
PREPARATION PROTOCOLS FOR STARCH NANOCRYSTALS

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Abstract

Starch is one of the most abundant biomass materials in nature. Starch nanocrystals of different shapes and sizes can be obtained depending on the starch origin and isolation process involved. Acid or enzymatic hydrolysis, regeneration, and mechanical treatment are three methods used to prepare starch nanocrystals..

Key words: Starch, Nanocrystal, Enzymatic hydrolysis, Starch nanocrystal

Introduction

Starch is one of the most abundant biomass materials in nature. Many plants store starch as a source of energy, as a natural, biocompatible, renewable, and biodegradable polymer. Starch is obtained from various classes of plants such as cereals or grains, roots, tubers, legumes, and fruits. Cereals have the highest percentage of starch¹.

Plant cells synthesize starch in 2 forms of polymers: amylopectin and amylose. Amylopectin is made of linear chains of glucose units linked by α -1,4 glycosidic bonds. It is highly branched at the α -1,6 positions by small glucose chains at intervals of 10 nm along the molecule's axis and represents between 70% and 85% of common starch².

Amylose is a linear chain of α -1,4 glucans with partial branching points at the α -1,6 positions and constitutes 15–30% of common starch. Wet grinding, sieving and drying are major ways of extracting and refining starch in the industry. It can be utilized in the natural

form after extraction from plant “native starch” or undergo 1 or more modifications (physical, mechanical and/or chemical) to achieve targeted properties and become “modified starch”.

Starch nanoparticles Several components such as quantum dots, liposomes, starch, and other newly developed materials were used in the development of nanoparticle-based delivery systems³⁻⁹. Nanoparticles nanocolloids and nanocrystals have been prepared from starches. Starch nanocrystals are derived from starch granule crystallites as a result of disrupting the semicrystalline structure of granules at temperatures below gelatinization temperature¹⁰⁻¹⁴.

Starch nanocrystals are also called starch crystallite, starch microcrystalline and hydrolyzed starch. It is important to note that starch nanocrystals are different from starch nanoparticles, which can be amorphous. While starch crystallite develops as a result of the disruption of non-crystalline domains from semi-crystalline granules by acid hydrolysis,

Starch nanoparticles are generated from congealed starch¹⁵. Both the crystalline and non-crystalline regions coexist in the structure of the native starch but it is noteworthy to underscore the responsibility of the amylopectin chains in the formation of the crystalline regions due to the stacking of the nanometric sub-units of the amylopectin chain blocks, which eventually form the starch nanocrystals¹⁶. As the native starch is subjected to acid hydrolysis, the starch granule begins to dissolve at the regions of low lateral order non-crystalline phases (amylose region), while changes do not occur at the highly crystalline water-insoluble lamellae¹⁷.

Le Corre et al. established that starches of the same amylose content but different botanical sources, e.g., maize, potato and wheat starches, show similar crystal size. However, differences in size were more pronounced with starches of the same botanical source but with different amylose content. This underlines the strong influence of the composition and molecular structure on the resulting crystallite dimensions that can be

obtained when preparing starch nanocrystals¹⁸. Low hydrolysis temperature ranging usually between 35°C and 45°C has been reported and is required to block starch congealing and consequent disruption of the starch crystalline structure¹⁹.

Starch nanocrystals of different shapes and sizes can be obtained depending on the starch origin and isolation process involved. Though the shape and size of the granules may vary between starches, there may be no discernable difference in the shape of starch nanocrystals from different sources²⁰. Xu et al. found that the structure and morphology of obtained nanocrystals are influenced by several factors, such as the type of crystalline, amylose relative portion, amylopectin, botanical origin, and morphology of starch granule. Nanocrystals with varying morphology and size have been reported. These include nanocrystals from plateletlike shaped waxy maize starch with 5–7 nm thickness and 15–40 nm diameter²¹ to those from potato starch granules with round and grape-like shape, with sizes ranging from 40 nm to 100 nm observed under transmission electron microscope (TEM)²².

Preparation protocols for starch nanocrystals

Three different methods are used to prepare starch nanocrystals and other nanoparticles: acid or enzymatic hydrolysis, regeneration and mechanical treatment²³. Different protocols for the preparation of nanocrystals from starches are employed with their attending challenges, chief of which is low or limited percent yield even after prolonged (days) acid hydrolysis. Another challenge is related to the onion-like structure of starch which hinders its hydrolysis.

Acid hydrolysis kinetic

Starch is hydrolyzed using either dilute HCl or dilute H₂SO₄ at 25–55°C for different durations to manufacture acid-thinned starches, microcrystalline starch or starch nanocrystals²⁴⁻²⁷. All types of starch experience a 2-stage hydrolysis profile, comprising an

initial fast hydrolysis step, presumably due to the hydrolysis of the non-crystalline regions of starch granules, and a 2nd slower step, presumably due to the hydrolysis of the crystalline regions²⁸. The dense packing of the starch in the crystalline region, which does not allow for the penetration of H₃O+A, leads to the slower hydrolysis rate of the crystalline region. A theory suggested that the dense packing of the starch in the crystalline region which does not allow for the penetration of H₃O+A led to the slower hydrolysis rate of the crystalline region. The other theory is based on the hydrolysis of the glycosidic bonds requiring a change or transformation from the chair to half-chair conformation which is required for hydrolysis of the bonds. This is an action that occurs slowly due to immobilization of the sugars in the starch crystallites^{29,30}.

