

SYNTHESIS AND CHARACTERIZATION OF CASSAVA STARCH
NANOCRYSTALS BY HYDROLYSIS METHOD

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ABSTRACT

Cassava starch nanocrystals (CSN) has not been reported in open literature, although other starches such as rice, corn, potato and bean were widely used as the main material. Thus, the objective of this research was to investigate the possibility of synthesizing high yield of CSN at different concentrations of sulphuric acid (H_2SO_4). The physical, chemical and thermal properties of synthesized CSN was also investigated. Synthesized CSN was prepared by hydrolysing native cassava starch (NCS) with several concentration of H_2SO_4 (2.8 M, 3.0 M, 3.2 M and 3.4 M). The acid hydrolysis process took 5 days with continuous stirring speed of 300 to 400 rpm, with constant temperature of 37 °C. The hydrolysed solution of CSN underwent a centrifuging process with distilled water until it was neutralized to make sure that no acid residues remain in the CSN solution. The CSN precipitate was dried in an oven over night at 60 °C. The highest yield (1.1 %) produced was from 3.4 M CSN. Morphological test by transmission electron microscopy indicated that the samples have been destructed and degraded to be nanocrystals with a size range of 5 - 20 nm. X-ray diffraction (XRD) and ^{13}C nuclear magnetic resonance (^{13}C NMR) were used to indicate the type of crystallinity for both NCS and CSN. XRD illustrated that NCS was a C – type patterns, however after hydrolysed (CSN), the crystallinity changed to B – type crystalline starch. This result was also proven in ^{13}C NMR resonance pattern. Fourier transform infrared (FTIR) spectra showed a few peaks shifted after hydrolysis, most of the changes were due to the hydrogen bonding in starch molecules which proved that there were alteration in the inter and intra-molecular hydrogen bonding of starch molecules. The thermal properties showed that glass transition temperature (T_g) was gradually increased as the concentration of the acid increased. This was due to the more energy needed to disrupt crystalline structures which were formed during synthesis process.

ABSTRAK

Kanji ubi kayu nano kristal (CSN) masih belum dilaporkan di sorotan kajian, walaupun kanji lain seperti beras, jagung, kentang dan kacang telah digunakan secara meluas sebagai bahan utama. Oleh itu, objektif kajian ini adalah untuk mengenalpasti kebarangkalian pensintesisan kadar hasil tinggi CSN pada kepekatan asid sulfurik (H_2SO_4) yang berlainan. Ciri-ciri fizikal, kimia dan terma CSN yang disintesis juga dikaji. CSN yang disintesis dihasilkan dengan menghidrolisis kanji ubi kayu asli bersama beberapa kepekatan asid sulfurik (2.8 M, 3.0 M, 3.2 M dan 3.4 M). Proses hidrolisis asid ini mengambil masa 5 hari dengan kelajuan kacauan berterusan 300 hingga 400 rpm, pada suhu malar 37 °C. Larutan terhidrolisis CSN menjalani proses emparan dengan air suling sehingga ianya dineutralkan untuk memastikan tiada sisa asid yang tertinggal di dalam larutan CSN. Mendakan CSN dikeringkan di dalam ketuhar pada suhu 60 °C semalaman. Hasil tertinggi (1.1%) diperolehi dari 3.4 M CSN. Ujian morfologi penularan mikroskop elektron pancaran menunjukkan CSN telah dihancurkan dan didegradasikan menjadi nano kristal dengan saiz antara 5 – 20 nm. Belauan sinar X (XRD) dan ^{13}C resonans magnet nukleus (^{13}C NMR) digunakan untuk mengenal pasti jenis kristal untuk kedua-dua NCS and CSN. XRD menggambarkan bahawa NCS adalah kristal corak jenis C, walau bagaimanapun setelah dihidrolisis (CSN), penghabluran bertukar kepada kristal kanji jenis B. Penemuan ini juga telah dibuktikan oleh corak resonans ^{13}C NMR. Spektra infra merah transformasi Fourier menunjukkan anjakan puncak selepas hidrolisis, kebanyakan perubahan disebabkan oleh ikatan hidrogen pada molekul kanji yang membuktikan perubahan antara dan dalam molekul ikatan hidrogen pada molekul kanji. Sifat terma menunjukkan suhu peralihan kaca (T_g) semakin meningkat dengan pertambahan kepekatan asid. Ini kerana tenaga yang lebih diperlukan untuk mengganggu struktur kristal yang terbentuk ketika proses sintesis.

