CHARACTERIZATION OF LOTUS STEM STARCH

A thesis submitted in partial fulfilment of requirements
For the degree of

BACHELOR OF TECHNOLOGY IN

BIOTECHNOLOGY & MEDICAL ENGINEERING

By

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NATIONAL INSTITUTE OF TECHNOLOGY
ROURKELA

CERTIFICATE

This is to certify that project entitled “Characterization of lotus stem starch” submitted by Manish Rout in partial fulfilment of the requirements for the award of the degree of Bachelor of Technology in Biotechnology Engineering at National Institute of Technology, Rourkela is an authentic work carried out by him under my supervision and guidance. To the best of my knowledge the matter embodied in this project report has not been submitted in any college/institute.

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ABSTRACT

Here we have reported the isolation of starch from lotus stem (a novel source) and its physico-chemical and biological characterization. Lotus starch was found poorly soluble in water but solubility increased significantly with an increase in solution temperature. The rheological analysis revealed that viscosity of lotus starch solution was higher than corn starch. XRD and FT-IR studies together confirmed that chemical composition of both compounds is similar. However glucose analysis showed that glucose content was higher in lotus starch. DSC analysis showed that thermal stability of lotus starch was less in comparison to corn starch. Biocompatibility of the samples were examined using HaCaT cells. Result revealed that lotus starch support viability and growth (up to an optimal concentration of 100 μg/ml) of human keratinocytes. The result confirmed that lotus starch can find its potential application in drug delivery and tissue engineering.

CHAPTER 1

INTRODUCTION

1.1 Lotus: an overview

Lotus whose scientific name is *Nelumbonucifera* is a palatable aquatic enduring herb with nutritional value worth fitting in with the monogeneric family *Nelumbonaceae* generally developed all through the world. Lotus stem's involves ash-1.10%, Nitrogen-1.36%, Protein-8.48%, Total Sugars-19.08% and free Amino acids-0.78% [1]. The lotus rhizome was discovered to be a poor source of raw petroleum give or take 2.68%. In the Indian subcontinent lotus plant purportedly develops in all lakes and other water bodies, both at high elevation territories, for example, 1400 m Kashmir, Himalayas, North India and low heights, for example, KaniyaKumari, Southern India.

However, the lotus from the Kashmir valley is of prime significance attributable to its geographic area and the height at which it develops. Starch influences viscosity, texture, gel formation, binding, adhesion, film formation, moisture retention, and product homogeneity. It is utilized fundamentally as a part of soups, sauces, flavors, pastry shop items, dairy, confectionery, snacks, players, coatings and meat items. Non-sustenance utilizations of starch incorporate the zone of pharmaceuticals, adhesives, alcohol-based fuels and textiles. Native starch is a decent composition stabilizer and controller in sustenance systems, yet there are confinements, for example, low shear resistance, minimal thermal resistance, thermal decay and high retro gradation inclination, are not ideal in some mechanical nourishment applications.

Starch modification, which requires the regulation of the physical and chemical elements of the native starch to improve its characteristics, can be used to tailor starch to particular sustenance applications. Chemical change is broadly upheld, yet there is similarly a creating enthusiasm for the physical modification of starch, particularly in nourishment applications. The physical change of starch by radiation has been getting more extensive acknowledgement on the grounds that no side effects of chemical reagents are available in the adjusted starch [2]. A noteworthy point of interest of physical change is that starch is thought

to be a characteristic material and an exceptionally safe fixing, so its vicinity and measure of nourishment is not bound by enactment.

The radiation of sustenance items is a physical treatment including direct presentation to electron or electromagnetic beams for their long term protection and for the change of wellbeing and quality. Irridation medications don't get a noteworthy increment in temperature, oblige insignificant specimen planning, are quick and are non-dependent on whatever sort of catalysts. The utilization of ionizing radiation (gamma and electron bar) is depicted to produce free radicals that are fit for prompting atomic changes and discontinuity of starch.

This extraordinary property has been proposed to be one of the fundamental components basic physicochemical changes in starchy nourishment, similar to lessening of thickness and high water solubility. Aside from nourishment commercial enterprises, high measurements of gamma illuminated starch are likewise utilized as a part of paper and material businesses. Throughout radiation treatments (as with gamma beams), the glycoside bonds (at chain endings) are separated into starch granules, which are later joined by the disintegration of macromolecules and the formation of macromolecules with small chains.

Overviews have likewise shown that there is a diminishment in the crystalline phase content and in addition in the circulation request of amylose and amylopectin in starch granules. Lotus stem has by and large distinctive developing conditions than grain and tuber crops which are viewed as the essential wellsprings of starch for sustenance applications. Adequate work has been accounted for light of sustenance grain and tuber starches, in that regard is a deficiency of data with respect to illumination of starches from oceanic sources. The present work was attempted to break down the physicochemical properties of lotus stem starch to expand its utilization as biomaterials.

CHAPTER 2

LITERATURE REVIEW

2.1 Starch:

Starch is a granular, natural substance that is produced by every green plant. Starch is a cushy, white, bland powder that is insoluble in cool water, liquor, or different solvents. The fundamental chemical formula of the starch molecule is (C6H10O5)n. Starch is a polysaccharide containing glucose monomer joined in α 1,4 linkages. The most basic sort of starch contains the direct polymer amylose though amylopectin is in charge of the branched state of starch as shown in the **Fig.1**.

