PAPER • OPEN ACCESS

Ultrasound Pre-treatment for Intensification of Hydrothermal Process in Reducing Sugar Production from Cassava Starch

To cite this article: Sumarno *et al* 2019 *IOP Conf. Ser.: Mater. Sci. Eng.* **543** 012085

View the article online for updates and enhancements.

Ultrasound Pre-treatment for Intensification of Hydrothermal Process in Reducing Sugar Production from Cassava Starch

Sumarno*, A Bramantyo, P Febriyati, I Gunardi and P N Trisanti

Departemen of Chemical Engineering, Faculty of Industrial Technologi, Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia

E-mail : onramus@chem-eng.its.ac.id

Abstract. Cassava is an abundant natural resource in Indonesia and mostly used as food. Starch, as the main component of cassava, can be converted to many of its derivative product such as glucose, fructose, and other reducing sugar. Conventionally, reducing sugar is produced from starch by acid and enzyme hydrolysis. A hydrothermal process is sophisticated biomass processing method which makes it possible to hydrolyze the starch by water only. However, the reducing sugar productivity of the hydrothermal process still not as high as the conventional process. By ultrasound pre-treatment, a hydrothermal process can be intensified, so that increase its productivity. This study set out to find the effect ultrasound pre-treatment to intensify production of reducing sugar from the hydrothermal process of cassava starch. The experiment was conducted by treating cassava starch suspension using ultrasonic irradiation with various operation time (0-120 minutes) and under a temperature of 40°C. For combination process, the starch suspension is processed under 15 minutes ultrasonic irradiation at 40°C, then continued to the hydrothermal process at 100°C and 100 bar using carbon dioxide as the pressurizing gas. The research found that the combination process can produce 0.693 mg/mL of reducing sugar

1. Introduction

Cassava is an abundant natural resource in Indonesia and mostly used as food. Indonesia produced about 21.8 million tons of cassava per year [26]. Starch, as the main component of cassava, can be converted to many of its derivative product such as glucose, fructose, and other reducing sugar. Reducing sugar is a kind of sugar that has the potential to reduce other compounds. This potential characterized by a hemiacetal bond in the Haworth structure of sugar. This bond can result in an open ring form which has open aldehyde or ketone functional group. Reducing sugar have good fermentability so that making it has more value for pharmaceutical, food and fermentation industries [6].

Cassava starch is composed by amylose (15-30%) and amylopectin (85-70%) [27]. Amylopectin is a branched chain that linked at α-(1,4) and α-(1,6) glycosidic bond. Amylopectin is a branched polymer which builds the amorphous structure, but it has strong hydrogen bonding among its molecule in native starch granule. This made its structure forms double helical chains and tends to build a semicrystalline structure of starch. Amylose is a linear chain linked at α-(1,4) glycosidic bond. It tangled at amorphous regions of the semicrystalline structure of starch. Therefore, their existence in starch constructs tight granule structure which is difficult for water to penetrate inside [5; 12; 21]. These become the inhibition factor for its degradation process.

Strong structure of the starch makes the conventional method need a higher concentration of acid, to optimize the whole process [15]. The application strong acid in degradation that allows high solubilization of starch in mostly solvent (mainly in water) lead to low in processing time. But, this process is to give serious problems in product purification, cause a negative effect on the apparatus because of the corrosion dangerous, and the difficulty in wastewater treatment handling.

Acid and enzyme hydrolysis have been accepted as a common degradation method for starch conversion to reducing sugar process [8; 13; 14]. Hydrolysis of starch has been modeled by Hoover. An interaction between hydronium ion with a glycosidic bond of starch can induce electron transfer between them. The electron from glycosidic bond transferred to hydronium ion, leaving unstable oxygen atom of the glycosidic bond. Unstable oxygen atom gets easily release its bond with D-glucose monomer and associated with OH ion. This glycosidic bond cleavage resulting in lower molecular weight polysaccharide.

For the industrial scale, the conventional acid method need a huge amount of acid to set pH around 1.65. This method also needs high-temperature solubilization around 120°C to ensure starch dispersion. Otherwise, the enzymatic method can overcome the acid and heat demand, but this method operates much longer operating batch time [15]. This encourages the development of non-conventional methods in reducing sugar production for recent years.

