and Nakamura 2000, Li *et al.*, 2007), and the stages of development (Briones *et al.*, 1968, Li *et al.*, 2007). Starch can be readily converted chemically and biologically into many useful and diverse products such as paper, textiles, adhesive beverages, confectionaries, pharmaceutics and plastics (Agbo and Odo, 2010).

Molecular and structural characteristics of starches have great importance to understand possible applications of these polymers in diverse system. Many different techniques can be used to elucidate these characteristics and these include X-ray diffraction (XRD), which measures level of crystallinity; Fourier transform infrared (FT-IR) microscopy, used to determine the short-range order in a sample while Differential scanning calorimetry (DSC), is used to monitor changes occurring upon heating to the bulk of the sample.

Mangrove trees have a variety of morphological, physiological and reproductive adaptations in common that enable them to grow in a tropical and subtropical coastline marine environment. *Heritiera littoralis* Dryand (Sterculiaceae) is prominent mangrove and it has various applications in traditional folk medicine. A tree is a slow-growing, branched, evergreen with a wide, dense crown of thick, horizontal branches. The species is more at risk along the west coast of Maharashtra because of coastal development, extraction at the extremes of their distribution and due to the habitat conversion. The beautiful tree is to be seen in the forest of mangroves, commonly known as 'Sundari'. Some-times, this tree has found solitary or in the group around sandy areas. The fruit of *H. littoralis* is egg shaped, woody with a ridge along the centre of one side so that they resemble boats with a sail. Pale green initially after ripening glossy brown

in colour and consists of 1-2 seeds. Large crabs, monkeys and wild boar eat the seeds. The fruits and seeds are used in treating diarrhoea and dysentery (Tewtrakul *et al.,* 2010).

In recent years, non-conventional starches have become of increasing importance because of their potential application in the development of new products. In this sense, the seed starch of *H. littoralis* has been studied to determine structural properties. However, no studies concerning the molecular as well as structural characterization have been reported to date. Therefore, the present work highlights the structural properties of seed starch of *H. littoralis* by using different techniques.

## II. MATERIAL:

**2.1Collection:** Fruits were collected from west coast of Maharashtra (Sindhudurg District). For this, collections were made by visiting the sites season to season as and when needed. Only the fruits that have fallen onto the ground were collected and used for analytical work.

**2.2 Sample Preparation:** Fruits were collected by locating the species after visiting the sites on west coast of Maharashtra and brought to the laboratory for further analysis. The seeds were washed and air-dried, then ground to a fine powder. Powder was stored in airtight containers prior to further analysis.

## III. METHODS:

**3.1 Starch Isolation:** Starch was isolated from seeds of *H. littoralis* following the method of Isao *et al.,* (2004) with modifications. Seeds were cut into small pieces and pulverized with an electric mill. The pulverized powder was combined with 5 vol. of cold water and then homogenized. The suspension was allowed to settle down overnight at 4°C. After decantation, the settled granules were re-suspended in cold water. The starch granules were then collected by centrifugation and partially purified by treating with acetone. Defatted starch was prepared by replicate dissolutions in dimethyl sulfoxide (DMSO) and precipitations with ethanol. Starch granules were allowed to repeat washing of 70% ethanol to attain maximum purity.

**3.2 Estimation of starch:** The extracted starch content was estimated by the method of Benesi *et al.,* (2004). The estimated starch was measured as follows:

**Starch content = Weight of isolated starch / Weight of dry powder × 100**

### 3.3 CONFIRMATION OF STARCH:

**Starch- $I_2$KI Test:** Isolated starch was confirmed by potassium iodide ($I_2$KI) test, which is given by Daniel (1954). 1g starch sample was mixed with 5 ml of distilled water in a test tube. The mixture was heated in boiling water bath for 2-3 min. After heating, it was cooled and settles the starch powder for 6-8 hours. Neutralized the solution with 0.1% HCL drop by drop and then phenolphthalein indicator was added one drop extra. The solution was mixed properly and then 0.2% $I_2$KI solution was added drop by drop until the blue colour develops.

