Effect of shear rate in high shear mixing process on the structure of cassava starch granule and reducing sugar product

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Abstract. Cassava starch is one of the biopolymers which can be degraded to many useful products such as reducing sugar, non-reducing sugar and modified starch. However, it has a solid granule structure that can inhibit its degradation process. High shear mixing (HSM) is a well-known method for dissolving biopolymers. Shear effect of HSM created by a narrow slit between rotor and stator which can be increased by the increase of rotational speed. By increasing its shear effect, HSM is able to cut the glycosidic bond of starch to produce reducing sugar. Therefore, this study aims to study the effect of high shear rate to granule structure and reducing sugar product. The HSM process was carried out on the suspension of cassava starch with a concentration of 1/20 (g/mL) for 15 min at various stirring speeds (5000-15000 RPM). Products produced from the process are separated between solid and liquid for analysis. Solid products were analyzed using Scanning Electron Microscopy (SEM), Particle Size Analysis (PSA), and Viscometer Ubbelohde. While liquid products were analyzed using UV-Vis Spectrophotometry with the DNS method. Based on the experimental results, HSM produced reducing sugar up to 0.1972 mg/mL. Granule breakage was also observed by SEM and increasing of the nanoscale granule.

1. Introduction
Starch is one of the most abundant biopolymers, which has various applications in the non-food and food industry due to its unique functional properties and relatively low costs [1,2]. Starch has a complex morphological polymeric substance in the form of granules, consists of loose amorphous regions interspersed with very regular crystal regions, which are produced from formation of hydrogen bonds between starch molecules [2]. Because it is composed of glucose monomers, starch can be degraded to produce reducing sugar such as glucose, fructose, and galactose [3,4].

The degradation processing in starch can be done conventionally or non-conventionally to produce reducing sugars. Conventional processes carried out using acids and enzymes which have some disadvantages. In the process of using acid, it creates the undesired side product that requires corrosion-resistant equipment. Meanwhile, the enzymatic process requires a long processing time and sterile equipment. It also needs more strict process control due to its high sensitiveness [5].

The development of technology in non-conventional processes in the past few decades has provided many benefits for researchers by applying environmentally friendly technology. The process of non-
conventional starch degradation has been done using microwave processing [6], hydrothermal processing [7], sonication [8] and high shear mixing [9] give a different effect on starch.

The application of high shear mixing technology will have the effect of homogenizing the dissolution process of starch. This process causes the size distribution to narrow and converge towards the maximum stable size. It is also known that shear degradation of polymer molecules is affected not only by molecular size but also by the branching structure. High branching density and short branch length are associated with a higher susceptibility to shear degradation. The degradation process is not significantly selective for the length of individual branches when the polymer is in a liquid state, but it is preferable to break down the branches again when starch polymers are in the semicrystalline granular form[10]. By dissolving the starch in water, it enables the contact between water and starch molecule so that optimizing the cutting process of the glycosidic bond. Therefore, the increase of shear effect can increase the granule breakdown to raise water-starch interaction and produce reducing sugar.

Previous studies have shown shear treatment that causes an increase in amylose content and viscosity, as well as a decrease in gelatinization temperature and granule size Wang, et al. has been observed shear effect using corn starch. They stated that shear degraded molecule to dissolve starch and lower enthalpy changes [11]. In addition, also the degradation of starch with the effect of shear forces at higher temperatures causes damage to the starch gel texture [12]. Other research performed at high temperature, was able to decrease the viscosity of starch. These results may be relevant for use with the type of starch to be used. High shear mixing with various levels has been studied to modify the functional properties of starch granules [13]. It also decreased the granule crystallinity and increased its solubility [9].

From the explanation above, HSM considered as an effective method for starch physical degradation. By increase its shear effect, HSM can disperse enough energy to increase the production of reducing sugar from starch. In this paper, we studied the degradation method of cassava starch using HSM processing. This study provides valuable new insight into the impact of high shear rate effects on changes in morphology, average molecular weight, and average particle size distribution during HSM processing.

2. Experimental
2.1. Sample preparation
In this work, we used commercial tapioca flour (Budi Acid Jaya, Indonesia) and distilled water. Then the starch-water suspension was made with a ratio of 1/20 (g starch/mL water) in 200 mL reactor. For normal mixing variable, the suspension was stirred by a magnetic stirrer with maximum speed at operating conditions of 35 °C for 15 minutes. For the HSM process variable was carried out at a temperature of 35 degrees Celsius with a predetermined stirring speed (5000, 10000 and 15000 RPM) for 15 minutes. Then the products were separated between liquid and solid by centrifugation. The solid product was freeze-dried before analyzed by SEM, PSA, and Ubbelohde viscometry. Meanwhile, the liquid product was analyzed using UV-Vis spectrophotometry through the DNS method.