It has been an interesting topic to optimize its degradation process through pre-treating and processing with non-conventional methods such as hydrothermal, ultrasound, microwave, and other [2; 3; 17; 25]. Each method has several advantage and limitation. Hence, the combination among non-conventional has the potential to wield more advantage in the starch degradation process. For example, hydrothermal has been combined with several methods to yield more reducing sugar. Hydrothermal has been combined with acid and enzyme. This method can produce more reducing sugar yield than the conventional acid and enzyme process [10].

Ultrasound is one of the non-conventional methods of starch degradation which utilize ultrasonic irradiation. Its wave transmits in bulk liquid resulting acoustic pressure with a sinusoidal cycle. It induces cavitation and produces microbubbles. The microbubble compressed and decompressed, resulting in a hot spot with high temperature and high pressure inside it. The microbubble can be collapsed, then producing high local temperature and high local pressure. It generates radical ion that can help glycosidic bond cleavage then producing oligosaccharide and monosaccharide. The explosion of the microbubble also produces microjet effect which can vigorously impact the starch granule surface [18; 22; 30].

Ultrasonic irradiation can increase granule porosity, decrease the size and disarray crystalline region of starch [7]. These will make water easier to penetrate inside granule. In the hydrothermal process, the reducing sugar production rate increased because more ion product reacted with starch in one time. Therefore, it can increase its reducing sugar production with lower operating temperature as in the hydrothermal process. By lowering operating temperature this also decreases the potential of reducing sugar to degrade further into furfural or 5-HMF [25].

Hydrothermal has become one of the sophisticated methods for polysaccharides degradation since it has the potential to substitute acid and enzyme [1; 2; 20; 23]. It utilizes water at high temperature and keeps it in a liquid phase by operating at high pressure. Higher temperature exhibit increasing of molar volume, then it will weaken hydrogen bonding among water molecule. By weaker hydrogen bonding, water can produce more ionic product concentration, enhancing its ability to degrade polysaccharides. This also decreases polarity of the water so that make water become less polar solvent [9; 16]. By hydrothermal process, water properties can be tuned to a suitable value to achieve the application need. Modification of the water as a solvent can also conduct to increase solvent power properties and able to become a specific reaction media.

One of the possible methods to increase the production of reducing sugar in the hydrothermal process is increasing its process temperature. However, this increases its energy demand and risk to trigger further degradation of reducing sugar into 5-HMF [20] Temperature rises not only increase the production of reducing sugar but also increase its further degradation rate. Consequently, the total reducing sugar

concentration decreases at a specific time and temperature. Controlling the reaction rate at high temperatures will save from the occurrence of further records, and also avoid unwanted products.

The aim of this work was set to perform degradation characteristics study of ultrasonic pre-treatment to intensify hydrothermal process in reducing sugar production from starch. Both the ultrasonic process only and the combination process was carried at various process time (15-120 minutes). For the combination process, 15 minutes of the ultrasonic process was applied before the hydrothermal process. The characterization was divided into granule degradation and reducing sugar production. The product was separated into a solid product and liquid product. The solid product was freeze dried and characterized using SEM and XRD to know granule degradation. The liquid product was analyzed by the DNS method to measure total reducing sugar concentration.

2.   Methods

2.1.   Material

The native cassava starch prepared from fresh cassava. Fresh cassava obtained from Mojokerto Regency, East Java, Indonesia. It shredded to reduce its size. Then it was separated from other residues by soaking in distilled water then it was squeezed. The separation process did three times. The obtained starch then was dried and sorted to 40 mesh.

Sodium hydroxide (NaOH), distilled water was purchased from SAP Chemical, Indonesia. dinitrosalicylic acid (DNS), D-Glucose, and potassium sodium tartrate (PST) was purchased from Merck, Germany. Ultra high purity carbon dioxide gas (CO_2) was purchased from Samator, Indonesia.