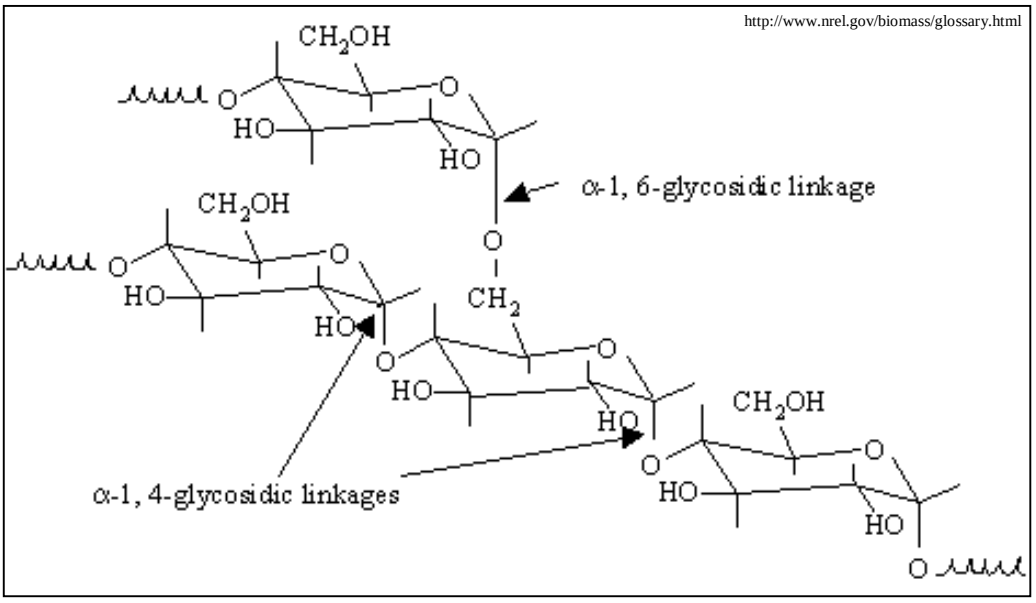


Figure.1 Basic Starch structure comprising Amylose and Amylopectin

Starch is synthesized via photosynthesis in plants containing the green pigment. Starch is deposited in chloroplasts as granules and in such organs as the foundations of the tapioca plant; the tuber of the potato; the stem essence of sago; and the seeds of wheat, corn, and rice. Starch is separated, in the point of specific catalysts and water, into its constituent monomer glucose units, which diffuse from the cell to keep the plant tissues [2]. In people and different creatures, starch is separated into its segment sugar particles, which then supply vitality to the tissues.

2.2 Starch as Disintegrant and Binder:

This study exhibits the distinctions acquired when utilizing distinctive corn starch items as both cover and disintegrant in pharmaceutical tablets. Arrangements made with Fluftex W, Tablet White and Purity 21 starches were looked at. Also, Avicel PH101 was utilized as a part of this study as a benchmark segment whose properties are extensively read. Four test plans containing hydrochlorothiazide were arranged by wet granulation. Starch was fused in both powder and glue structure. All granulations were found to have comparable characteristics when assessed based upon geometric mean measurement, molecule size dispersion, mass/tap densities, powder stream rate and surface attributes.

Pills produced using these granulations were indicated to be comparative when assessed for level of variability, weight and substance consistency. All starch plans deteriorated inside of 30 minutes and created comparative disintegration profiles. Tablets created with Avicel, then again, were situated up to show fundamentally more crumbling times than the starch definitions. In summation, these tablets showed a disintegration profile than was fundamentally unique in relation to the starch details, particularly amid the prior periods of the separation process. At the point when observing pressure and discharge powers needed to create tablets of the same level of hardness (≈6kg), Fluftex W and Tablet White granulations were found to utilize essentially lower strengths than the Purity 21 granulation. This may be demonstrative of Fluftex W and Tablet White's predominance over Purity 21 as far as binding capacity is concerned.

2.3 Applications of Starch:

Starch contains the greater part of the dry matter collecting in the plant structure, alongside cellulose and chitin. Starch comprises of two polysaccharides: amylose which is a linear polysaccharide and amylopectin which is a branched polysaccharide. The atoms of amylose comprise of very weak spreaded polysaccharide chains made from residues of

glucose connected by valence bonds. Amylose breaks up in warm water with the moulding of a clear unstable arrangement. Amylopectin has a more entangled social arrangement of expanded chains. The substance of this polysaccharide in starch changes inside of expansive limit points, running from 30 to 100%. It is not just the primary wellspring of supplement for the world, yet can in like manner be considered as renewable asset that may be utilized as a part of numerous mechanical application.

Complete study of modified starches in nourishment and modern segments give inside and out experiences into the part and potential for modified starches. Sugar economy is running forward with the pace of new information and innovations advancing at an extraordinary force. A few improvements in progress is utilizing the biotechnology that will show new innovations and items that will conceivably change the scene for modified starches. Most remarkable are characteristic high phosphate starch that can possibly substitute chemical changes, particularly beginning with paper and other modern application then in sustenance.

National Starch, driving changed starch maker has drawn out another genealogy of regular starches to supplant artificially altered starches. New half breeds by means of biotechnology will further upgrade normal starches to be connected to meet shopper requests. Modified starches are used in hundreds or even a large number of sustenance, modern, biofuels, bioplastic applications.

