Cutting Technique Chain Structure of Amylopectin as Macro-initiator for Biodegradable Copolymers by ATRP

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Abstract. The polymer backbone of cassava starch waste can be used as a source of hydrocarbons as a biodegradable polymer materials backbone. The polymer will then be used as a graft copolymer macro-initiator based on starch and synthetic polymers. For some applications, starch has a lack of properties than synthetic polymers. Starch modifications needed to improve the physical and chemical properties of starch. As a macro - initiator, starch polymers designed needed to be shorter to facilitate the grafting with synthetic polymers. Molecular structure of amylopectin is a very important determinant of starch functional properties.

Amylase and amylopectin were isolated from cassava starch by α-amylase and β-amylase enzyme to reduce degree of polymerization (DP = 2 - 20). The purpose of this study was to shorten the structure of amylopectin chains with enzymatic cutting technique. Cutting technique used to reduce the branch chain of amylopectin. Starch debranching by α-amylase occurred sporadically to straight chain molecules. Debranching of starch by β-amylase happened at alpha 1,6 D-glucose from external side. Amylopectin shorter branch will be converted by hydroxyl groups of amylopectin with Ethyl α - Bromoisobutyrate in homogeneous mild conditions to an ATRP macro initiator. DMF will be used as the solvent of amylopectin ATRP macro initiators with a well-controlled degree of functionalization. The product contained about 12% of water as determined by thermo gravimetric analysis. The versatility of this method allowed us to prepare copolymer based on amylopectin macro initiator with synthetic polymer like propylene a wide range of properties (amphiphilic, ionic, and thermo responsive) by simply changing the solvent composition and the catalyst. This could make possible the synthesis of new interesting biomaterials starting from a wide range of amylopectin.

Keywords: amylopectin structure; α-amylase; β-amylase; biodegradable amylopectin; Atom transfer radical polymerization

Introduction

Starch is the mayor storage of polysaccharide in plants. It can be used for various applications, such as for food, pharmaceutical and other technical applications. It is

composed of two types of glucose polymers; amylose (15-30%) and amylopectin (70-85%). Amylopectin is the highest polymer branches that are composed of glucose residues that linked together with α– (1,4)– and α– (1,6)– D-glucose linkages. While amylose composed of mainly linier glucose chains containing α– (1,4)– D glucose linkages [1]. Form of these two components are semi crystalline structure in the starch granules [2]. Starches degree of crystalline depend on the starches sources, its range about 15-45% [3].

Starch derivative is produced chemically or biologically processed into a variety of different products such as starch hydrolyzed, glucose syrups, fructose, other starch form or maltodextrin derivatives. In spite of the large number of plants able to produce starch, only a few plants are important for industrial starch processing. The major industrial sources are maize, tapioca, potato and wheat.

Starch granules exhibit hydrophilic properties and intermolecular strong associations with hydrogen bonding through the hydroxyl group formed by the granule surface. Because of hydrophilic properties, internal interactions and morphology of starch would be easily modified by water molecules. In addition, the hydrophilic of the starch can be used to increase the degradation rate of some polymers that not easily to degrade. Starch is a biodegradable material in several of environments. Such as: starch is easily to hydrolyze by microorganisms to glucose, and then metabolized into carbon dioxide and water [4]. α-amylase can be used as an enzyme to hydrolyze starch polymers. However for retrograded starches, α-amylase does not work properly. These type of starches are not completely hydrolyzed by α-amylase [5]. Cutting technique to reduce the branch chain of amylopectin can be used by α-amylase and β-amylase. Starch debranching by α-amylase occurred sporadically to straight chain molecules. Debranching of starch by β-amylase happened at alpha 1,6 D-glucose from external side [6].

