Research Article
Volume 1, Issue 1, 2025

Structural and Optical Characterization of Starch Granules Due to the Effect of α-Amylase

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Received: 11 December 2024; Accepted: 05 January 2025; Published: 21 January 2025

Abstract: Starch, one of the most prevalent polysaccharides in plants, is a key component of the human diet. It's morphology and chemical composition can vary depending on the plant species from which it is derived. In this work, we examined starch granules from potato and corn using a range of techniques, including biochemical Dinitrosalicylic Acid Test, optical imaging, and various spectroscopic methods. We also explored the impact of the starch-digesting enzyme α-Amylase on potato and corn starch under different temperature and pH conditions. We observed basic morphology through optical microscopy, analyzed chemical composition using Fourier Transform Infrared Spectroscopy, assessed crystallinity with X-Ray Diffraction and detailed results are reported in this study.

Keywords: Starch, α-Amylase, optical microscopy, X-Ray diffraction, FTIR spectroscopy

1. Introduction

Starch is a natural polysaccharide composed of numerous glucose subunits linked by glycosidic bonds. It is the second most abundant biomolecule in nature, following cellulose, and is synthesized in plants during photosynthesis within the amyloplasts [1]. A single amyloplast may contain multiple starch granules. While starch is also produced by other plant organs, such as the meristem and root cap, it is primarily stored in seeds, fruits, and tubers. The shape and size of starch granules vary across different plant species. For example, starch granules in cereals like maize and rice are polygonal and range in size from 5 to 50 µm, whereas potato starch granules are oval and range from 30 to 100 µm in diameter [2-3]. Each starch granule has a central core region known as the hilum—around which growth rings are arranged in concentric circles [4]. These growth rings are formed by complex structures of amylose and amylopectin. Amylose constitutes about 20% of the starch granule and is the amorphous region of the starch [5]. This molecule is made up of α-1,4 linked D-glucose units. Since these molecules are rigid and hard, it does not dissolve in water and is less absorbed in the body. However, it can store more energy when compared to amylopectin.

Amylose has a property of binding with itself to form a double helix and it can also bind to other molecules such as iodine.

Amylopectin on the other hand constitutes about 80% of the starch granule and is the crystalline part of the starch [6]. It is highly branched and is joined by α-(1-6)-glycosidic bonds. When compared to amylose, it is easily digested. Amylopectin can be explained in terms of three chains. A chain is linear and unbranched and consists of 15 glucose units. B chain supports the A chain and consists of about 15-45 glucose units. C chain on the other hand terminates into a single reducing end. Amylopectin is widely used in making lubricants and adhesives due to the retrogradation property of starch which is the physical conversion of starch from liquid to a gel like form which occurs due to the arrangement of glucose molecules [7]. Another property of starch is the gelatinization of starch. In the presence of heat and water, the intermolecular bonds between glucose units are broken down which results in the amorphous region of starch to engage more water which in turn leads to the swelling of the starch granule and leads to the dissolution of amylose and amylopectin [8]. Starch can be classified into three main types depending upon its digestion property. Slow digesting type of carbohydrates are digested very slowly and thus, provides our body with enough energy without increasing the blood glucose level. Examples include sweet potato, black beans, brown rice etc. [9]. Rapidly digested starch is carbohydrate which is broken down very fast in the small intestine. This type of starch has higher amylopectin content. Examples of food include white sugar, candy etc. which rapidly increase the blood glucose level [9]. Resistant starch is carbohydrate which is converted into short chain fatty acid by the intestinal bacteria and is therefore not fully digested. These fatty acids are used for colonic bacteria for energy and also stimulate the flow of blood to the colon and aid in absorption of minerals [10].

Our study focuses on the effect of α-Amylase on starch at various conditions. It acts on the internal α-1,4-glycosidic bonds [11]. Since α-Amylase acts on random locations in the glucose chain, it is acting faster than the rest of the amylases. It is found in various life forms including humans, plants, bacteria and fungi. The human pancreatic and salivary amylases are also of α-type [12]. α-Amylase is a glycoprotein which consists of a single polypeptide chain and consists of about 475 glucose units. It has four disulphide bridges, two thiol groups and tightly bound calcium. It is also found to have three domains out of which two of them are joined whereas the third domain varies in each type of amylase. α-Amylase is used in various industries like detergent [14], food [15], paper and textile industry etc [16]. This study focuses on the characterization of starch granules using various methods such as optical microscopy, X-Ray Diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR) to investigate the action of α-Amylase on starch granules.