Unmodified starches have constrained use because of its intrinsic shortcoming of hydration, swelling and basic association. To improve viscosity, surface texture, steadiness among numerous necessary useful properties wanted for some nourishment and mechanical applications, starch and their subordinates are adjusted by compound, physical and biotechnology implies. Because of progress in business interest and fast monetary development explore on generation of adjusted starch and starch subordinates grew rapidly

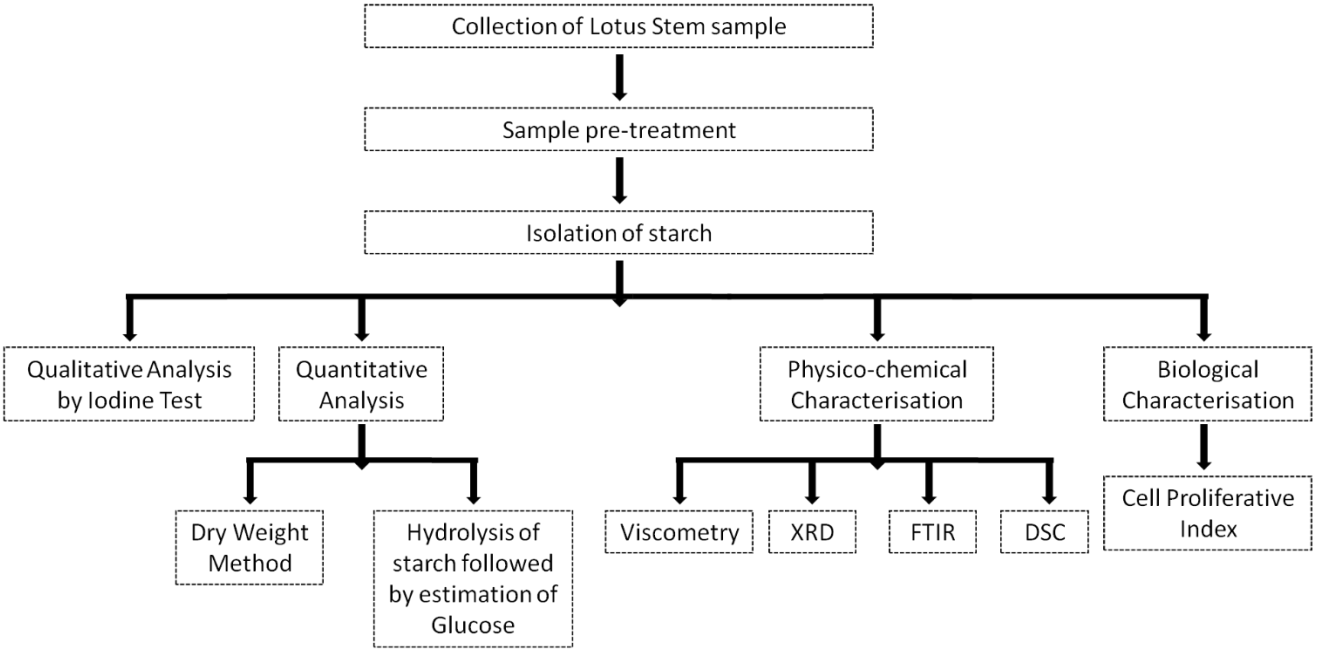
around the globe. Local starch is utilized as surface stabilizer and controller in the sustenance business, yet limit, for example, minimal thermal resistance; thermal decay and shear resistance farthest point its utilization in mechanical application. Modification of starch, includes the adjustment of the physical and compound qualities of particular mechanical applications.

Starch modification is by and large accomplished through derivatization, for example, esterification and etherification, cross linking, acid hydrolysis, enzymatic hydrolysis heat treatment and uniting of starch. The late investigate changed starches and its future degree anticipated that aggregate utilization will develop to very nearly 75 million tons by 2012 and the interest for starch by nourishment and non-sustenance commercial ventures in Asia is liable to develop by 4 - 6 percent for each year in low and centre salary nations in this district. Modified starches are used in hundreds or even a huge number of nourishment, mechanical, biofuels, bioplastic applications. Unmodified starches have restricted utilization because of its inborn shortcoming of hydration, swelling and auxiliary association. To improve viscosity, composition, strength among numerous coveted useful properties wanted for some sustenance and modern applications, starch and their subordinates are modified by chemical, physical and biotechnology implies.

OBJECTIVE

- Isolation of Lotus stem starch
- Physical, chemical and biological characterization of the isolated starch sample

WORK PLAN



CHAPTER 3

MATERIALS AND METHODS

3.1 Materials:

Lotus stem starch, Corn starch (Himedia), 0.1 N NaOH (Himedia), 2.5 N HCl (Himedia), Mesh cloth, Motor & Pestle, Falcon tubes (Tarson), Iodine crystals, Methylene chloride (Himedia), Pottasium iodide, 5% Phenol, Sulphuric acid, Standard glucose, Acetone, Starch-iodine solution, Sodium carbonate, Distilled water.

3.2 Methods:

3.2.1 Collection of sample:

Lotus stems were collected from a lotus plant situated in a local pond from Koel River. The stems were cut from top to bottom without harming the leaves and flowers. The samples were placed in a sterile bag and were taken to lab for further processing.

3.2.2 Pre-treatment of sample and Extraction of starch:

The Lotus stems were cut into roughly even with little pieces and washed with distilled water. The pieces were peeled to evacuate the top meagre layer covering of the stem, the slight layer of the stem has high lignin content which is viewed as bioplastic in nature that traps segments like starch, phytochemicals, and water and so on. Along these lines, it is essential to uproot the slim layer. The stems were washed distilled water and ground to glue like consistency utilizing a motor and pestle mechanical assembly. The resultant slurry was sieved into a beaker using mesh cloth. The starch suspension was left overnight and separated by washing with distilled water four times. The resultant slurry was permitted to settle down where the base stage was starch. At that point it was washed 2-3 times with distilled water and incubation is done in 0.1 N NaOH (50 ml) to eliminate starch bonded impurities. The resultant slurry was centrifuged at 3200 rpm for 10 minutes. The starch isolated was kept for drying in hot air oven at 400 for 24 hours.