Solid wastes of industrial Cassava are abundance and might be polluting the environment. Based on experiences, biological changes of waste convert it into other products, such as energy, food, animal feed, organic fertilizer and others. Solid waste of cassava has not utilized yet into product that has more economic benefit. The Indonesian Agency for the research and assessment of technology reported that the solid waste of tapioca starch content in the pulp of 67.8%, the analysis of the carbohydrate content in dry cassava waste by 68%, protein 1.57%, fat 0.26%, crude fiber of 10% and a water content of 20% [7].

The polymer backbone of cassava starch waste can be used as a source of hydrocarbons as a biodegradable polymer materials backbone. The polymer will then be used as a graft

copolymer macro-initiator based on starch and synthetic polymers. For some applications, starch has a lack of properties than synthetic polymers. Starch modifications needed to improve the physical and chemical properties of starch. Of various modifications, like a starch debranching and starch acetylation can be performed with relative ease to improve significantly the physical-chemical and functional properties of starch [8]. As macro-initiator, starch polymers designed to be shorter to facilitate the grafting with synthetic polymers [9-12]. These modification with enzyme hydrolyzed depend on prevalent environmental factors within the reaction system, such as the pH, reaction time, presence of catalyst and concentration [13].

The purpose of this study was to shorten the structure of amylopectin chains with enzymatic cutting technique.

Experimental

Material

Solid waste of tapioca was used as the cellulose material; α-Amylase and β-Amylase Enzymes were purchase from Liquid Sunshine Distillery (Novozyme produced); Na_2CO_3, NaOH, HCl, H_2SO_4, Iodine and Luff Schoorl reagent standard were purchase from Merck.

Equipment

Equipment's used were shaker incubator (Barnstead/Lab-line MaxQ 4000), spectrophotometer (UV/VIS spectrophotometer SP-3000 Plus OPTIMA JAPAN), pH meter (Dakton pH 510 Series), micropipette, centrifugation, hot plate (Hot plate Stirrer), oven (Memmert) and glassware.

Procedure

This research was divided into 2 stages; (1) the liquefaction process with the addition of α-amylase enzyme to find the starch solvent ratio and the reaction time to produce straight chain for 2 h, and (2) the Saccharification reaction time estimation to form shorten amylopectin in order to use the debranching products (straight chain) as substrates.

The liquefaction process with the addition of α-amylase enzyme at pH range 4.1-4.5. Samples were taken after 45 min at a temperature of 80-90^oC. Then proceed with the Saccharification at 60-62^oC with β-amylase enzyme at various concentrations of enzyme and reaction time. Product was neutralized by the addition of Na_2CO_3. Glucose concentration analyzed by using UV-VIS and FTIR.

Isolation of amylase and amylopectin begins with preparing the three types of samples based on ratio of starch and water solvent, in which the ratio of each experiment consist of 1:30 w/v, 1:45 w/v, and 1:60 w/v. Furthermore, each sample was heated at various temperatures is 60^{o}C, 70^{o}C, and 80^{o}C. Each subsequent is cooled to 5°C. Precipitate of amylopectin was filtered in order to separate from amylase. 5 ml of the sample solution is taken dissolved in 1 M acetic acid, distilled water and Iodine solution, and then left a few minutes. Furthermore concentration of amylase were measured using a UV-VIS at a wavelength of 575.4 nm

Characterization

Amylase and Amylopectin Content characterization

12.5 grams Acetylated starch diluted with 100 ml of 0.1 N HCl and 15 ml of n-butanol. The solution heated at 85^{o}C for 35 min and cooled for 24 h at room temperature. The solution centrifuged, to obtain amylase content of starch. After centrifugation the precipitate was dried and then weighed to get amylase content (%). Residue from centrifugation was weighed then added to 200 ml of methanol and then decanted to get amylopectin content (%).

UV-VIS Spectrometer

The UV–Vis spectra of the starch measured at 575.4 nm (measurement range 200-800 nm) were obtained using a Shimadzu UV-Vis spectrometer (Model 2550).