### 3.4 GRANULE MORPHOLOGY:

**Photomicrography:** Photomicrography of each starch was done by using the Biological Research Microscope (LYNX- LM-52-1803).

**Scanning Electron Microscopy (SEM):** SEM analysis was done by using JEOL JSM-6360A Analytical Scanning Electron Microscope**.**

**Granule Size analysis:** SEM images were used for determination of size of starch granules by using TS View version 7.3.1.7 software.

### 3.5 CHARACTERIZATION OF ISOLATED STARCH:

**Thermal Properties of isolated starch:**

The thermal characteristics of isolated starches were studied by using a differential scanning calorimeter (DSC, model SDT Q600 V20.9 Build 20), equipped with a thermal analysis data station. Starch (∽3mg dry weight) was loaded into aluminium pan and distilled water was added by micro syringe, to achieve

a starch-water suspension. Samples were thermetically sealed and allowed to stand for 1 h at room temperature before heating in DSC. Empty aluminium pan was used as a reference. Sample pans were heated at a rate of 10°C/min. from 10 to 150°C. Thermal transitions of starch samples were defined as $T_0$ (onset temperature), $T_p$ (peak of gelatinization temperature) and $T_c$ (conclusion temperature) and $\Delta H_{gel}$ referred to the enthalpy of gelatinization. Enthalpies were calculated automatically. The gelatinization temperature range (R) and peak height index (PHI), was calculated as $2(T_p - T_0)$ and $\Delta H/ (T_p - T_0)$, as described by Krueger, Knutson, Inglett and Walker (1987). After conducting thermal analysis, the samples were stored at 4°C for 7 days, for retrogradation studies. The sample pans containing the starches were reheated at the rate of 10 C/min. from 10-150°C after 7 days to measure retrogradation ($\Delta H_{gel}$) were evaluated automatically and percentage of retrogradation (%R) was calculated as,

**% Retrogradation= (enthalpy of retrogradation/enthalpy of gelatinization) X 100**

**Fourier transform infrared spectroscopy (FT-IR) Study:**

In order to determine the structure of both the starches, the FT-IR spectra were obtained using FT-IR (Perkin Emler Spectrum 100). The spectra were recorded in transmission mode from 5,000 to 400 $cm^{-1}$.

**X-ray diffraction (XRD) Analysis:**

X-ray diffraction patterns were analysed using Bruker D8 Advance. The continuous scan was recorded from 2Θ (5-50) on diffractometer.

## IV. RESULTS AND DISCUSSIONS:

The starch obtained from seeds of *H. littoralis* was creamy, crystalline, non-hygroscopic powder with a yield of about 31.5%, which is considered to be appreciable. Isolated starch was further confirmed by $I_2KI$ test and microphotography. Granule microstructure is the inherent feature of botanical starch, which is often the basic underlying factor affecting its physicochemical and functional properties. Cereal starch granules such as maize, oat, and rice have polygonal or round shapes (Blanshard, 1987; Pomeranz, 1985; Hoover, 2001.). Here, also the seeds of *H. littoralis* showed oval and polygonal shaped starch granules (Fig. 1).

SEM images were used for determination of actual size of starch granules and for this TS View version 7.3.1.7 software was used. For present study, granule size was expressed as average length for oval and polygonal shape of starch granules. Average length of starch granules of *H. littoralis* was 1-5µm which showed small granules (Fig. 2).