3.2.3 Qualitative analysis of starch by iodine method:

We took 0.5 grams of I_2 crystals in a hawk tube and added with 5ml of distilled water. As iodine is not dissolvable in cold water, we added 1ml of methylene chloride to the solution which solubilized the iodine. The arrangement was vortex for 1 min and a pinch of potassium iodide was added to the arrangement in spatula which reacts with I_2 and forms I_3 which is soluble in distilled water at room temperature. The solution was shaked till I_3 got solubilized in distilled water totally. At that point the tube containing the arrangement was left for 10 minutes at room temperature, so that the unrequired methylene chloride will settle down. The top layer of the solution which was brown in shade was our required iodine arrangement. Then again, we made two starch solutions by including 0.5 grams of lotus starch and 0.5 grams of corn starch to 5ml of distilled water in two falcon tubes. A couple drops (100µl) of previously prepared starch-iodine arrangement was added to the starch arrangements which changed the solution color to dark blue. Then lastly we permitted both the solutions for settling down at room temperature for 30 minutes.

3.2.4 Quantitative analysis of starch by Dry weight method:

We prepared two solutions by taking 0.5 grams of extracted lotus starch and 0.5 grams of corn starch with 5 ml of distilled water in two falcon tubes. After mixing it well, we added a few drops (100µl) of previously prepared starch-iodine solution, the colour of the solution changed to deep blue. After proper mixing both the solutions were left to settle down at room temperature for 30 minutes. The precipitated solutions were centrifuged at 3000 rpm for 2 minutes. The dry weights of the precipitates were measured.

3.2.5 Solubility of Starch:

The starch granules of lotus and corn (110 mg) were suspended in 8 ml of water in six distinct falcon tubes and autoclaved at 1210C for 30 min, cooled and diluted to 10 ml and centrifuged at 10,000g for 20 minutes at 200 C. The supernatants were took away and diluted to 10.0 ml and 4-Vol of ethanol were added for precipitating the solubilized starch, which was centrifuged [3]. The supernatants were took away and the two precipitates, the starch that did not go into solution and the solubilized starch which was precipated with the 4-Vol of ethanol, were dried by treating them 4 times with 1 ml of CH3)2CO and 1-time with 1 ml of ethanol, trailed by drying in a vacuum oven at 40 0C for 10–15 h [4].

3.2.6 Glucose estimation of acid modified starch sample:

100 mg of the powdered lotus starch and corn starch were taken in two falcon tubes and were hydrolized with 5 ml of 2.5 N HCl for 3 hours in a boiling tube at 100^0C and was neutralized by adding Na_2CO_3 till effervescence ceases. The volumes were made to 100 ml using a 500 ml beaker for both the tubes and divided into 4 falcon tubes (50 ml) containing 50 ml each, 2 tubes containing lotus starch solution and 2 tubes containing corn starch solution. We took 0.4 ml and 0.8 ml solution from each 50 ml tubes, made 8 tubes and added 1 ml of Phenol and 5ml of sulphuric acid to each tube very carefully.

A series of volumes of0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, 1.0 ml were taken from the standard glucose solution and were made to 1 ml using distilled water. Then 1 ml of Phenol and 5 ml of sulphuric acid was added to each of the 5 tubes. Standard glucose was made by a solution of known glucose (dextrose) in the concentration of 1 mg/ml and we made 14 ml of the solution. A blank was also made using 1 ml of distilled water and adding 1 ml of phenol and 5 ml of sulphuric acid. Finally we took all the 5 tubes containing the standard glucose

and 8 tubes containing the samples to the spectrophotometer for measuring the absorbance at 490 nm and total carbohydrate content was estimated from standard glucose curve [5].

3.2.7 Viscometery :

Viscometry was done to study the viscous properties of our samples w.r.t increasing shear rates. We prepared four different starch solutions of 20 ml in four 50 ml beakers by adding 10% and 20% of extracted lotus stem starch in two beakers and 10% and 20% of corn starch in the other two beakers. Then we continuously mixed and heated the solutions using a magnetic stirrer with heater at 3000 rpm and 90^0C for 45 minutes still the solutions gets completely homogenous. Finally, we took the solutions to the viscometer instrument for obtaining the values of viscosity w.r.t the changing shear rates [6].

3.2.8 X-Ray Diffraction:

XRD is a non- destructive analytical technique that is used to predict the crystal information about the material. It is based on the scattering intensity of X-Ray light by the atoms of a crystal for generating diffraction pattern to produce interference.3-5 mg of extracted lotus starch and corn starch were took in two different glass slides for XRD analysis using Expert High Score X-ray Diffractometer [7].

3.2.9 Fourier Transformed Infra-red (FTIR) Spectroscopy:

FTIR analysis was done to determine the functional groups present in the samples. It was performed using an Alpha Bruker FTIR Spectrophotometer instrument. Samples were prepared using the pellet method. In this method, a few milligrams of the extracted lotus starch and corn starch were mixed with approximately 0.5-g of potassium bromide (KBr) which was being used as control. These mixtures were placed in a KBr press machine one by

one and were subjected to a pressure of 20 psi for approximately 5 minutes each to make them as pellets of 13 mm size. These pellets were placed one by one in the FT-IR spectroscopy. Infrared light source generates a wavelength from 4000 to 400 cm^{-1} 32 times per sample with a resolution of 4. Infrared spectrum was Fourier transformed and recorded in the transmittance mode and later was converted to absorption values [8].

3.2.10Differential Scanning Calorimetry:

It is used in the thermal analysis in which the difference in amount of heat required to increase the temperature of the lotus stem starch (sample) and corn starch (control) reference is measured as a function of temperature. Here both sample and reference were took at the same temperature.7 mg of the extracted lotus starch and corn starch samples were placed in a crucible one by one with change in the heat flow was 0.1-10 mW and were placed in the DSC instrument. The temperature was varied from 40 ^{0} C (ambient) -240 ° C [9].

3.2.11Biological characterization:

The cell viability of the HaCaT cells in the presence of test starch samples was studied via MTT assay. The cells were maintained in complete DMEM media (10% FBS and 1% antimycotic-antibiotic solution) at 37°C, 5% CO_2. Upon attaining 80% confluence, cells were harvested by trypsinization and 1 x 10^4 cells were added to each well of a sterile 96-well plate and incubated for 24 hrs to ensure proper cell adhesion.