Fourier Transform Infra-Red Spectrometer

Fourier transforms infrared spectrometer (FTIR) was performed using a Bio-RAD FTS-165 spectrometer by KBr disk.

Differential Scanning Calorimeter (DSC)

The glass transition temperature (Tg) of the product was conducted by the differential scanning calorimeter (DSC) analysis with a Perkin-Elmer system under liquid nitrogen. 21-33 mg of Starch sample inserted into the crucible 40 μl. Samples were measured in a crucible with sequence variations in the temperature of 30^{o}C - 90^{o}C - 0^{o}C - 230^{o}C, with a first heating rate of 20°C/min, cooling -20°C/min and second heating 15°C/min. N_2 purging gas at 50 ml/min.

Results and Discussion

Amylase and amylopectin composition in cassava starch

The isolation of amylase and amylopectin result illustrated that during the reaction, the higher enzyme concentration (20, 40, 60 w/v), the reduce amylase and amylopectin concentration (Fig. 1 and Fig. 2).

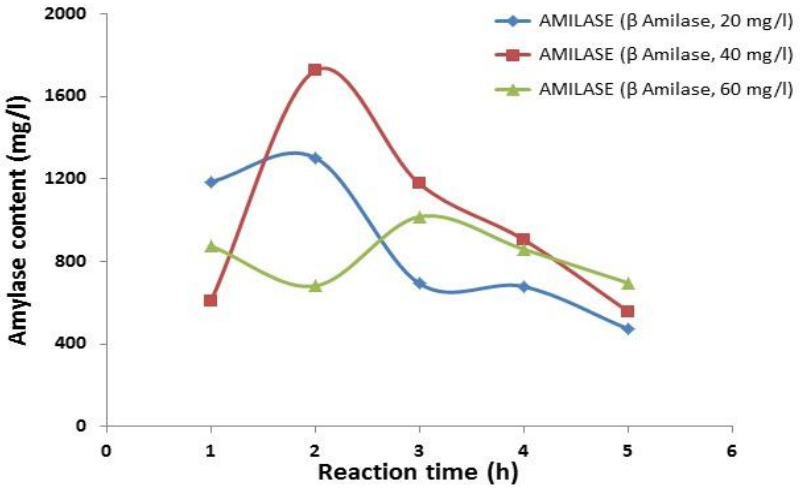

Figure 1. Amylase content after saccarification versus reaction time.

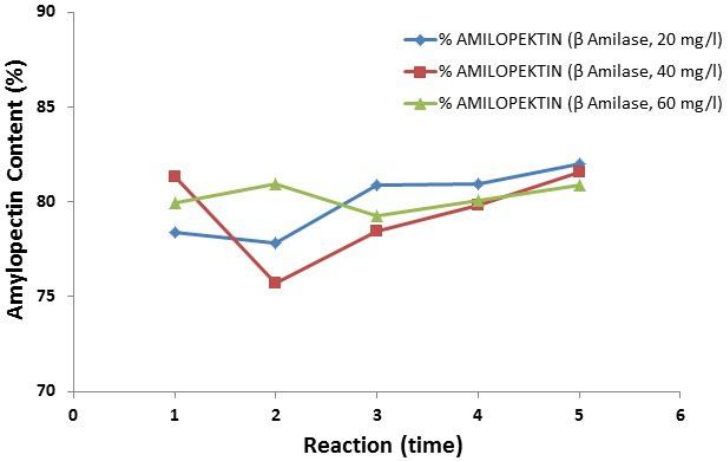

Figure 2. Percentage of Amylopectin content in cassava starch.

Analysis of chain length distribution of Amylopectin by Mark and Houwink equation

Intrinsic viscosity can be used to measure the molecular weight through mark and Houwink equation:

$$[\eta] = K.M^{a} \qquad (1)$$

Where the intrinsic viscosity, K and a are constants typical for polymer - solvent systems at a certain temperature. For amylase-water solvent, used log K = -5.1 and a = 0.9.