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Among naturally polymers from renewable resources, starch is one of the most favourable materials. Starch is the economical biopolymer and is totally biodegradable. These two main reasons lead to growing interest in the non-food usage starch based products for industrial applications such as medicine, nanofiber, and cosmetic (Liu *et al.*, 2009).

Starch is a biodegradable polysaccharide produced by many plants as an energy storage polymer. It is found in plant roots, stalks, crop seeds, and staple crops such as rice, corn, wheat, tapioca, and potato. The starch industry extracts the refines starches by wet grinding, sieving and drying. It is either used as an extracted from the plant and is called “native starch”, or it undergoes one or more chemical modifications to reach specific properties and is called “modified starch”.

Composite materials are solid materials composed of a binder or matrix that surrounds and binds together fibrous reinforcements. In the nanocomposites industry, a reinforcing particle usually considered as a nanosize where at least one of its linear dimension is smaller than 100 nm (Dufresne, 2010).

Over the past decade, many academic and industrial researchers have incorporated nanocomposites technology to enhance the properties of these polymers: thermoplastics, thermoplastic elastomers, thermoset and biodegradable polymer. For example, many kinds of thermoplastic based nanocomposites have been prepared such as polyetherimide/clay (Morgan *et al.*, 2001), polyethylene/silica (Monteil *et al.*, 2006), polypropylene/clay (Morlat *et al.*, 2004; Tang *et al.*, 2005), polypropylene/cellulose whiskers (Njungberg *et al.*, 2005) and polystyrene/silica (Schmid *et al.*, 2006) etc. Chen (2008) who conducted waterborne polyurethane/potato nanostarch nanocomposites, found simultaneous enhancement in mechanical properties, such as tensile strength, elongation at break and Young's modulus. With a starch nanocrystals content of only 2%, the strength and elongation of the nanocomposites were improved over those of the genuine waterborne polyurethane.

Starch hydrolysis products are industrially produced by either acid or enzyme reaction. Generally, the enzymatic hydrolysis of granular starches results in poorly hydrolysed starch products due to their low degree of conversion to fermentable sugars (Mu *et al.*, 2013). Xia *et al.* (2012) hydrolysed *Pueraria thomsonii* Benth (PTB) starch by glucoamylase at different lengths of and found that the crystallinity has reduced (43.5 to 20.9 %), which is not a preferable option considering the objective of this thesis is to gain better crystalline region.

Wang *et al.* (1995) hydrolysed sago starch by enzyme hydrolysis method. Raw sago starch mixed with 0.1 M acetic acid – sodium buffer, the solution was incubated at 35 °C with constant shaking. After that, 1 % (weight of enzyme/weight of starch) of isoamylase enzyme was added. Based on the morphology image of untreated and treated sago starch, there were no obvious changes in the size dimension of starch granules. The isoamylase enzyme alone could not attack the sago starch granule. Enzyme hydrolysis method is a risky option where it may contribute to failure and the process steps is very complicated.

Synthesize starch into nanocrystals by acid hydrolysis method has been discovered since early 1996 (Le Corre *et al.*, 2010). Starch acid hydrolysis depends on several factors such as the botanic source (crystalline type, relative proportion of amylose and amylopectin, and granule morphology), conditions of acid hydrolysis (acid type and concentration), temperature and hydrolysis duration (Liu *et al.*, 2009; Le Corre *et al.*, 2010; Lin *et al.*, 2010).

There are two types of inorganic acid that have been widely used to modify starch granule structure and produce “soluble starch”, which are hydrochloric acid and sulphuric acid (Hoover, 2000; Le Corre *et al.*, 2011). Hoover (2000) reported the treatment of native potato starch in water with 15 % sulphuric acid (H_2SO_4) for 30 days at room temperature. He obtained an acid resistant fraction that readily soluble in hot water. This suspension to be known as Nægeli amyloextrin (Kainuma and French, 1971; Gidley and Bociek, 1985). This suspension has been shown is a mixture of low molecular weight, linear, and branched dextrans, with an average degree of polymerization (DP) of 25 – 30 (Hoover, 2000).

Song *et al.* (2008) managed to synthesize corn nanostarch by H_2SO_4 hydrolysis for 7 days. It was then grafted into polystyrene to produce amphiphilic starch-g-polystyrene nanocrystals. X-ray diffraction image showed that there was no crystalline disruption occurred while grafting, but there were some new peaks appeared due to the addition of polystyrene. The study did not compromise the original properties of corn starch, even though polystyrene was grafted upon it. Starch – g – polystyrene can be used as filler of polymer matrix such as natural rubber, polylactic acid and other synthetic polymer.