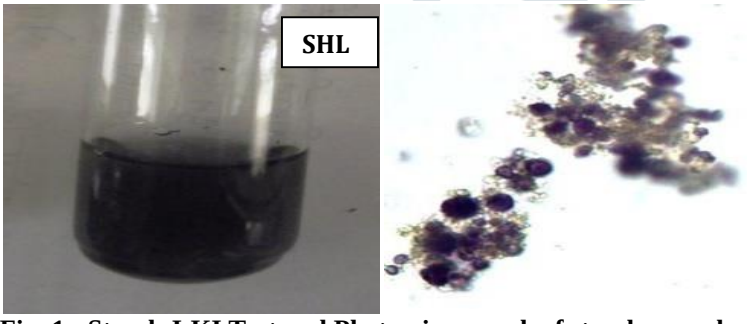


**Fig. 1: Starch-$I_2KI$ Test and Photomicrograph of starch granules of *H. littoralis***

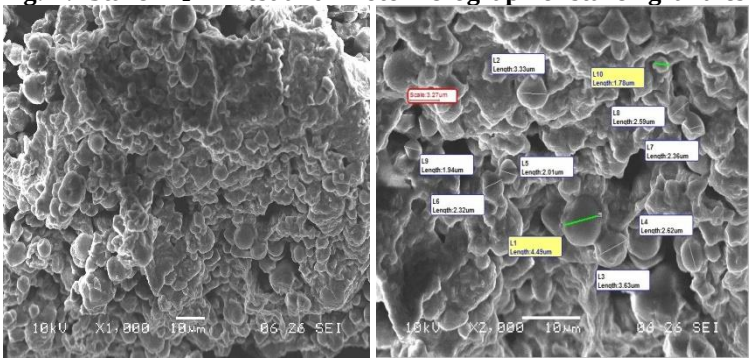

**Fig. 2: SEM images of starch granules of *H. littoralis***

**Thermal properties of starches:**

A thermal property of the starch was measured by Differential scanning calorimetry (DSC). Thermal properties were measured in terms of gelatinization and retrogradation.

**Gelatinization:**

The transition temperatures ($T_0$), range 2($T_p - T_0$), enthalpies of gelatinization ($\Delta H_G$), and peak height indices (PHI) of starch from seeds of *H. littoralis* depicted in table 1. Peak Height Index (PHI), is a parameter, which represents the uniformity of gelatinization. The variation in transition temperatures is related to the granule size, microstructure, proportion, form of crystalline organization and the ratio of amylose and amylopectin (Ahmad *et al.*, 1999 and Singh *et al.*, 2001). Peak Height Index (PHI) is the ratio of $\Delta H_G$ for gelatinization to the gelatinization temperatures range and is a measure of uniformity in gelatinization. The PHI value (0.44) is found lower for starch of *H. littoralis*.

**Table 1: Gelatinization properties of *H. littoralis* starch**

| Sr. No. | Gelatinization Parameters | *H. littoralis* Starch |
|---|---|---|
| 1. | $T_{0G}$ (℃) | 88.84 |
| 2. | $T_{PG}$ (℃) | 114.07 |
| 3. | $T_{CG}$ (℃) | 127.98 |
| 4. | $T_{PG}$ -$T_{0G}$ (℃) | 25.23 |
| 5. | $\Delta H_G$ (J/g) | 11.053 |
| 6. | $R_G$ 2($T_{PG}$ -$T_{0G}$) | 50.46 |
| 7. | PHI | 0.44 |

The transition temperatures observed for isolated starch was higher than those earlier observed for corn, wheat, potato and rice (Singh *et al.*, 2003). Starch of *H. littoralis* with high $T_o$, narrow R, high PHI has small sized granules.

**Retrogradation:**

Retrogradation is the gelatinized, amorphous starch molecules tend to recrystallize in a double helical structure during storage. The retrogradation properties of isolated starch are presented in Table 2. The gelatinization temperature of retrograded starch was higher than that of native starch. This might result from improper alignment of the starch chains during re-association. For starch of *H. littoralis*, percentage of retrogradation was 83.51%. The thermal behavior of starches is much more complex because this is depend upon the moisture content and the water contained in starch is not stable during heating. Retrogradated starch showed lower enthalpy than their native counterparts. The amylopectin and the intermediate materials play a significant role in retrogradation during refrigerated storage. Recrystallization of amylopectin branch chains has been reported to occur in a less ordered manner in stored starch gels than in native starches (Ward *et al.*, 1994). ). Enthalpy of retrogradation ($\Delta H_R$) for isolated starch was found 9.231 J/g, which was found to be higher.