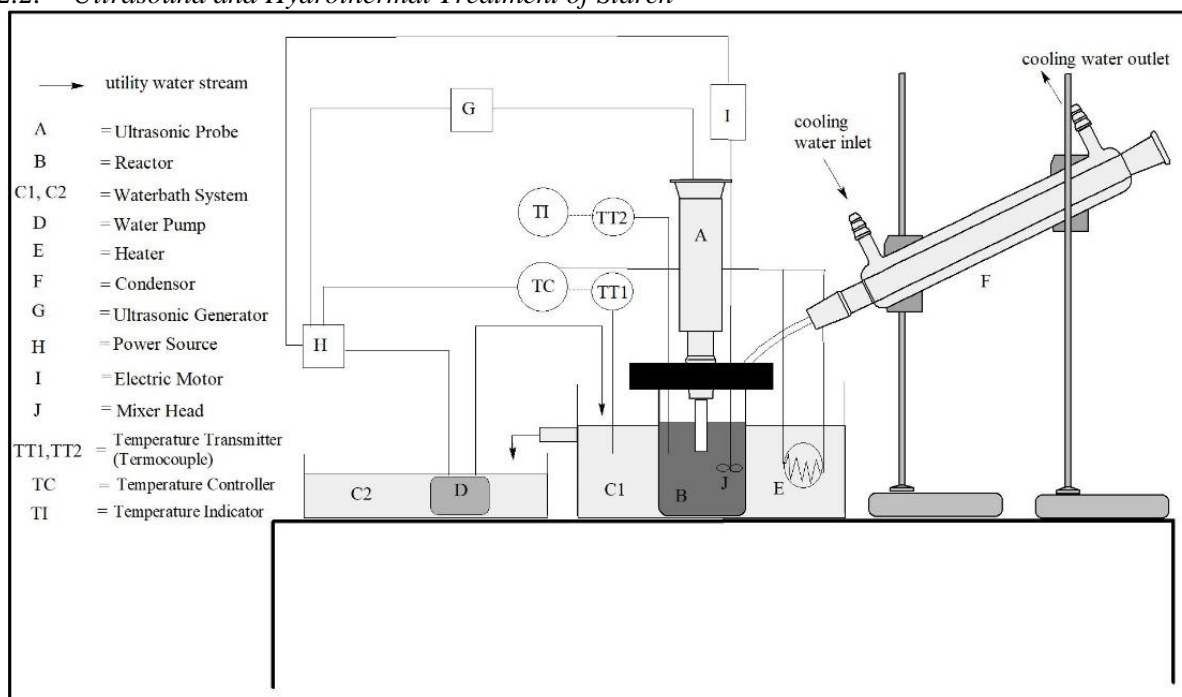### 2.2.   Ultrasound and Hydrothermal Treatment of Starch

Figure 1. Schematic of ultrasound treatment

Direct ultrasonic probe VCX500 (Sonics & Materials, Inc.) with a frequency of 20 kHz and power of 500 watts was used in this experiment. Reactor capacity was 1000 mL. Ultrasound equipment arranged in figure 1. The initial starch suspension used was 1/20 (g/mL) with a volume of 500 mL. Ultrasound treatment operated at 50% amplitude and initial temperature of 40°C. Impeller was used to assist suspension mixing. The condenser was also used to avoid a concentration change effect by vaporization.