After 24 hours of incubation, the cells were treated with starch samples at a concentration of 100µg/ml. MTT assay was carried out by adding 100µl of MTT reagent (MTT reagent and DMEM complete media in the ratio 1:10) to each well, and kept for 4 h of incubation. After completion of incubation, the formazan crystals formed were dissolved in

100 µl of DMSO. The absorbance of DMSO solution was then measured at 595nm and the cell viability correlated to OD value obtained at 595nm [10].

CHAPTER 6

RESULTS AND DISCUSSIONS

4.1 Extraction of starch:

Finally, after appropriate treatment and drying of the sample collected from the lotus plant we obtained the required lotus starch.

4.2Qualitative analysis of starch:

After adding the iodine solution to the lotus and corn starch samples the colour of the solutions changed to deep blue colour. These solutions were left to settle down for one hour which allowed the settling of starch at the bottom of the falcon tube confirming the presence of starch.

4.3 Quantitative analysis of starch:

The dry weight of the precipitate for extracted lotus and corn starch was found to be 0.42 grams and 0.48 grams respectively.

4.4 Solubility of starch:

The solubility of the autoclaved native starches were found out to be 72mg.ml^{-1} and 75mg.ml^{-1} for Lotus starch and Corn starch respectively. This was obtained by weighing the precipitate of starch obtained from the supernatant of the autoclaved native starches.

4.5 Glucose estimation:

Figure.2 Increasing Concentration of Glucose taken for Standard Curve generation

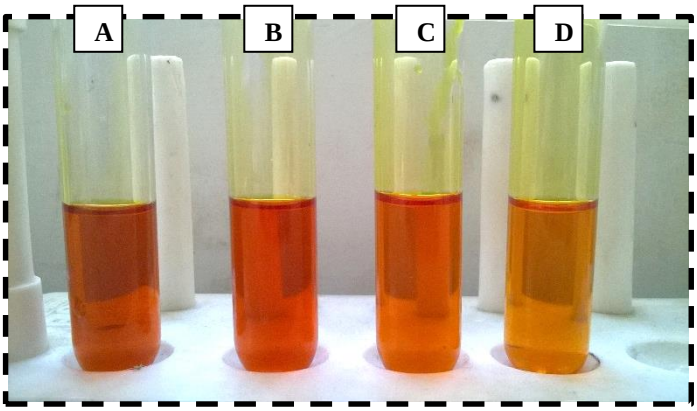


Figure.3 A) 0.8mg/ml **B)** 0.6mg/ml **C)** O.4mg/ml Acid treated Lotus Starch samples respectively and **D)** Blank

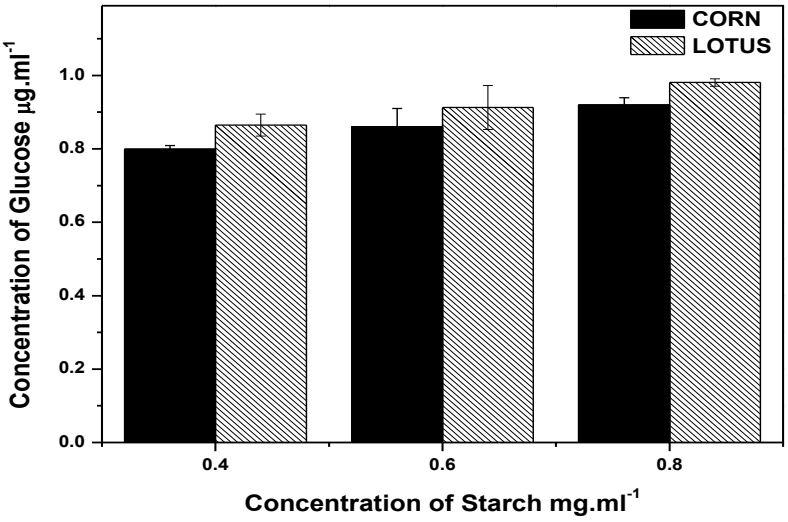


Figure.4 Graphical representation for Glucose estimation for various concentrations of acid treated starch samples.

From the **Fig.4** we can clearly observe that with increasing concentration of our acid modified starches for both, the lotus starch and the corn starch the concentration of glucose present is also increasing. We, also observed that the amount of glucose concentrations present in the lotus stem starch is higher by a very less microgram levels. So, we concluded that the glucose concentration of lotus stem starch and corn starch are almost equal.

