The effect of size and solid content in hydrolysis of sweet potato starch using endogenous beta-amylase enzyme

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Abstract. Sweet potato contains significant amount of endogenous amylase enzymes. The endogenous enzymes can be used in maltose production from sweet potatoes to carry out pre-hydrolysis of the starch to some extent, follow by complete hydrolysis using external enzymes. The main objective of the current study is to investigate the effect of particle size and solid to liquid ratio in hydrolysis process of sweet potato starch using endogenous enzymes. The extent of hydrolysis process is measured by dextrose equivalent of the crude maltose solution. Hydrolysis process consists of liquefaction and saccharification process. The liquefaction process is carried out at 71.5°C and pH 6 for 25 minutes while saccharification process is carried out at 53°C and pH 5.5 for 72 hours. Dextrose equivalent is measured using Lane-Eynon method. The results show that the access of enzyme to the potato tubers is the key to hydrolysis process. The smaller size of tubers gives bigger surface area while lower solid-to-liquid ratio increases enzyme mobility but decreases sugar concentration. Based on the results, a simulation shows that the pre-hydrolysis of sweet potatoes using endogenous enzymes can save almost a half of enzymes required for traditional hydrolysis process.

1. Introduction

Maltose is a disaccharide sugar formed from two units of glucose molecules. Even though maltose is less sweet than table sugar and fructose, it has been commonly used as ingredients in production of hard candies and frozen desserts due to its unique tolerance to heat and cold conditions. Nowadays, maltose is frequently used as sweetener replacing sucrose and fructose. Food companies have attempted to avoid an increasing negative public perception of fructose by replacing high-fructose syrup with high-maltose syrup. Maltose syrup can be produced by starch hydrolysis using β-amylase enzymes [1–3].

Sweet potato (Ipomoea batatas) is a tropical dicotyledonous plant and has large, starchy, sweet-tasting tuberous roots. Sweet potato starch is widely used in manufacture of food starch and sweetener; sizing of paper; as well as in textile industry [4]. The pulp is used for animal feed while the juice and spent process water is used for fertilizer or as a feedstock for biogas production. A number of study were also carried out in the use of sweet potato as a feedstock of the production of chemical precursors such as bioethanol and biobutanol [5–8]. In Indonesia, sweet potato is widely grown and the annual production exceed around 2 million tones and about 90% is used as food and its derivatives [9].

A sweet potato tuber contains around 22-28% of starch or about 70-80% in dry-basis [9]. The protein content of sweet potato is not more than 1% but a part of it forms as endogenous α-amylase and dominantly as β-amylase enzymes [1]. These enzymes can readily hydrolyze the starch into simple...
sugars in favorable conditions. A local famous snack called “ubi-cilembu” is made from a baked sweet potato where the potato is heated at 50-60°C prior to completely baked. The sweet taste arises from starch hydrolysis products. Since β-amylase enzyme is dominant in sweet potato, the hydrolysis product is dominated by maltose sugar.

As sweet potato is rich of starch as well as its endogenous amylase enzymes, the production of maltose syrup from sweet potato may be more suitable. Nevertheless, almost no publication reports the uses of sweet potato endogenous enzymes for carry out auto-hydrolysis in maltose syrup production.

In the current study, the utilization of sweet potato endogenous enzymes in carrying out starch auto-hydrolysis was investigated. Since hydrolysis reaction is heterogenous, thus contact area affects the reaction rate. Furthermore, enzyme mobility during hydrolysis reaction is also affect the reaction rate. Therefore, two process variables i.e., sweet potato particle size and solid to water ratio were examined to determine the effect of those variables on hydrolysis process.

2. Materials & Methods

2.1. Materials
Sweet potatoes (SPs) were bought from local market. The tuberous roots were washed thoroughly. About 3 portions of 300 g of SPs were prepared differently i.e.: chopped into 0.5 cm dices, grated using a kitchen grater, and milled in slurry using a kitchen blender. A group of sample contains 5 individual samples that every sample was taken out along the saccharification process for analysis. Thus, every portion was divided into 20 g samples and transferred into 15 ml falcon tubes. Every 5 samples were diluted with water with ratio of 1:1; 1:3 and 1:5. There were 9 sample groups for every SPs preparation and solid-to-liquid ratio. The samples were directly used to avoid an extensive bacterial deterioration.

2.2. Methods
The pH of all samples was adjusted to value of 6.0 by addition of 1 N HCl or 1 N NaOH. The samples were then stirred for about 2-3 minutes [10] using stirring rod. Furthermore, all samples were incubated at temperature of 71.5°C for 30 minutes to carried out liquefaction process.

After liquefaction process, all samples were readjustment to pH of 5.5 by addition of 1 N HCl. During pH readjustment, the samples were stirred for about 2-3 minutes using stirring rod. The saccharification process was carried out in temperature of 53°C for 72 hours. Every certain period of time, one falcon tube from every sample group was taken out for analysis. The analyses were carried out directly afterward.

2.3. Analyses
In every sample, the liquid was separated from the solid by filtration using ordinary filter paper. The solid was dried at temperature of 60°C for a night, while the liquid was centrifuged at 3000 rpm for 5 minutes to separate the starch from hydrolysate. The hydrolysate was then directly analyzed using Lane-Eynon method [11,12].