Retrogradation process is very sensitive to temperature (Zeleznak and Hoseney, 1981). The change in crystalline structure after pasting involves the formation of ordered double helical structure from amorphous glucans (Kulik and Haverkamp, 1997). Retrogradation is induced by low temperature, high amylose content and the presence of polar substances, such as salts (Nebesny 1991). The retrogradation properties of isolated starch were studied after storage of gelatinized starches at 4ºC for 7 days. The retrogradation peak of isolated starch was shifted in higher temperature regions.

**Table 2: Retrogradation properties of *H. littoralis* starch**

| Sr. No. | Retrogradation Parameters | *H. littoralis* Starch |
|---|---|---|
| 1. | $T_{0R}$ (℃) | 71.33 |
| 2. | $T_{PR}$ (℃) | 110.12 |
| 3. | $T_{CR}$ (℃) | 136.22 |

| | | |
|---|---|---|
| 4. | $T_{PR}$ -$T_{0R}$ (℃) | 38.79 |
| 5. | $\Delta H_R$ (J/g) | 9.231 |
| 6. | $R_r$ 2($T_{PR}$ -$T_{0R}$) | 77.58 |
| 7. | %R | 83.51 |

$T_{0R}$, onset temperature; $T_{PR}$, peak temperature; $T_{CR}$, conclusion temperature; $\Delta H_R$, enthalpy of Retrogradation; $R_r$ 2($T_{PR}$ -$T_{0R}$), retrogradation range; %R, ratio of enthalpy of retrogradation to enthalpy of gelatinization × 100.

## FOURIER TRANSFORM INFRARED (FT-IR) SPECTROSCOPIC ANALYSIS:

Fourier transform infrared (FT-IR) offers quantitative and qualitative analysis for organic and inorganic samples. FT-IR identifies chemical bonds in a molecule by producing an infrared absorption spectrum. The FT-IR spectrum for isolated starch was shown in figure 3. The information obtained from this technique is related to the short-range order in the starch molecule (Sevenou *et al.*, 2002)

The infrared (IR) spectrum of starch samples was described by seven main modes, with maximum absorbance peaks near 3,500, 3000, 1,600, 1,400, 1,000, 800 and 500 $cm^{-1}$ (Koksel *et al.*, 2008; Sitohy *et al.*, 2000). In present investigation, FT-IR spectra showed similar pattern for the isolated starch, with seven main modes with maximum absorbance peaks near to 3,500, 3000, 1,600, 1,400, 1,000, 800 and 500 $cm^{-1}$. The study was confirmed the observed spectra by FT-IR spectroscopy of starch sample. The peaks at 3398.10 $cm^{-1}$, 2926 $cm^{-1}$ in starch of *H. littoralis* could be attributed to O-H and C-H bond stretching. The ratio between the absorbance obtained at a wavenumber of 1022 $cm^{-1}$, related to the amorphous component (Van *et al.*, 1995) and that obtained at 1045 $cm^{-1}$ related to the ordered component (Smits *et al.*, 1998). The band at 1,023 $cm^{-1}$ in isolated starch was associated with the amorphous structures of starch. The bands at 951∼930 $cm^{-1}$ were attributed to D-glucopyranosyl ring vibrational modes and 766 ± 10 $cm^{-1}$ were attributed to D-glucopyranosyl ring stretching. The isolated starch was slightly less ordered in the external region of the granule.