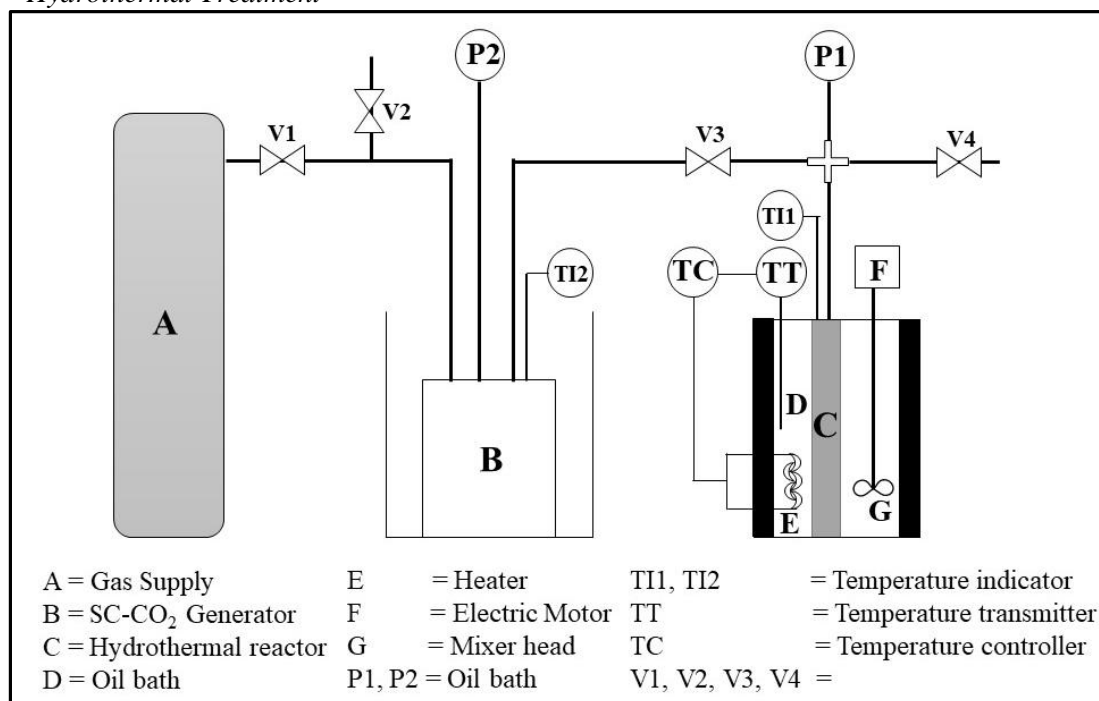## 2.3. Hydrothermal Treatment

Figure 2. Schematic of hydrothermal treatment

The hydrothermal reactor was made from super duplex stainless steel tubing. Tubing system is obtained from Swagelok and arranged as shown in figure 2. Dimensions of the reactor, among others, the outer diameter 1.270 cm, 2.387 cm. Inner diameter, 29 cm in length with a volume of 20 mL. In the reactor is installed K type thermocouple with a size 0.159 cm as a sensor for the temperature inside the reactor in accordance with the setpoint. As a pressure indicator used pressure gauge (Hanyo) with a maximum pressure reading of 343.233 bar (350 kg / cm^2).

The hydrothermal process was done after the sample ultrasonicated for 30 minutes. It was operated at a pressure of 100 bar and temperature of 100°C with the hydrothermal time of 15-120 minutes (interval 15 minutes). The operating temperature condition was set by the heater and the oil bath system. Supercritical CO_2 was used for the pressurizing gas. Supercritical CO_2 was generated by compression and adiabatic decompression in supercritical CO_2 generator (B).

2.4. Scanning Electron Microscopy (SEM)

SEM was conducted by Zeiss Evo MA-10. The sample was frozen by nitrogen liquid before it was snapped to get represent the image of granule morphology. The sample coated by PdAu before characterized. The magnification factor of 2000 was used.

2.5. X-Ray Diffraction (XRD)

XRD was performed by X'pert Pro PANalytical. The signal was processed by OriginPro 2018. Diffraction angle 2θ used at a range between 12° to 28°. The data obtained smoothed by Savitsky-Golay method, 11 points of window and 2nd-degree polynomial order. Relative Crystalline Index (RCI) is calculated according to the previous studies (Wu et al., 2016). It calculated as the ratio between the crystalline area (A_C) and the total area of the crystalline and amorphous region (A_T).

$$RCI = \frac{A_C}{A_T} \times 100\%$$

(1)

A_C was calculated by peak integration with manually select 7 base marker at relatively same diffraction angle on the curve and 5th-degree polynomial as the fitting method. By the same manner, A_T was determined by minimum constant baseline as the fitting method.

2.6. DNS method for total reducing sugar

Dinitrosalicylic acid (DNS) method was adapted from the experiment performed by Miller. Briefly, it utilizes a reduction-oxidation reaction between reducing sugar and DNS. Reduction of DNS form 3-amino, nitrosalycylic acid resulted in orange color. Rochelle salt or PST was used to prevent oxygen dissolving by DNS [19]. The fresh reagent was made by adding 1 gr DNS, 1.6 gr NaOH, PST 30 gr was mixed in 100 mL distilled water. Then 0.2 mL sample diluted into 1 mL solution, then this considered as dilution factor of 5. The standard curve solution was made by 0.05-0.5 mg/mL of glucose for each day of the experiment. After that, 3 mL reagent was added. The solution was heated in a 100°C water bath for 10 min, then it was drenched in cool water. Subsequently, it was diluted by adding 2 mL of distilled water. Afterward, the spectrophotometry conducted by Genesys 10S UV-VIS Spectrophotometer at 502 nm wavelength.