Some major factors affecting acid hydrolysis kinetics include time, acid type, acid concentration, and temperature. The rate of hydrolysis increases at a constant temperature of 50°C with increasing acid concentration. Another study assumed that the optimum temperature and acid concentration for starch nanocrystal production should be 40°C and 3.16M, respectively. Acid concentration, extent of hydrolysis and the separation technique used have all been implicated in the low yield of nanocrystals from acid hydrolysis of starches

Le Corre et al³⁰. showed that starch nanocrystals are mixed with other microparticles and some nanocrystals were converted to sugar at the end of the production process. This fact results in the observed low yields. They suggested that the amount of starch nanocrystals in the final suspension may depend on the extent of hydrolysis. The study concluded that during the course of hydrolysis both micro- and nanoscale particles are present in the system. Also, differential centrifugation was not suitable for fractionation due to hydrogen bonding and different densities within starch granules. A continuous extraction technique such as microfiltration was suggested as the appropriate separation technique³¹.

A dispersion of uniform starch nanocrystals was obtained using another technique which combined hydrolysis process with ultrasonication. Breaking up the aggregates of nanoparticles formed through hydrogen bonds was observed to be effective by ultrasonic treatment at 60% vibration amplitude for 3 min. However, the starch nanocrystals treated by ultrasonication may have decreased crystallinity. An innovative solution to address the limitations of the current processes for generating starch nanocrystals was developed by Le Corre et al³². It involved the use of a microfiltration unit equipped with ceramic membranes for the filtration of hydrolysates obtained from wheat starch.

Microfiltration limited the conversion of starch nanocrystals into oligo- or monosaccharides. During the 1st day of hydrolysis, the nanoparticles produced were more crystalline and mostly B-type particles. The critical factor in the development of nanocrystals from starch is to maintain the crystalline structure of the starch while completely removing the non-crystalline region of starch granules. Practical application of SNCs acquired using the usual acid hydrolysis method is limited because the conventional preparation method is time-consuming and results in low yield. Ball milling is a cheap and environment-friendly physical processing method that has been confirmed capable of changing properties of starch.

Enzymatic hydrolysis method

This method involves the use of enzymes, such as α -amylase, as a pretreatment to boost the efficiency of preparation. Starch nanocrystals were prepared from waxy rice starch with average diameters of 500 nm using selective enzymatic hydrolysis.

Recent developments are now focused on this method with an aim to mitigate the limitations of acid hydrolysis of starches in the preparation of starch nanocrystals. Enzyme treatment before acid hydrolysis has been proposed to in order decrease the duration of starch nanocrystals preparation. Studies have shown that nanocrystals with platelet-like

structure and length of 20–40 nm with a thickness of 4–7 nm were produced using this method.

Regeneration method

Starch nanoparticles have been obtained from starch solutions using precipitation with organic solvents. Ethanol was used to precipitate starch nanoparticles in the range of 50–100 nm from pre-cooked native starch³³.

Mechanical treatment method

Mechanical and thermal treatment of starch granules can be used to generate starch nanoparticles. A simplified technique involving high-pressure homogenization method was proposed. A specially designed microfluidizer was used through which a 5% starch slurry was passed several times under high shear pressure. Starch particles of sizes reduced into the range between 3–6 μm and 10–20 nm were obtained after 20 passes.

By combining a high-pressure homogenization technique with mini-emulsion cross-linking, sodium trimetaphosphate cross-linked starch nanoparticles were produced. This method is environment-friendly because the mechanical treatment procedure is carried out within a water system. However, the efficiency process is limited as only low concentration starch slurry can be use processed for homogenization, thereby giving a relatively low yield.

Common modifications to starch and starch nanoparticles

Starch has limited application in industry because of poor solubility and high viscosity. Improvement in certain properties and the addition of more functional groups to starch is possible using chemical modifications. Glucose units making up the chain provide sites to be modified with multifunctional groups due to the abundant hydroxyl groups. Improvement of specific functions of the polymer and consequent applications of starch nanocrystals is the main objective of chemical modification. Three approaches have been

commonly used for starch nanocrystals modification. These include modification with chemical reaction with small molecules, grafting onto polymer chains with coupling agents and grafting from polymer chains with polymerization of a monomer³⁴.

Elemental analysis, infrared spectroscopy analysis (FTIR) and X-ray photoelectron spectrometry (XPS) can be used to prove that modification occurred. Monitoring changes in the morphology and size, solubility or polarity, surface properties, and thermal properties can also be used to determine the effects of chemical modification of starch nanocrystals. The integrity of the starch nanocrystal crystalline structure must be intact regardless of the modification method used.

Conclusion

Starch nanocrystals are also known as starch crystallites, microcrystalline starch, and hydrolyzed starch. It is important to distinguish starch nanocrystals from starch nanoparticles, which can be amorphous. Acid or enzymatic hydrolysis, regeneration, and mechanical treatment are three methods used to prepare starch nanocrystals. Various protocols for the preparation of nanocrystals from starches are used, each with its own set of challenges, the most significant of which is low or limited percent yield even after prolonged (days) acid hydrolysis.

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