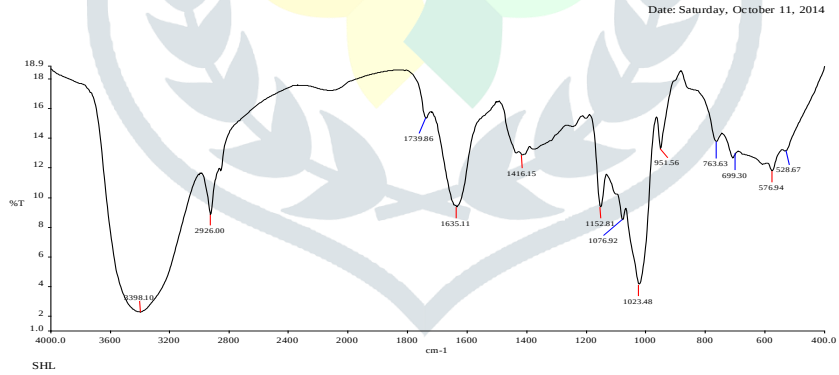


**Fig. 3: FT-IR spectra of isolated starch**

## X-RAY DIFFRACTION (XRD) ANALYSIS:

X-ray diffraction (XRD) provides information on structures, phases, preferred crystal orientations (texture) and other structural parameters, such as average grain size, crystallinity, strain and crystal defects. X-ray diffraction is a very important method for the determination of structure in molecule (Jayraman and Jayaraman, 1974). There are four major types of x-ray diffraction patterns of native starches, A, B, C, and V (Zobel, 1988). XRD analysis of isolated starches was done using Bruker D8 Diffractometer and further calculation was made by using X'pert Highscore plus software version 2.0a (2.0.1). Cu-Kα X-rays of wavelength (λ) =1.54056 Aº (0.1541 nm) and data was taken for the range of 5º to 50º. XRD analysis gave degree of crystallinity of the particles and its structural characterization. In the interpretation of XRD data,

various approaches have been considered. Thus, some studies identified the type of crystallinity by the position of the strong peaks while other studies considered the position of both the strong and weaker peaks. An XRD spectrum of isolated starch was analyzed for type of crystallinity and the peaks observed in XRD spectra are reported in Table 3.

The isolated starch is of C-type. X-ray diffraction pattern (Fig. 4), typically found in native legume starches. In general, legume starches and some tropical tuber starches display the C-type pattern, which represents a mixture of A –and B-type crystallinity within the granule (Spencer and Jane, 1999). The peak observed at a Bragg's angle value of 2θ = 15.4148º was much more prominent than that at 2θ = 17.9256º, and the peak at 2θ = 23.1685º was broader. All these differences indicate that the isolated starch of *H. littoralis* was a mixture of the A- and B-polymorphs.

**Table 3: XRD peak list of *Heritiera littoralis* starch**

| Pos. [°2Th.] | Height [cts] | FWHM [°2Th.] | d-spacing [Å] | Rel. Int. [%] |
|---|---|---|---|---|
| 15.4148 | 2194.63 | 1.7137 | 5.74833 | 73.72 |
| 17.9256 | 2976.80 | 1.8098 | 4.94843 | 100.00 |
| 23.1685 | 2402.00 | 1.5585 | 3.83913 | 80.69 |

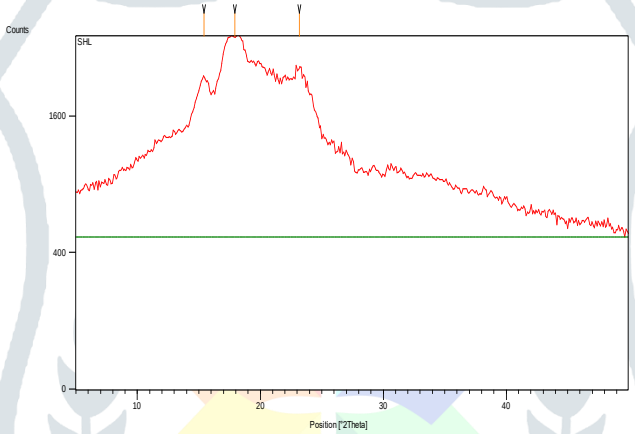


**Fig. 4: X-ray diffraction pattern of isolated starch**

## XRD: Degree of Crystallinity of isolated starches

A starch granule contains densely packed polysaccharides and a small amount of water, being composed of crystalline and amorphous domains. The internal structure of starch is formed by two regions-crystalline and amorphous lamellae, which together form the crystalline and amorphous growth rings. It is a semi-crystalline polymer, in which amylose forms the crystalline region and amylopectin forms the amorphous region (Wang *et al.*, 2005). The main crystalline peaks in the XRD patterns are attributed to the crystalline peaks of starch.