4.6 Viscometry:

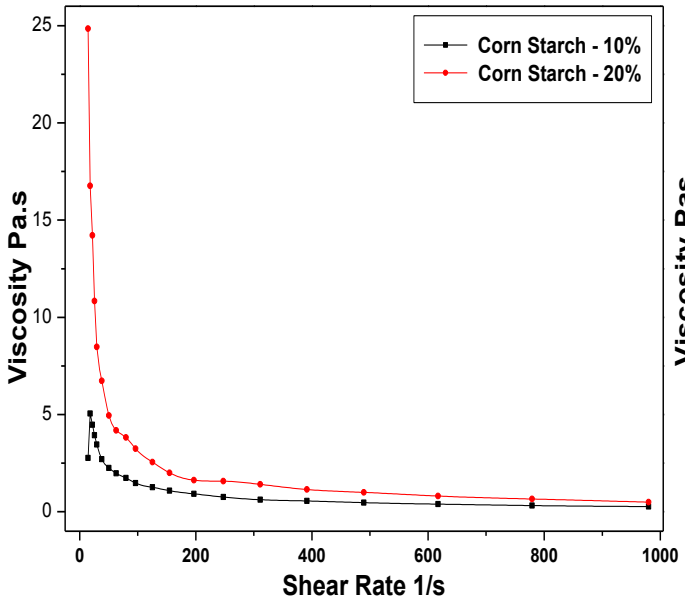


Figure.5 Corn Starch Viscosity Profile

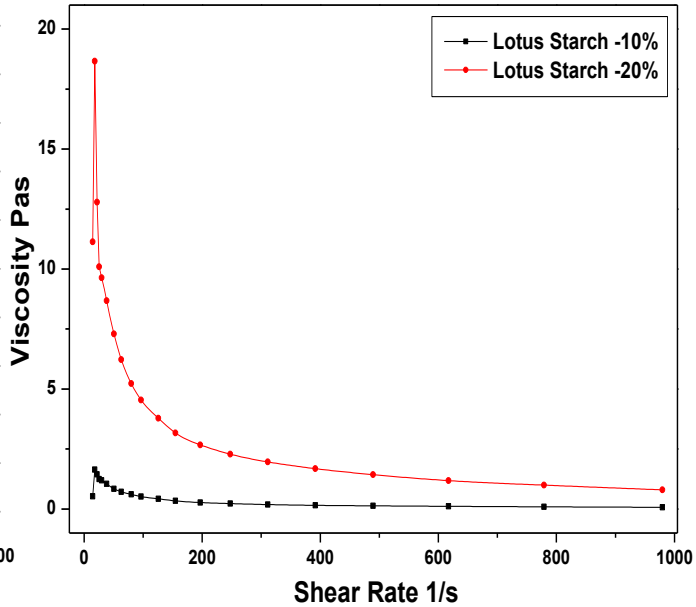


Figure.6 Lotus Starch Viscosity Profile

From the **Fig.5** and **Fig.6**, we can observe that the viscosity is decreasing with increasing shear rates for both, lotus stem starch and corn starch. Both the sample (lotus starch) and the control (corn starch) passed the criteria for being a Bingham fluid.

By applying the Bingham's equation, the viscosity values for 10% and 20% of the lotus stem starch sample were found to be 0.04 and 0.59 respectively. Similarly, the viscosity values for 10% and 20% of the corn starch were found to be 0.01 and 0.12 respectively. Hence, we concluded that viscosity of our sample (lotus stem starch) is higher in comparison to our control (corn starch).

4.7 X- Ray Diffraction analysis:

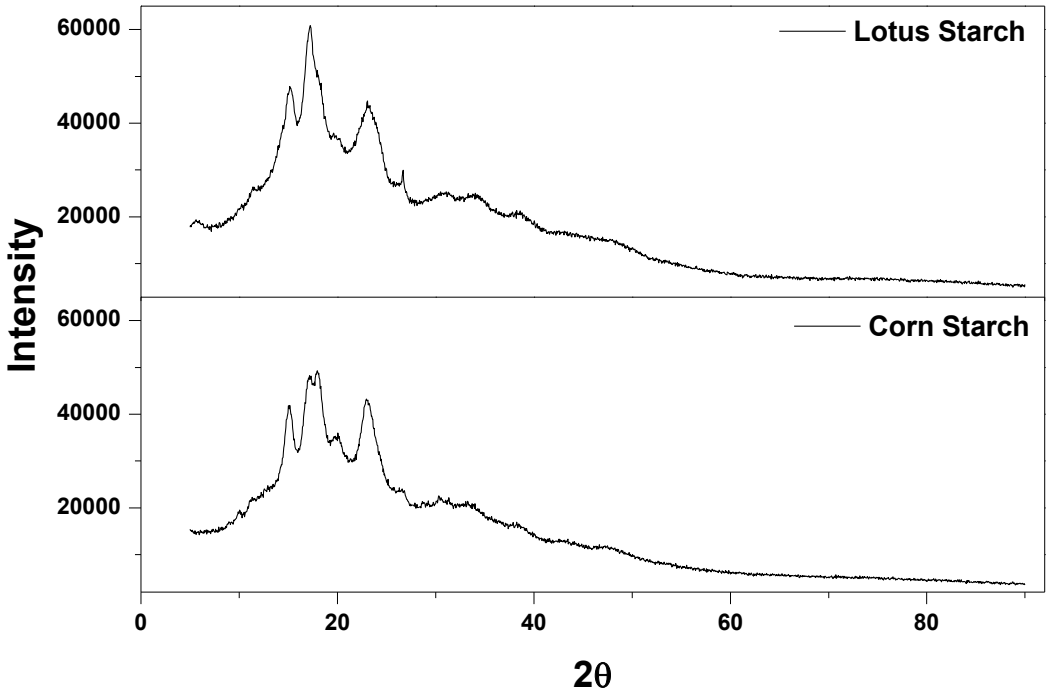


Figure.7 XRD pattern for Native Lotus and Corn Starch samples

X-beam diffraction is a standout amongst the best systems in considering the structure of native starch, particularly in deciding the crystalline type of starch. X-beam diffraction gives a clarification of the long-extend sub-atomic request, normally termed as crystalline, which is because of requested varieties of twofold helices framed by the amylopectin side

chains. Two diverse polymorphic structures are normally seen in native starches, in particular, A-sort and B-sort polymorphs, which comprise of parallel-packed, left-gave twofold helices. In the A-sort structure, left-sided parallel-stranded twofold helices are pressed in the monoclinic space grouping B2.

In the B-sort structure, nonetheless, the twofold helices are pressed in a hexagonal unit cell with the P61 space grouping. The primary distinction between A- and B-sort is that the previous receives a nearby close arrangement with water particles between every twofold helical structure, while the B-sort is more open, there being more water atoms, basically all of which are situated in a focal hole encompassed by six twofold helices. The separation between two linkages and the branching thickness inside every group are deciding variables for the improvement of crystallinity in starch granules. Groups having various short chains and short linkage separation create thickly packed structure, the A allomorphic sort. Longer ties and separations lead to a B-sort. C-sort starch example has been viewed as a blend of both A- and B-sorts in light of the fact that its X-beam diffraction example can be determined as a mix of the past two.