2.2. Analytical Method
2.2.1. Scanning Electron Microscopy (SEM). To investigate the structural changes and surface morphology of native and modified cassava starch after high shear mixing processing, starch was observed using a scanning electron microscope (SEM; Inspection s50). Modified starch then coated in a vacuum condition before analysis.

2.2.2. Nanosize – Particle Size Analysis (PSA). PSA preparation was conducted according to Kochkina et al. [14]. The sample of starch was dissolved in 1 M KOH so as to obtain a solution of 0.5 g/L of biopolymer. The hydrodynamic radii (Z-average) of the particles were then determined using a Zetasizer NanoZS analyzer (Malvern, UK) at 25°C. The analyzer employs a patented NIBS (noninvasive backscatter) technology that minimizes the optical path of the laser beam in a sample. The effect of multiple scattering by particles in the dispersion medium is thus levelled. The hydrodynamic radii of the particles were calculated using the Zetasizer NanoZS software.
2.2.3 Viscometer Ubbelohde. The viscometry produced adapted Nakamura et al. [15]. Weigh 0.4 grams of sample and put in a bottle that has been provided. Add 40 mL of aquadest and put the magnetic blade into the bottle. Stir the sample at speed 8 on the magnetic stirrer for 30 minutes at 50°C in water. Then raise the temperature to 100°C for 20 minutes while still stirring in the water bath. Cool the benefits of using running water for 2-3 minutes. Give the benefits of 20 mL of KOH 5 N into a 100 mL volumetric flask containing the sample. Do dilution by adding aquadest to the boundary mark, then filter it. After doing the dilution again for a concentration of 0.1-0.3% using the approved sample (sample concentration 0.4%). Take a time measurement with the Ubbelohde viscometer in a constant temperature bath at 25°C. From the reduced viscosity thus obtained, the intrinsic viscosity was calculated. Average molecular weight calculated using Mark-Houwink-Sakurada constant (K = 0.0000118 and α = 0.89) which obtained from previous research Ahmad, 1999.

2.2.4 Total Reducing Sugar. In order to determine the total reducing sugar, the liquid product from high shear mixing treatment was analyzed using the DNS method as described elsewhere [16]. The DNS reagent was prepared to make a DNS solution with dissolving 1 g of 3, 5-dinitrosalicylic acid in 50 mL distilled water. And then make a NaOH solution with dissolving 1.6 g of NaOH in 15 mL distilled water. After that, mixed the DNS solution and NaOH solution until homogeneous in a water bath at temperature 45°C. And then cooled to room temperature and put in 30 g of potassium sodium tartrate and distilled water in 100 mL. For prepared sample with dissolving 0.2 mL of the sample solution in 1 mL distilled water. And then added 3 mL of reagent DNS, heated for 10 min in a water bath with 100°C and cooled to room temperature for 10 min. After that, throw in 2 mL distilled water for solution. The absorbance of the final solution was measured using a GENESYS 10S UV-Vis spectrophotometer at a wavelength for 504 nm.

3. Results and Discussion
The high shear mixing process is intended to homogenize the suspension of cassava starch and produce reducing sugar. The product produced by the process is then transferred between the liquid product and the solid using a centrifuge for further analysis

3.1. Effect of High Shear Mixing Process on Patient Granule Morphology
The morphology of cassava starch granules before and after the high shear mixing process was observed using SEM (Scanning Electron Microscopy). Solid products produced from the process will be freeze-dried before the analysis process is carried out. Product characterization using SEM (Scanning Electron Microscopy) aims to determine the morphological changes of starch granules after the process. Product results from the high shear mixer process can be seen in figure 1.

Figure 1. SEM micrographs of the surface of granule cassava starch with the magnification of 3000x
(a) native cassava starch, (b) normal mixing, (c) HSM 5000 RPM, (d) HSM 10000 RPM and (e) HSM 15000 RPM

On the results of SEM micrographs, granule of cassava starch were reported to exhibit oval with a smooth surface, truncated shape and clustered with different sizes for native starch (figure 1 (a)) [17]. The SEM micrographs showed that the typical structure of native granules severely changed by
mechanical treatment. From figure 1 (c)-(e), shows that stretching between granules but did not change the granular morphology even though there was some breakdown to the surface of the granule. It might be the shear effect from the process was significantly not able to affect the granule morphology.

3.2. Effect of High Shear Mixing Process on Average Particle Size Distribution
Nanoscale PSA was done to detect the shear effect on nanoscale granule. Relative changes in the diameter of granules, compared to their original sizes were observed using Zetasizer. Solid products produced from the process will be freeze-dried before the analysis process is carried out. Product characterization using Zetasizer aims to determine the average particle size distribution changes of the original starch granules after HSM processing.

| Table 1. Particle size distribution parameter of cassava starch in various processes |
| --- |
| No. | Sample Product | Z-Average (nm) | PdI |
| 1 | Native Cassava Starch | 49.05 | 0.507 |
| 2 | Normal Mixing | 47.19 | 0.579 |
| 3 | HSM 5000 RPM | 114.4 | 0.560 |
| 4 | HSM 10000 RPM | 126.3 | 0.667 |
| 5 | HSM 15000 RPM | 181.3 | 0.479 |