2. Materials and Methods

2.1. Isolation of Potato Starch

The commercially available potatoes were peeled and soaked in 500 mL of distilled water and kept undisturbed for overnight. The potatoes were discarded and the residue was centrifuged at 3000 rpm for 10 minutes. The centrifugation process was repeated until white pellet was obtained. The sample was then lyophilized and stored at -80º C for further experiments [19].

2.2. Isolation of Corn Starch

Corn kernels were handpicked and soaked in sodium metabisulphate for 24 hours. These were then taken out and the endosperms were separated from the pericarp and the germ seed using forceps. This was then made into a paste using a mortar and pestle and centrifuged at 3000 rpm for 10 minutes. The centrifugation process was repeated until white pellet was obtained. This sample was then lyophilized and stored at -80º C for further experiments.

2.3. Estimation of amylose content

The amylose content was determined using the method outlined by Hassan, L.G. et al. Initially, 100 mg of starch was placed in a 100 mL volumetric flask, followed by the addition of 1 mL of ethanol and 9 mL of sodium hydroxide. The mixture was then heated for 10 minutes. After cooling to room temperature, the solution was diluted to the mark with distilled water. Next, 5 mL of this solution was transferred to a separate flask, to which 1 mL of acetic acid and 2 mL of iodine solution were added. The absorbance was measured at 620 nm using a spectrophotometer [20].

2.4. Rate of Hydrolysis

Rate of hydrolysis was performed using methods of Liu, X. *et al*. 50 mg of starch was incubated with 4.9 mL of sodium acetate and 100 µl of 500U α-Amylase (from *Bacillus subtilis*, MP Biomedicals, India) at 37^0 C for 48 hours. The sample was taken out after 48 hours and was centrifuged at 10,000 rpm for 10 minutes. The pellet was lyophilized and used for further analysis and the supernatant is used for DNS method [21].

1 mL of the supernatant was taken and 1 mL of DNS reagent was added to it. The mixture was heated for 7 minutes in a boiling water bath and the cooled down to room temperature. After the sample was cooled down 1 mL of distilled water was added to it and the absorbance was taken at 550 nm using a spectrophotometer.

$$Rh\,(\%) = (A1/A2) \times 100 \tag{1}$$

where, $A1$: amount of sugar in the supernatant after hydrolysis
$A2$: amount of raw starch before the reaction

2.5. Rate of Hydrolysis with Different Concentration of Amylase

50 mg of starch was incubated with different concentration of amylase. For potato, 50 mg of starch and 300U, 500U, 700U, 900U and 1100U of enzymes were used. For corn, 50mg of starch and the amount of enzymes used were 300U, 500U, 700U, 900U, and 1100U. The rate of hydrolysis was measured using DNS method.

2.6. Optical Microscopy

Optical microscopic images were taken using the Motic BA400 Microscope, United States of America (USA), which is equipped with a camera. Native starch and hydrolyzed corn and potato starch were mounted on a glass slide and covered by a coverslip. This was then observed under the microscope.

2.7 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra of native and hydrolyzed starch were obtained using a Bruker alpha FTIR spectrophotometer (Germany) with KBr optics. Starch powder was mounted on to the KBr pellet holder after mixing it with KBr. Spectra were obtained for native corn and potato starches and hydrolyzed sample after treatment with 500U of enzymes.

3. Results and Discussion

3.1. Degree of Hydrolysis

Starch granules are broken down by α-Amylase at 1-4 glycosidic bonds to produce simple sugars. 50 mg of starch was taken and different concentration of amylase was added. The control used was starch and acetate buffer. The amount of reducing sugar in the supernatant was determined using the DNS method. As the amount of enzyme concentration increases, the rate of hydrolysis also increases up to a certain enzyme concentration after which the rate of hydrolysis decreases. For potato very little hydrolysis took place with 300U, 500U, 700U, 900U and 1100U of enzymes. In corn starch, the maximum hydrolysis was observed at 900U Table 1.