3.   Result and Analysis

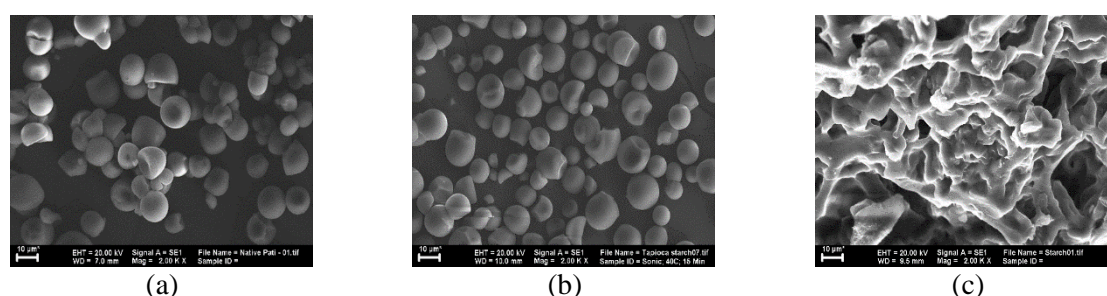### 3.1. Granule breakage effect

Figure 3. SEM result of starch: native or before processed (a), ultrasonicated for 15 minutes (b) and ultrasonicated for 15 minutes continued with hydrothermal process for 15 min (c)

Starch granule consists of amylopectin and amylose which formed as a semicrystalline biopolymer. Native starch has granules which close each other as shown as SEM result in figure 3(a). It forms a tight structure that can prevent the penetration of water inside the granule.

After ultrasonicated as shown in figure 3(b), it exhibited a small portion of surface destruction. No significant granule size reduction can be seen from the figure. However, ultrasonic treatment at 50% Amplitude and 40°C dispersed the granule arrangement. This result was similar to experiment using 170 W ultrasonic treatment of potato starch that only shows some fissure over its external surface without significant granule size reduction [28; 29].

Figure 3(c) show that the granule was severely disrupted. Heat effect conducted by 100°C hydrothermal inducing swelling of the starch granule. As porosity of starch granule grew larger, more water penetrates inside it. Then, at some point granule was not able to maintain its shape. It disrupted. This process often called starch gelatination. This process can extract most part of starch into liquid, then lead to starch solubilization. Solubilization will make glycosidic cleavage easier. By enhancing this process, conversion of polysaccharide into oligosaccharide or monosaccharide will be also increased. Unconverted polysaccharides were reassociated each other and resulting gel-like image like Figure 3. This phenomenon called retrogradation [31].
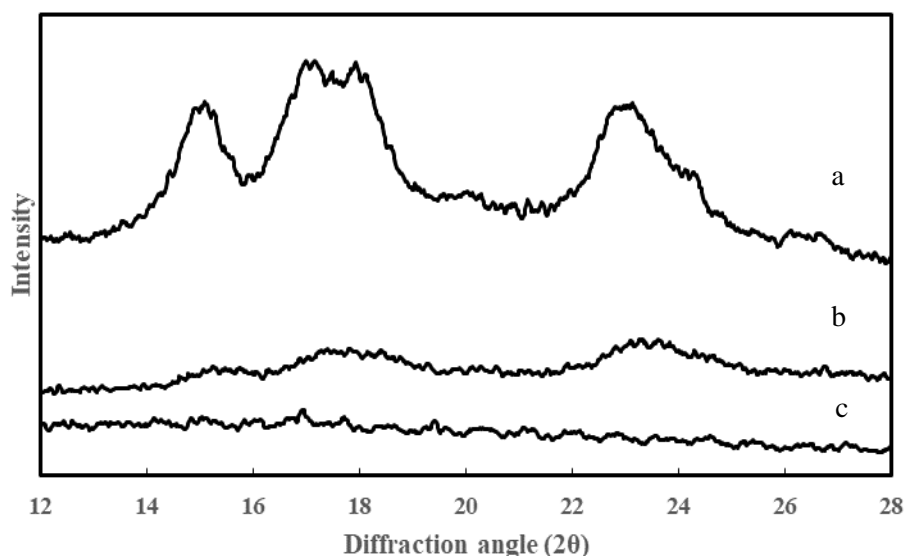
Figure 4. XRD result of starch: native (a), ultrasonicated for 15 min (b) and ultrasonicated for 15 min continued with hydrothermal process for 15 min (c)