Degree of crystallinity is calculated by using empirical method of Segal *et al.*, (1959) by using following equation,

$$\mathbf{CrI = 100 \times \frac{I_{002} - I_{Amorph}}{I_{002}}}$$

Where, CrI = Degree of crystallinity, $I_{002}$= Maximum intensity of the lattice diffraction and $I_{Amorph}$ = Intensity diffraction at 2θ degrees. It is noted here that, the first 2θ position at 15º was assumed corresponds to the amorphous region. The method that is based on peak intensity has been truly accepted and used to quantify the CrI in many occasions (Holladay, 2006; Fan *et al.*, 1980).

**Table 4: Intensity of XRD peaks of *H. littoralis* starch**

| Peak No. | Position (º2θ) | FWHM (º2θ) | Height (cts) | Relative intensity (%) |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| **1.** | 15.4148 | 1.7137 | 2194.63 | 73.72 |
| **2.** | 17.9256 | 1.8098 | 2976.80 | 100.00 |
| **3.** | 23.1685 | 1.5585 | 2402.00 | 80.69 |

**Degree of crystallinity = 100 × $\frac{100 - 73.72}{100}$ = 26.28%**

For starch of *Heritiera littoralis*, XRD pattern showed degree of crystallinity 26.28%. A general polymer X-ray spectrum will have a broad amorphous peak, and if the polymer has crystallinity, it will show up as sharp peaks on the top of large amorphous peak. The spectrum is the sum of crystalline peaks and an amorphous peak.

**XRD: Morphology Index of isolated starches**

The use of isolated starches as a raw biomaterial is derived from its unique structural, physical and chemical properties. A XRD morphology index (M.I.) is developed from FWHM (full width at half maximum) of XRD data to understand the relation between particle morphology and size (Theivasanthi and Alagar, 2011). M.I. relates the FWHM of two peaks to its particle morphology (peak having highest FWHM and a particular peak's FWHM for which M.I. is calculated).

**Morphology Index (MI) = $FWHM_h$ /( $FWHM_h$ + $FWHM_p$)**

Where, M.I. = Morphology index, $FWHM_h$= highest FWHM value obtained from peaks and $FWHM_p$= value of particular peak's FWHM for which M.I. is to be calculated.

**Table 5: Morphology index of starch of *H. littoralis***

| Peak No. | Position (º2θ) | FWHM (º2θ) | M.I. |
|---|---|---|---|
| **1.** | 15.4148 | 1.7137 | 0.514 |
| **2.** | 17.9256 | 1.8098 | 0.5 |
| **3.** | 23.1685 | 1.5585 | 0.537 |

1) **Morphology Index (MI) = 1.8098/( 1.8098+ 1.7137)**
**= 0.514**
2) **Morphology Index (MI) = 1.8098/( 1.8098+ 1.8098)**
**= 0.5**
3) **Morphology Index (MI) = 1.8098/( 1.8098+ 1.5585)**
**= 0.537**

Starch of *H. littoralis* has M.I. range from 0.5 to 0.537.

The degree of crystallinity for isolated starch was found to be 26.28%. Lower percentage crystallinities were obtained from different varieties of wheat starches and their values ranged between 13 and 18% (Yoo and Jane, 2002). This could be related to the different botanical source of starch but will also be dependent on the method of calculating the percentage crystallinity.

It is generally observed that, M.I. has direct relationship with particle size and an inverse relationship with specific surface area. The observed results of the M.I. confirm the uniformity and fineness of the prepared granules.

## V. CONCLUSIONS:

The isolated starch granules of *H. littoralis* were found to be oval to polygonal in shape. Small sized granules showed prominent structure in light as well as in Scanning electron microscopy, This indicated that the isolation procedure yielded intact granules. Also, the isolated starch was with highest temperature and

enthalpy of gelatinization, a parameter important in starch applications. The highest gelatinization temperatures were found due to agreement with the different XRD pattern, which is mixture of A- and B-type of starches. Isolated starch contained similar amount of short-range order that could influence on some other physicochemical and functional properties. The results obtained in structural characterization provide information about the possible behaviour of the starch when being used in certain applications.

## VI. ACKNOWLEDMENT:

Financial support from UGC, New Delhi is gratefully acknowledged.

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