Table 1. Degree of hydrolysis of corn starch at various enzyme concentrations

Enzyme conc. (U)	O.D. (Corn)	Degree of hydrolysis [Corn (%)]
Control	0.0730	
300 U	0.4135	7.21%
500 U	0.6193	15.73%
700 U	0.4781	9.97%
900 U	0.8327	37.99%
1100 U	0.6723	16.8%

3.2. Amylose Content

The average amylose content in corn and potato starch is 1.4 - 70% and ~29%, respectively. In our study, we found that potato starch contained ~24% of amylose whereas corn starch contained about ~28% of amylose. The amylose content was also determined for the commercial corn and was found to have an average of ~S22% of amylose. Detailed results are presented in Table21.

Table 2. Amylose content % in commercial corn, isolated corn and potato

Starch	Reading 1	Reading 2	Reading 3	Average
Commercial corn	21.97	22.52	23.58	22.69
Corn	27.84	27.85	27.84	27.84
Potato	23.86	24.34	24.56	24.25

3.3 Optical Microscopy of Starch Granules

Optical microscopic images provided basic morphology of starch granules (Figure 1). The central hilum was clearly visible in optical microscopic images which is surrounded by concentric circles known as the growth rings. Potato starch appeared to be bigger than corn starch and was found to be more oval in shape. Corn starch on the other hand, was polyhedral in shape. The optical images after hydrolysis of both the samples at similar conditions showed that corn starch was hydrolyzed at a much higher rate than potato starch.

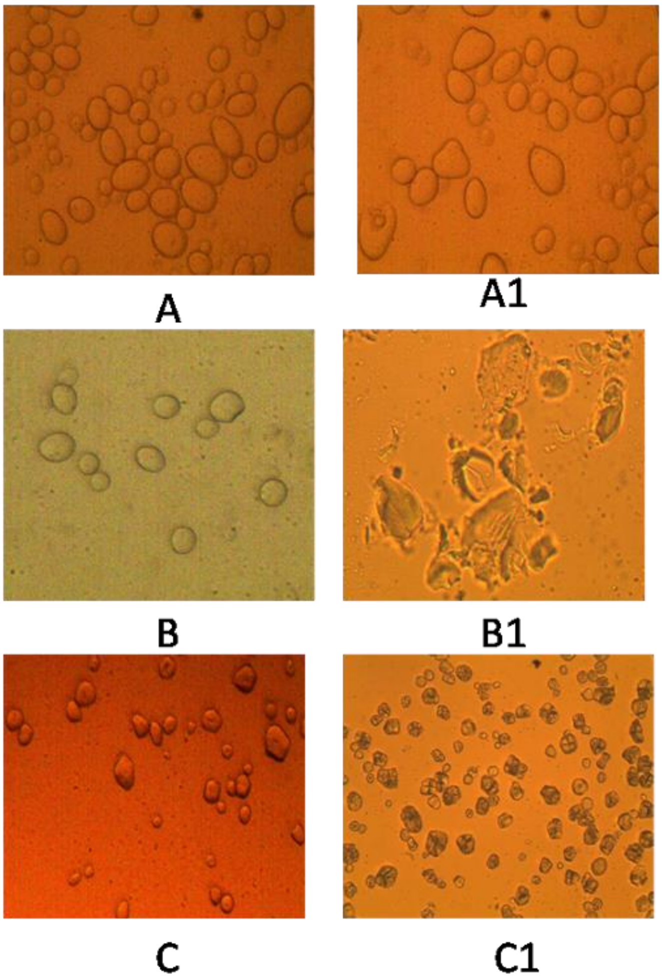

Figure 1. Native and hydrolyzed starch under optical microscope at 40X magnification: (A) Native potato; **(A1)** Hydrolyzed potato; **(B)** Native corn; **(B1)** Hydrolyzed potato; **(C)** Native Commercial corn starch; **(C1)** Hydrolyzed commercial corn starch