From XRD result in figure 4(a), it has four characteristics peak which located diffraction angle (2θ) of 13°, 17°,18° and 23°. Those peaks were formed as four sloppy peaks which represented semicrystalline areas of the starch granule. After ultrasonicated, as represented in figure 4(b), the peak reduced significantly. There was crystalline region degradation by ultrasonic although there is no significant destruction as shown in figure 3(b). Microjet effect pushed water inside the granule. Cavitation might be generated inside the granule, then degrading the whole granule from inside. Some part of the crystalline region was disarrayed. Lost of the crystalline region could decrease starch thermal resistance, then it might increase degradation in the hydrothermal process.

Combination process resulted in the XRD curve as shown in figure 3(c). The process was followed by the disorientation of the main peak of the starch granule (13°, 17°, 18° and 23°). It extracted amylose from the whole structure and disrupted the semicrystalline structure of amylopectin. Disrupted of amylopectin might be made it turned into amorph form as figure 3(c).

Table 1. Relative crystallinty index calculation result for each variable

Variable	RCI
Native (a)	37.47%
Ultrasonicated for 15 min (b)	29.61%
Ultrasonicated for 15 min continued with Hydrothermal Process 15 min (c)	6.41%

The relative crystallinity index (RCI) can be calculated to represent the percentage area occupied as the crystalline area. Commercial cassava used in the experiment has an initial RCI of 37.47% as shown in Table 1. This result quite agreed with other experiment using ACS standard cassava starch which shows result relative crystallinity of 37% [11].

Starch relative crystallinity index slightly decreases to 29.61%. This supported by the XRD result in figure 4(b). The main peak of starch intensity was considerably decreased indicating some crystalline part of starch was broken and converted into amorphous form.

Combination process relative crystallinity index become 6.41% as shown in Table 1. This value represent 82% of crystallinity reduction. These crystallinty reduction show more satisfactory than ordinary Heat Moisture Treatment (HMT) at 100 °C which experimented by Gunaratne and Hoover. HMT only provided 2% of crystallinity reduction [11]. For the combination process, starch suspension

treated with 30 minutes ultrasonic irradiation at 40°C. By ultrasonication, the starch granule had more pore and lower crystallinity index. It became easier to have water penetrate inside. After that, the sample was put in a hydrothermal reactor and processed at 100°C and 100 bar by supercritical CO_2 as the pressurizing gas. The starch granule excessively disrupted and converted most parts of the semicrystalline region become amorphous regions. This was agreed in figure 4(c) which show the intensity of its peak significantly decreased. The combination ultrasound treatment and hydrothermal can greatly decrease the degradation process.

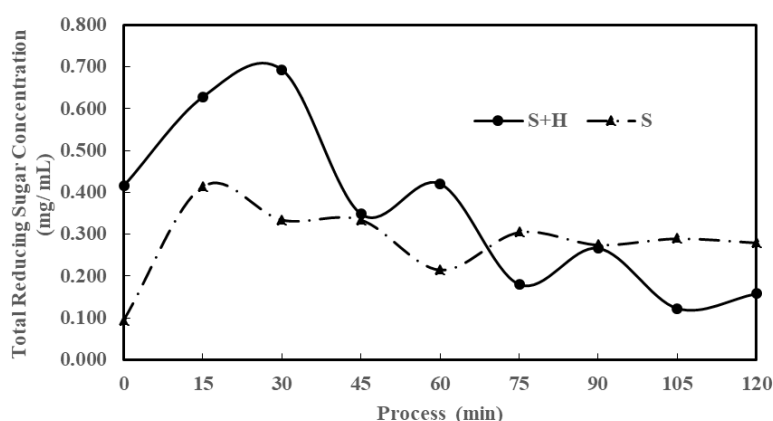*3.2.   Reducing sugar production effect*

Figure 5. Total reducing sugar concentration analysis result on ultrasonicated (S) and ultrasonicated continued with hydrothermal process (S+H)

In figure 5, total reducing sugar production by ultrasound treatment was increased up to 0.415 mg/mL for 15 minutes of the process. Afterward, its total reducing sugar concentration tended to stable in around 0.291 mg/mL. From previous studies, ultrasound has a limitation for degrading polymer and tend to reach a stable point [24]. It also has possibilities to have similar phenomena at starch degradation. Physical degradation by microjet effect extracted some part of amylose and amylopectin. Then, radical ion product generated from ultrasonic irradiation cleaved the glycosidic bond similar mechanism to conventional hydrolysis.