Results of the analysis indicate that the different enzymes (20%, 40%, 60%) produce intrinsic viscosity,-0.1381, -0.1395 and -0.1175 respectively. Molecular weight obtained 51,443 gr/mol. 52,022 gr/mol and 42,990 gr/mol respectively. The average lengths of the chains are 285.47, 288.57, and 238.5 respectively. For enzyme concentration of 60% obtained shortest chain length of amylase, it's caused that the highest concentration of the enzyme, which resulted in an enzyme molecule with sporadic break the chains of 1.4 and 1.6 d-glucose contained in amylase and amylopectin simultaneously.

Thermal stability of amylopectin debranching

TGA is widely used as an appropriate method to investigate thermal stability of different organic compounds, especially for carbohydrates polymers [14]. Knowledge of the degradation and decomposition of the model under heat treatment effects is recommended in optimization. Thermo gravimetric curve (TG) and the corresponding derivatives (DSC) to process cassava starch degradation in 30^{o}C min^{-1} is shown in Fig. 3. This figure shows the thermo grams of heat-flow rate at various temperatures for starch on heating and cooling by standard DSC. Rate of heating, and cooling as well, as long as the experiment were 10 C/min. during the first heating 30-500^{o}C.

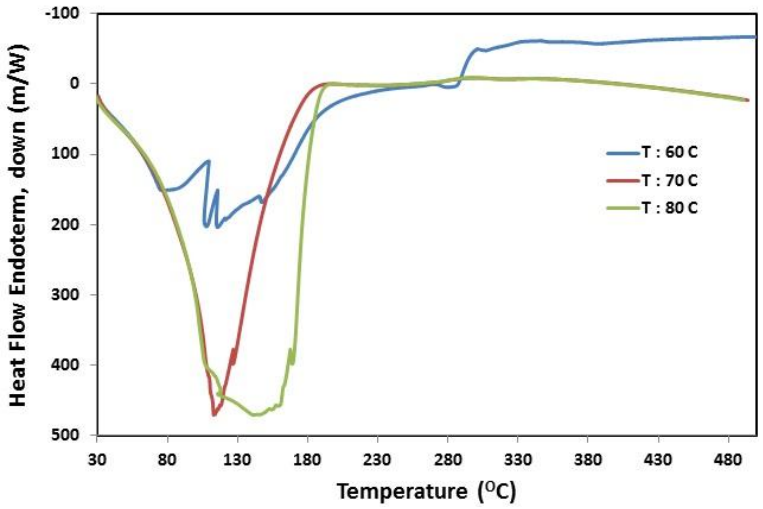

Figure 3. Response DSC and TGA spectrum of the ratio of water / starch 45:1 at difference temperature heating.

Enthalpy is one of the most important parameters during gelatinization to determine the energy input and primarily reflects the loss of molecular order.

Starch with lower crystalline and a less than perfect gelatin has a lower T gelatin. High transition temperatures associated with high crystalline degree, which provide structural

stability and make the granules more resistant to gelatinization. From Fig. 3 shows that, the T saccharification 80^{o}C showed the higher gelatinization temperature compared with the T 70^{o}C and 60^{o}C.

TGA has proved to be suitable method to investigate the thermal stability of different organic compounds especially for carbohydrate [14]. Knowledge of degradation and decomposition under the influence of heat are recommended in the optimization process. TG curve show three main zones (Fig. 4.) in degradation process of starch waste. The first mass loss was Zone 1 between 30–112^{o}C, which is attributed to dehydration that occurs in single step (T 70^{o}C, 2 steps (T 60^{o}C) and single step (T 80^{o}C). after dehydration, the investigated sample is stable up to 130^{o}C for T 80^{o}C, up to 200^{o}C for T 70^{o}C and for T 60^{o}C have two stable zone are zone up to 180^{o}C and up to 380^{o}C.