The native starch granules show a well-dispersed average particle size of 49.05 nm. Normal mixing has similar nano-size granule with native form. Meanwhile, significant change happened in HSM treatment variable. Increase of rotational speed resulted in larger size of the nanoscale granule. From the results table 1, it also shows that the increasing rotation speed at 15000 RPM gives the shape of the granule size to be uniform and produced an average granule size of 181.3 nm. It is contrary with SEM image which shows no significant change on microscale granule. It implied that HSM could only trigger significant swelling on nano-scale granule. It might be the shear rate resulted by HSM was able to make a portion of surface degradation. Higher stirring speed produced a higher shear rate that was able to create more surface degradation. It made the granule become more viable to water intrusion. Therefore the granule was swelled and the size was increased.

3.3. Effect of High Shear Mixing Process on Average Molecular Weight
The Ubbelohde viscometer is used to determine the intrinsic viscosity and molecular weight of starch after the high shear mixer process. From Table 2, 5000 RPM and normal mixing variable showed a similar value of average molecular weight which lower than native starches. However, increase the rotational speed increased the average molecular weight of starch up to 841.221 Da. Based on the results of the study, it was found that there was an increase in the value of average molecular weight. It might indicate the possibility of smaller molecular weight degraded. Shear rate is known to cause of high molecular weight compounds in starch. Therefore, intrinsic viscosity can be used as a measure of average molecular weight.

| Table 2. Average Molecular Weight and Intrinsic Viscosity Parameters Cassava Starch as Determine by Viscometer Ubbelohde |
| --- |
| No. | Sample Product | Intrinsic Viscosity (dL/g) | Average Molecular Weight (Da) |
| 1 | Native Cassava Starch | 1.7927 | 663.832 |
| 2 | Normal Mixing | 1.5937 | 581.636 |
| 3 | HSM 5000 RPM | 1.5663 | 570.405 |
| 4 | HSM 10000 RPM | 2.0459 | 770.056 |
| 5 | HSM 15000 RPM | 2.2133 | 841.221 |
Several studies have shown that crystal parts of starch are not susceptible to mechanical treatment. Amylose, which is stored as an amorphous region, have smaller molecular weight than amylopectin. The degradation of smaller molecular weight to soluble sugars can increase the average molecular weight of solid product. This argument supported by the distribution of covalent bonds in amylose molecules can occur almost instantaneously if the force exceeds the bond strength \[2\]. The average molecular weight of starches tend to increase by higher rotational speed was able to degrade some amylose content of starch granule. This supported by several statements that amylose has a smaller molecular weight than amylopectin. Hence, loss of amylose content increased the average molecular weight of solid product.

3.4. Effect of High Shear Mixing Process on Production Reducing Sugar

Cassava starch has the main composition, amylose and amylopectin which is composed of glucose monomers so that it can be used to become reducing sugars through the degradation process. To be able to determine the concentration of sugar after a high shear mixing process, analysis is needed using UV-Vis Spectrophotometry. The liquid product produced from the high shear mixing process was analyzed using UV-Vis spectrophotometry with the DNS method to determine the total concentration of reducing sugars produced after the process.

| No. | Sample Product       | Total Reducing Sugar Concentration (mg/mL) |
|-----|----------------------|-------------------------------------------|
| 1   | Normal Mixing        | 0.0938                                    |
| 2   | HSM 5000 RPM         | 0.1226                                    |
| 3   | HSM 7500 RPM         | 0.1652                                    |
| 4   | HSM 10000 RPM        | 0.1519                                    |
| 5   | HSM 12500 RPM        | 0.1812                                    |
| 6   | HSM 15000 RPM        | 0.1972                                    |

Based on the results of the analysis carried out in figure 2, shows the process of using a stirrer at the maximum speed obtained by the total reducing sugar concentration of 0.0938 mg/mL. While in the high shear mixing process with operating speed increases, an increase in total reducing sugars is produced. This shows that the increasing stirring speed will affect the simultaneous contribution directly to shear for the production of reducing sugars resulting in an increasing product. The highest production of total reducing sugar concentration in the high shear mixing process carried out at the stirring operating speed of 15000 RPM at 35°C for 15 min was obtained at 0.1972 mg/mL. The high shear mixing process can produce more products when compared to conventional processes using a stirrer mixer. On the other hand, this process showed a trend increasing shear rate of HSM can raise the reducing sugar production.
The high shear rate that applied to starch granule increased leached amylose and the ability to cleave the solubilized polysaccharide chain.

4. Conclusions
Degradation of starch carried by HSM affect the granule structure and reducing sugar production. No significant change was observed by the SEM image for all treatment. HSM can increase the average size of nanoscale granule and the average molecular weight of granule. However, HSM can increase reducing sugar production up to 0.1957 mg/mL.

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