3.4 Fourier Transform Infrared Spectroscopy

FTIR spectra was obtained for native and hydrolyzed corn and potato starch samples (Figure 2). 3100-3700 cm^{-1} bands represent the complex with intermolecular hydroxyl groups which were found to be similar in all the starch samples. Bands were sharp at 2920-2928 cm^{-1} for hydrolyzed sample when compared to the native starch. This is related to the CH_2 stretching vibration. No peak was obtained at 1640 cm^{-1} in all the samples which implies that no firmly bound water was present in all the samples. The peaks at 920-980 cm^{-1} represent the α-(1-4) glycosidic linkages (C-O-C) and were absent in native potato. The peaks at 1081, 1156 and 1020cm^{-1} were characteristics of the anhydrous glucose rings C-O stretch and were absent in native potato. Representation of the various wave numbers and respective bonds in FTIR spectra are presented in Table 3.

4. Discussion

Starch has wide range of applications in many industries due to its cheap price and availability [17]. Some examples of industries which utilizes starch as a raw material includes fuel alcohol production, paper industry and textile industry. Optical imaging gives information about the finest arrangement and organization of starch which is due to the arrangement of amorphous amylose and crystalline amylopectin molecules [18]. The effect of α-Amylase on starch was verified using FTIR and XRD. Potato starch was resistant to enzymatic degradation even at a higher concentration (2500U) of enzyme. Thus, the rate of hydrolysis of potato starch was much lower compared to corn starch. This is due to the large size of potato starch which makes them hard to digest. Even though starch is made up of amylose and amylopectin, the degradation takes place in the amorphous part *i.e.*, in amylose. This can be explained due to the fact that after degradation of starch, amylose content decreases whereas the crystalline region increases as α-Amylase acts better on the amorphous region of the starch granule. The shape and size of the starch granules also vary according to different botanical origins. According to previous literature, the average size of starch granule ranges from 1-100 µm. Due to the fact that potato starch showed less hydrolysis even at a higher enzymatic concentration, the optical images of potato starch before and after hydrolysis did not show much difference when compared to corn starch. Corn starch on the other hand showed degradation even with a much lesser enzymatic concentration.