From DNS (figure 5) and XRD (figure 4 and Table 1) analysis result, it shows that the degradation process through pre-treated hydrothermal by ultrasound (S) reducing sugar concentration reached higher maximum concentration than ultrasound only (S+H) for early process time. It possible since the S+H process utilize better crystalline region reduction that S process. Better crystalline region reduction can make starch more viable to water penetration inside granule. The increasing of water viability of starch enhanced gelatinization process brought by the hydrothermal process. The ionic product generated by water dissociation at hydrothermal process cleaved glycosidic bond like conventional hydrolysis. The decreasing of dielectric constant at the hydrothermal process also converting water into a less polar solvent. Water becomes less polar and became more suitable for starch which less polar compound. Both properties assist water to degrade starch become reducing sugar without the presence of an additional solvent.

Therefore it can increase the number of starch reacted at the same time and increase its total reducing sugar concentration to 0.693 mg/ mL. After that, the total reducing sugar concentration tends to decrease over time. This further degradation phenomenon has identical to previous research [20]. It may the hydrothermal process has transferred enough energy so that the produced reducing sugar degrade further into furfural and 5-HMF. This result had much reducing sugar than experiment conducted by Ayoola

et.al. using 1-hour acid process using 1 M H_2SO_4 which resulting about 0.06 mg/ mL of reducing sugar [4].

4. Conclusion

Ultrasonic treatment of 50% Amplitude and 40°C can disperse the granule arrangement and produce reducing sugar up to 0.291 mg/mL. Granule dispersion was followed by reducing the crystalline area without resulting in significant granule size. Combination ultrasonication as pretreatment of hydrothermal at 100°C and 100 bar can completely disrupt starch granule, reducing the crystalline area and increase the produced total sugar concentration up to 0.693 mg/mL.

5. Acknowledgement

References

[1]   Albarelli J, Paidosh A, Santos D, Marechal F and Meireles M 2016 Environmental, energetic and economic evaluation of implementing a supercritical fluid-based nanocelulose production process in a sugarcane biorefinery *Chem. Eng. Trans.* **47** 49-54.

[2]   Alcázar-Alay S C, Osorio-Tobón J F, Forster-Carneiro T, Steel C and Meireles M A A 2017 Polymer modification from semi-defatted annatto seeds using hot pressurized water and supercritical CO_2 *J. Supercrit. Fluids* **129** 48–55.

[3]   Audrey H V 2013 Catalytic depolymerisation of starch-based industrial waste: use of non-conventional activation methods and novel reaction media PhD Thesis, University of Oulu, Oulu, Finland.

[4]   Ayoola A, Adeeyo O, Efeovbokhan V and Olasimbo D A 2013 Optimum hydrolysis conditions of cassava starch for glucose production *J. Adv. Res. IT Eng.* **2(1)** 93–101.

[5]   BeMiller J and Roy W 2015 Starch Chemistry and Technology, 3rd Edition, Academic Press, Oxford, UK.

[6]   Bertolini A., 2010, Starches: Characterization, Properties, and Applications, CRC Press: United States of America.

[7]   Boufi S, Bel Haaj S, Magnin A, Pignon F, Impéror-Clerc M and Mortha G 2018 Ultrasonic assisted production of starch nanoparticles: structural characterization and mechanism of disintegration *Ultrason. Sonochem.* **41** 327–36.

[8]   Cardenas-toro F P, Alcazar-alay S C, Forster-carneiro T and Meireles M A 2014 Obtaining oligo- and monosaccharides from agroindustrial and agricultural residues using hydrothermal treatments *Food Public Health* **4** 123–39.

[9]   Cocero M J, Cabeza Á, Abad N, Adamovic T, Vaquerizo L, Martínez C M and Pazo-Cepeda M V 2018. Understanding biomass fractionation in subcritical & supercritical water *J. Supercrit Fluids* **133** 550-65.

[10]   Gaewchingduang S and Pengthemkeerati P 2010 Enhancing efficiency for reducing sugar from cassava bagasse by pretreatment *World Acad Sci Eng Technol* **46** 727–30.