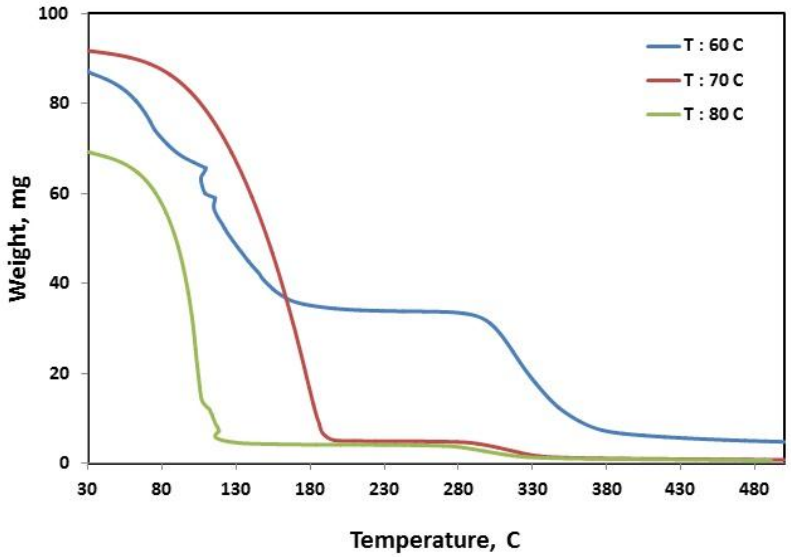

Figure 4. Response of TGA at difference temperature heating.

Fourier Transform Infra Red (FTIR) Analysis

Increasing of the ratio of water/starch provides opportunities for amylopectin to be dissolved in water. This resulted in the reduction of the amount of amylopectin. It was confirmed by an increasing of percent transmission with the addition of water on starch (Fig. 5).

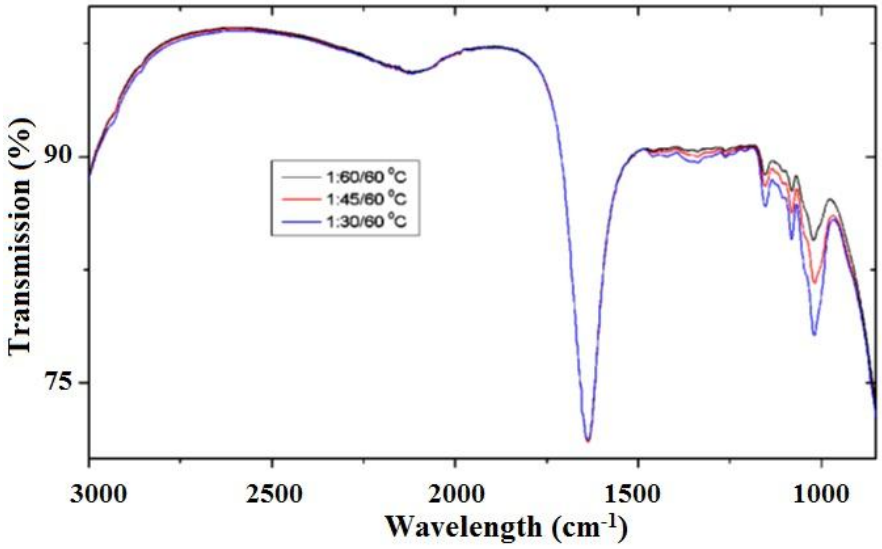

Figure 5. FTIR Spectra of starch waste isolation.

Conclusion

In this study, we evaluated amylopectin content in tapioca solid waste, from this research can be concluded that the Carbohydrate content in tapioca solid waste as a backbone has prospect as degradable polymer material. For the Cutting technique of amylopectin using enzymatic methods can be cut–off amylopectin molecular weight 51,443 gr/mole. 52,022 gr/mole and 42,990 gr/mole respectively. The average lengths of the chains are 285.47, 288.57 and 238.5 respectively

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