From the **Fig.7**, we can observe that the maximum peaks for the lotus stem starch (sample) were at 15.5 ^{0}, 17.25 ^{0} and 23.05 ^{0} and for corn starch (control) the maximum peaks were at 15.15 ^{0}, 17.85 ^{0}, 17.95 ^{0} and 22.9 ^{0} as we can notice that the middle peak for the corn starch is divided into two consecutive peaks due to some lignin impurities. Hence, by studying the graphs thoroughly, we concluded that both, the lotus stem starch and corn starch samples showed B-type pattern of starch.

4.8 FTIR spectrophotometer:

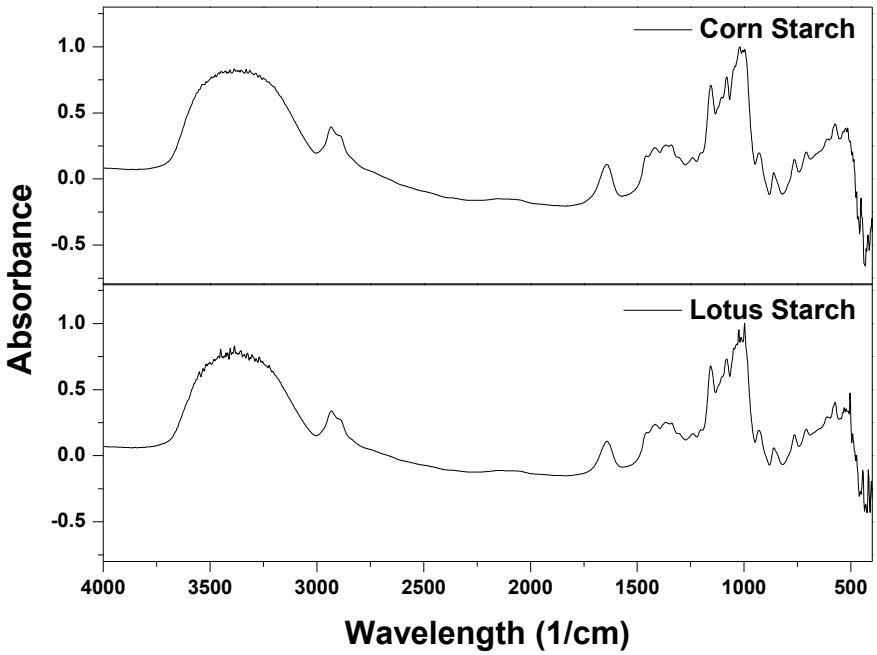


Figure.8 FTIR peaks observed for Corn and lotus starch respectively

The subsequent range speaks to the sub-atomic ingestion, making a sub-atomic unique fingerprint of the example. Like a fingerprint no two extraordinary atomic structures deliver the same infrared range. This makes infrared spectroscopy valuable for a few sorts of examination. The mid infrared range (4000–400 cm−1) is more or less separated into four districts. The way of a gathering frequency is controlled by the area in which it is found. The areas are summed up as takes after: the X–H stretching area (4000–2500 cm−1), the triple-bond area (2500–2000 cm−1), the double-bond area (2000–1500 cm−1) and the fingerprint area (1500–600 cm−1).The principal vibrations in the 4000–2500 cm−1 area are because of O–H, C–H and N–H stretching. O–H stretching delivers a wide band that happens in the extent 3700–3600 cm−1.

From previous studies, N–H stretching is typically seen somewhere around 3400 and 3300 cm−1. This retention is by and large way more sharp than O–H extending and accordingly be separated. C–H stretching groups from aliphatic compounds happen in the reach 3000–2850 cm−1. On the off chance that the C–H bond is contiguous a twofold bond or sweet-smelling ring, the CH extending wave number expands and assimilates somewhere around 3100 and 3000 cm−1.

The key groups in the 2000 – 1500 cm−1 area are because of C=C and C=O extending. Carbonyl extending is one of the most effortless retentions to perceive in an infrared range. It is generally the most extraordinary band in the range and relying upon the sort of C=O bond, happens in the 1830–1650 cm−1 locale. The metal carbonyls ingest over 2000 cm−1. C=C extending is much weaker and happens at around 1650 cm−1, however this band is frequently truant for symmetry or dipole minute reasons. C=N stretching also appear in this area and generally have more strength.

From **Fig.8** we can observe that the spectrum formed when infrared radiation passed through both, the sample (lotus stem starch) and the control (corn starch) were exactly the same except a trace of noise was found in the lotus stem starch. There were several absorbance peaks at 1159, 1082, 1014 cm^{-1} due to the C=O bond stretching. Additional characteristics absorption bands appeared at 992, 929, 861, 765, 575 cm^{-1} due the entire anhydrous glucose ring stretching vibrations. An extremely broad band due to H_2 bonded hydroxyl groups appeared at 3421 cm^{-1} for the lotus stem starch and corn starch both.