[11]   Gunaratne A and Hoover R 2002 Effect of heat-moisture treatment on the structure and physicochemical properties of tuber and root starches *Carbohyd Polym* **49** 425–37.

[12]   Hancock R D and Tarbet B J 2000 The other double helix—The fascinating chemistry of starch *J. Chem Educ* **77** 988-92.

[13]   Hernández I P, Pérez-Pimienta J A, Messina S and Saldaña D C E 2012 Dilute sulfuric acid hydrolysis of tropical region biomass *J. Renew Sustain Ener* **4** 021201.

[14]   Hoover R 2007 Acid-treated starches *Food Rev Int* **16:3**, 369-92

[15]   Hull P 2010 Glucose syrups: technology and applications United Kingdom: John Wiley and Sons

[16]   Kruse A and Dahmen N 2015 Water-A magic solvent for biomass conversion *J. Supercrit Fluids* **96** 36–45.

[17]   Martína C, Wei M, Xiong S and Jönsson L J J 2017 Enhancing saccharification of cassava stems by starch hydrolysis prior to pretreatment *Ind Crop Prod* **97** 21–31

[18]   McDonnell C and Tiwari B K 2017 *Comprehensive Analytical Chemistry*, ed E. Ibanez and A. Cifuentes (Amsterdam, Elsevier) p 111-26

[19]   Miller G L 1959 Use of dinitrosalicylic acid reagent for determination of reducing sugar *Anal. Chem.* **31** 426–28.

[20]   Nagamori M and Funazukuri T 2004 Glucose production by hydrolysis of starch under hydrothermal conditions *J. Chem. Technol. and Biotechnol.* **79** 229–33.

[21]   Pérez S and Bertoft E 2010 The molecular structures of starch components and their contribution to the architecture of starch granules: A comprehensive review *Starch/Staerke* **62** 389–20.

[22]   Pingret D, Durand G, Fabiano-Tixier A S, Rockenbauer A, Ginies C and Chemat F 2012 Degradation of edible oil during food processing by ultrasound: Electron paramagnetic resonance, physicochemical, and sensory appreciation *J. Agric. Food Chem.* **60** 7761–68.

[23]   Posmanik R, Cantero D A, Malkani A, Sills D L and Tester J W 2017 Biomass conversion to bio-oil using sub-critical water: Study of model compounds for food processing waste *J. Supercrit. Fluids* **119** 26–35.

[24]   Price G J and Smith P F 1993 Ultrasonic degradation of polymer-solutions: 2. the effect of temperature, ultrasound intensity and dissolved-gases on polystyrene in toluene *Polymer* **34** 4111–17.

[25]   Rogalinski T, Liu K, Albrecht T and Brunner G 2008 Hydrolysis kinetics of biopolymers in subcritical water *J. Supercrit. Fluids* **46** 335–41.

[26]   Statistics Indonesia 2015 *Production of food crops* Subdirectorate Food Crop Stat Indonesia, Jakarta, Indonesia

[27]   Srichuwong S, Sunarti T C, Mishima T, Isono N and Hisamatsu M 2005 Starches from different botanical sources I: Contribution of amylopectin fine structure to thermal properties and enzyme digestibility *Carbohydr. Polym.* **60(4)** 529–38.

[28]   Sujka M 2017 Ultrasonic modification of starch–Impact on granules porosity *Ultrason. Sonochem.* **37** 424–29.

[29]   Sujka M and Jamroz J 2013 Ultrasound-treated starch: SEM and TEM imaging, and functional behaviour *Food Hydrocoll.* **31** 413–19.

[30]   Suslick K S, Didenko Y, Fang M M, Hyeon T, Kolbeck K J, McNamara III W B, Mdleleni M M and Wong M 1999 Acoustic cavitation and its consequences Philos. *T. R. Soc. A* **357(1927)** 335–53.

[31]   Wang S, Li C, Copeland L, Niu Q and Wang S 2015 Starch retrogradation: A Comprehensive Review. *Compr. Rev. Food Sci. Food Saf.* **14(5)** 568–85.

[32]   Xue Z, Liu L and Zhang M 2017 Study on optimization of reducing sugar yield from corn stalk based on microwave-assisted acid pretreatment process *Chem. Eng. Trans.* **62** 1315-20