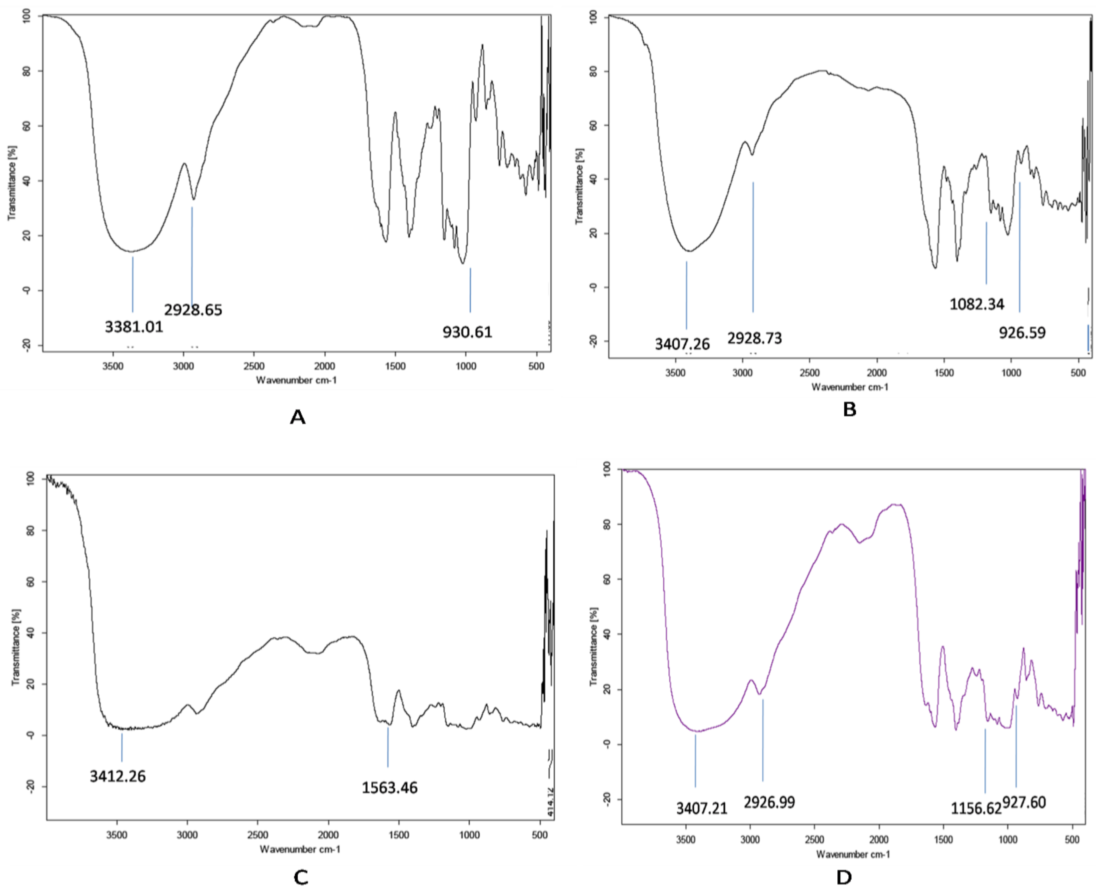

Figure 2. FTIR spectrum of native and hydrolyzed starch granules: (A) Native corn; (B) Hydrolyzed corn; (C) Native Potato; (D) Hydrolyzed potato

Table 3. Representation of the various wave numbers and respective bonds extracted from FTIR spectra (+ represents “present” and – represents “absent”)

Wavenumber (cm^{-1})	Bonds	Native corn	Hydrolyzed corn	Native potato	Hydrolyzed potato
3100 - 3700	O-H stretch	+	+	+	+
2920 - 2928	C-H stretch	+	+	+	+
1640	O-H bending Water	–	–	–	–
1485	H_2O	–	–	–	–
920 - 980	C-O-C	+	+	–	+
1081, 1156 and 1020	C-O	–	+	–	+

5. Conclusions

In this study, the structural and optical properties of starch granules from potato and corn were analyzed, and the effect of the enzyme α-Amylase on these starches was characterized under varying conditions. Corn starch, which contains higher amylopectin and smaller granule size, exhibited greater susceptibility to enzymatic degradation compared to potato starch, which is more resistant due to its larger granule size and higher amylose content. Enzymatic erosion of starch granules occurs in two forms: exoerosion and endoerosion. Exoerosion involves the digestion of the entire surface or parts of the granular structure, while endoerosion occurs when digestion starts from specific channels and moves toward the center of the granule. The type of starch granule determines the nature of digestion. B-type crystalline starches, such as those found in potatoes, undergo exoerosion, whereas A-type crystalline starches, like those in corn, are digested through endoerosion. The chemical composition is investigated by FTIR. The crystalline region of the starch can be classified into A, B and C chain. A chain is linear and unbranched and consists of about 15 glucose units and is associated with cereal starches. B chain supports the A chain and consists of about 15-45 glucose units and is found in tubers. C chain on the other hand terminates into a single reducing end and can be found in bean starch. This study enables detailed understanding of the effect of α-Amylase on corn and potato starch granules thereby helping to develop novel applications of these starches.

Funding

This research was funded by Global Innovation and Technology Alliance (GITA), Department of Science and Technology (DST), India [Project Number- GITA/DST/TWN/P-95/2021], Government of India.

Acknowledgments

We thank Global Innovation and Technology Alliance (GITA), Department of Science and Technology (DST), India [Project Number- GITA/DST/TWN/P-95/2021], Government of India, for financial support. We also thank Manipal School of Life Sciences, Manipal Academy of Higher Education, Manipal, Karnataka, India for the infrastructure and Faculty of Life and Environmental Sciences, Shimane University for the support provided during the course of this study.

Conflicts of Interest

The authors declare that they have no conflict of interest with the contents of this article.

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