4.9 DSC analysis:

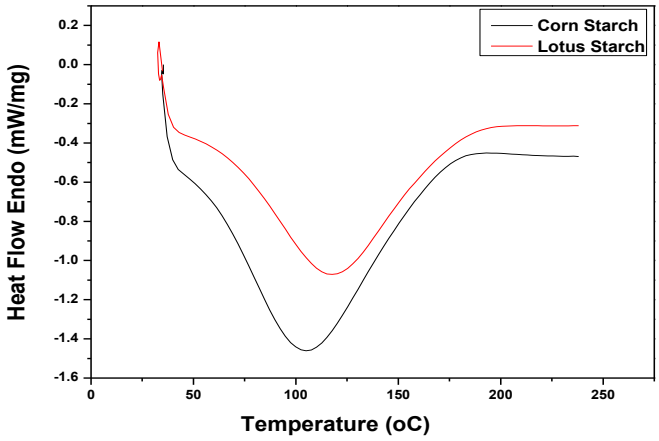


Figure.9 DSC Curve obtained for Corn and Lotus Starch

As observed in **Fig.9** the endothermic peak of corn starch (control) and extracted lotus starch showed a broad region of evaporation of water in a range of 80-130 ^{0}. Peak temperature of the control is nearly at 105 ^{0}C but the peak temperature of the extracted lotus starch shifted to higher regions of 117 ^{0}C. The area under the curve for both endothermic graphs explains the enthalpy of the formulations. As referring the literature more the area under the curve, more is the enthalpy. Thus, we can observe that enthalpy of the control is more is more than the sample. Entropy of the system is also calculated from the endothermic of the formulations which explains that the lotus starch extract has a higher entropy value than the control.

Table.1 Values of temperatures, Enthalpy and Entropy in DSC analysis

Formulations	**$T_{onset,m}$**	**T_m**	**$T_{e,m}$**	**Area under the curve**	**Enthalpy (ΔH)**	**Entropy (ΔS)**
	(°C)	**(°C)**	**(°C)**		**J/g**	**J/g/°C**
Corn Starch	48.33	105	180.51	135.065	135.065	290.153
Lotus Starch	46.04	117	198.04	101.222	101.222	294.847

4.10 Biological characterization:

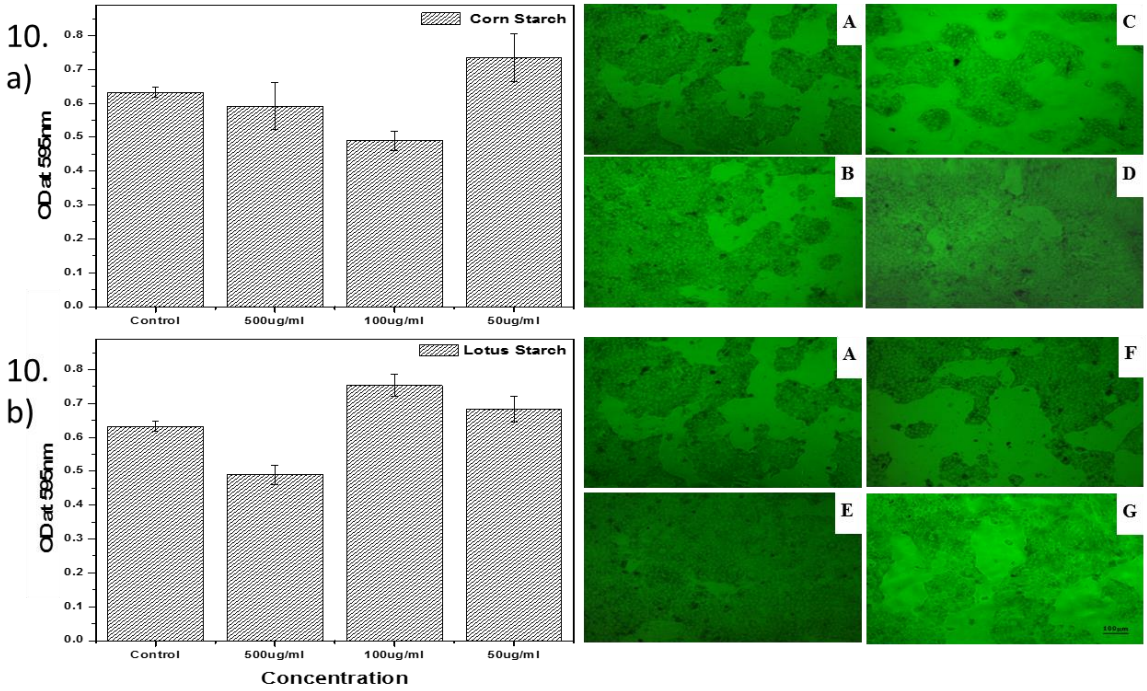


Fig.10 A graphical representation of cell viability measured via MTT Assay using HaCaT **Fig.10.a)** of Corn starch treated cells and **Fig 10.b)** of Lotus treated cells. **Fig A** showing untreated HaCaT cells. Lotus starch treated cells **Fig.B** 500µg.ml^{-1} **Fig.C** 100µg.ml^{-1} **Fig.D** 50µg.ml^{-1} respectively. Corn starch treated cells **Fig.E** 500µg.ml^{-1} **Fig.F** 100µg.ml^{-1} **Fig.G** 50µg.ml^{-1} respectively.

From the **Fig.10** we can observe that for 50µg/ml, the cell showed maximum viability as compared to other concentrations for corn starch but in case of lotus stem starch for 100µg/ml, the cell showed maximum viability as compared to other concentrations. Therefore, lower concentrations of starch are favourable for cell survival as compared to control.

CHAPTER 5

CONCLUSION

5.1 Conclusion

In this study, the starch was successfully isolated from the stem of lotus. Further, the solubility of the starch was analysed in water. The rheological properties and the physico-chemical properties of the isolated starch were studied using Viscometry, XRD, FTIR, DSC and glucose estimation analysis. The results showed that lotus starch was comparable to the commercial corn starch in case of physico-chemical properties. Later, biocompatibility of the samples were analysed with HaCaT cells. However, in case of cell viability lotus starch at an optimal concentration (100 μg/ml) showed better cell viability and supported cell growth in comparison to control tissue culture plate and corn starch. The result confirmed that lotus starch can find its potential application in *in vivo* drug delivery and tissue engineering. Further biological and drug release studies along with degradation analysis should be performed to understand the potential of lotus starch for *in vivo* application.

CHAPTER 6

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