Similar studies also have been done by Le Corre *et al.* (2011) using waxy maize, sulphuric acid hydrolysed starch sample produced a convincing increment of crystallinity (45 – 55 %). For the first 24 hours of acid hydrolysis, starch nanocrystals were detected. As the hydrolysis time increased, both microscaled and nanoscaled particles were found coexist. The properties of the starch nanocrystals was great, but the yield of the product was too low (0.4 %).

Lintnerized starch was described as an acid treatment of native potato starch in which granules were modified in an aqueous suspension with 7.5 % (w/v) hydrochloric acid (HCl) for 7 days at room temperature (Murh *et al.*, 1984; Bertoft, 2004). The product was a high molecular weight starch, which form clear solution in hot water (Hoover, 2000). However, lintnerization does have its imperfection in term of suspensions stability. Even though the synthesise of cassava starch nanocrystals only took a week, the resulting product showed less stable suspensions compare to H₂SO₄ which is an important characteristic for composite materials processing (Angellier *et al.*, 2004).

Acid hydrolysis shows remarkable result in reducing size and enhancing the amount of crystalline region of starch. However there are few draw backs in which the treatment needs 7 – 40 days (Wang *et al.*, 2006; Song *et al.*, 2006; Wang *et al.*, 2008) to perform, and it produced a very low yield (~ 0.5 wt %) (Angellier *et al.*, 2004).

This research introduces the synthesis of cassava starch nanocrystals which has not been reported in literature using H₂SO₄. It is interesting to investigate the effect of varying acid concentration on the physical, chemical and thermal properties of cassava starch nanocrystals such as crystallinity, morphology, chemical structure by Nuclear Magnetic Resonance (NMR), Fourier Transmission Infrared (FTIR), Transmission Electron Microscopy (TEM), X-Ray Diffraction and Differential Scanning Calorimeter (DSC) analysis.

1.2 Problem Statement

Several studies have been conducted to synthesis starch by acid hydrolysis method such as pea (Chen *et al.*, 2008; Zheng *et al.*, 2009), corn (Song *et al.*, 2008) to produce nanocrystals. They have succeeded in producing starch nanocrystals with high amount of crystalline fraction. However, synthesis of cassava starch nanocrystals has not been reported in literature. Comparing concentration of acid to

gain higher yield and the properties of nano starch produced are an interesting aspect that needs to be investigated.

For this study, the main research question that needs to be answer is:

What are the effects of H₂SO₄ concentration on the yield of cassava starch nanocrystals on physical, chemical and thermal properties?

1.3 Objective of Studies

The objective of this study is to develop a more stable and high crystalline content of cassava starch nanocrystals. The formulations were based on the different concentrations of sulphuric acid in each of cassava starch synthesis process.

The two main focus objectives are:

1. To synthesis cassava starch by sulphuric acid hydrolysis method.
2. To study the effect of sulphuric acid concentration and the yield of cassava starch nanocrystals on physical, chemical and thermal properties.

1.4 Scopes of Study

- 1) Native cassava starch was used to synthesis nanocrystals starch by sulphuric acid hydrolysis. The concentration for each samples were varied at 2.8 %, 3.0 %, 3.2 % and 3.4 % respectively. Duration of hydrolysis was fixed for 5 days which has been suggested by Angellier *et al.* (2004). To obtain a stable

solution, the concentration of H_2SO_4 could not be too mild or too strong. The best range was around 3.0 % to 3.2 % also reported by Chen *et al.* (2008).

- 2) The speed and temperature for centrifuge process (Successive Centrifugal) was set at 11000 rpm and 10 °C to neutralize the solution. Le Corre *et al.* (2011) suggested that the suspension washing (centrifuge) process should be carried out at high rotational speed to avoid loss of material. The suspension was dried overnight in an oven at 60 °C (lower than gelatinization temperature which is at 72.6 °C).
- 3) Scanning electron microscopy (SEM) was used to observe the microsize of NCS at accelerating voltage of 20 kV. For CSN, Transmission electron microscopy (TEM) was tested at accelerating voltage of 100 kV.
- 4) X-ray diffraction (XRD) with diffraction angle (2θ) between 5° - 60° was performed to obtain type of crystallinity of the starch.
- 5) Nuclear magnetic resonance (NMR) and Fourier Transmission Infrared (FTIR) were applied to indicate the starch chemical structure and the characteristic of the component after acid hydrolysis.
- 6) Differential scanning calorimetry (DSC) was used to imply thermal properties of native and hydrolysed cassava starch. Temperature between 40 °C to 140 °C was applied with heating rate of 10 °C/min.

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