3. Results and Discussions
Sweet potato contains number of endogenous enzymes such as α–amylase and β–amylase, while the β–amylase enzyme is about 5% of total protein contained in sweet potato [1]. These enzymes carry out hydrolysis reaction to break down its starch polymer into simple sugars such as monosaccharides (e.g. glucose) and disaccharides (e.g. maltose). The hydrolysis reaction is a heterogenous reaction where the enzymes must diffuse from bulk of solution into a polymer surface to carry out a hydrolysis reaction. Certainly, the hydrolysis process is affected by the mobility of enzymes as well as the surface area that enzyme can be attached [13].

The mobility of enzymes usually is affected by its dilution in water. The more dilute the enzyme, the more mobile the enzyme. On the contrary, more water is unfavorable to sugar solution as the main
product. The product concentration is preferred to be high enough to minimize the further separation process. In addition, the large surface area is usually provided by smaller particle size. However, preparation of smaller particles needs more energy, thus increase production operating cost. In this study, the effect of dilution and particle size of sweet potato to the hydrolysis process using endogenous enzymes is investigated.

This study carried out hydrolysis processes of sweet potatoes in different particle sizes and dilution ratios. The results of these hydrolysis processes are shown in figure 1. The dextrose equivalent (DE) indicates sugar contain in the solution. Along the time, the hydrolysis reaction was carried out in every samples and resulted increasing DE values. However, the hydrolysis of chopped and grated sweet potatoes resulted low dextrose equivalent (DE) values in every dilution ratio, while hydrolysis of sweet potato slurry in 1:3 and 1:5 dilution ratio results more than 3 times higher.

Diced and grated sweet potatoes have less surface area thus hinder the transportation of enzymes into the starch surface even though in dilute solutions. Figure 1 shows that the particle sizes of diced and grated sweet potato were still big enough thus hinder the enzymes to access the inner starch polymer. In

**Figure 1.** The profile of dextrose equivalent (%) during saccharification process of (▲) milled; (●) grated; and (■) chopped sweet potatoes with solid-to-water ratio of (a) 1:1; (b) 1:3; and (c) 1:5.

**Figure 2.** Comparison of sugar production at different dilution ratio from hydrolysis of slurry, grated and chopped sweet potato using its endogenous amylase enzymes.
the hydrolysis of sweet potato slurry especially in high dilution ratio, the enzymes could easily access the starch polymer and yielded higher hydrolysis product with high sugar concentrations [14]. Nevertheless, the sweet potato slurry could not be hydrolysis successfully in low dilution ratio such as 1:1. Low dilution ratio leaded to low mobility of sugars produced from hydrolysis reactions thus stimulated an accumulation of sugars around enzymes. The high sugar concentration around enzymes might inhibit the hydrolysis reactions [15]. The product inhibition is usually occurred in enzymes mechanism due to its allostERIC property.

The hydrolysis of sweet potato slurry in higher dilution ratio yielded higher DE values compare with that of hydrolysis of chopped and grated sweet potatoes. Even though the DE difference was trivial, the product volume made them different. This means that hydrolysis of potato slurry in dilution ratio of 1:5 produced more sugars in the solution (figure 2) and degree of hydrolysis is much higher.

According to International Starch Institute [4], maltose syrup has DE value of 50-55%. If this DE value is a target, endogenous enzymes in sweet potato can serve an adequate preliminary hydrolysis prior to ordinary enzymatic hydrolysis using external enzymes. Lin [16] shows that 0.033 mg amylase enzymes with activity of 750 IU/mg protein could increase the DE value of 87 point. These data were then used to develop a simple proportional calculation to compare the enzyme requirement of an ordinary hydrolysis process using sole external enzymes with that of a hydrolysis process with a help from endogenous enzymes. The simulation show that the use of endogenous enzymes can save about half of external enzymes (figure 3).

**Figure 3.** Comparison of external enzymes requirement in starch hydrolysis using sole external enzymes with that using endogenous enzymes followed with external enzymes addition

In conclusion, this study has shown that endogenous enzymes contained in sweet potato is very useful for preliminary starch hydrolysis with significant results. Furthermore, it also shows that endogenous enzymes in sweet potato may reduce the requirement of external enzymes needed to complete the hydrolysis to certain extend. These results are very useful for sweet potato hydrolysis process design which includes preliminary hydrolysis using endogenous enzymes. In addition, these results also contribute data and knowledge for further research and development.

**References**

[1] Nakamura K, Ohto M A, Yoshida N and Nakamura K 1991 Sucrose-induced accumulation of β-amylase occurs concomitant with the accumulation of starch and sporamin in leaf-petiole cuttings of sweet potato *Plant Physiol*. 96 902–9

[2] Das R and Kayastha A M 2019 Enzymatic hydrolysis of native granular starches by a new β-
Amylase from peanut (Arachis hypogaea) *Food Chem.* **276** 583–90

[3] Pereira C R, Resende J T V, Guerra E P, Lima V A, Martins M D and Knob A 2017 Enzymatic conversion of sweet potato granular starch into fermentable sugars: Feasibility of sweet potato peel as alternative substrate for α-amylase production *Biocatal. Agric. Biotechnol.* **11** 231–8

[4] International Starch Institute 2006 *Starch Technology: Sweet potato starch production* vol 1

[5] Zhang L, Chen Q, Jin Y, Xue H, Guan J, Wang Z and Zhao H 2010 Energy-saving direct ethanol production from viscosity reduction mash of sweet potato at very high gravity (VHG) *Fuel Process. Technol.* **91** 1845–50

[6] Weber C T, Trierweiler L F and Trierweiler J O 2020 Food waste biorefinery advocating circular economy: Bioethanol and distilled beverage from sweet potato *J. Clean. Prod.* **268**

[7] Virgínio e Silva J O, Almeida M F, da Conceição Alvim-Ferraz M and Dias J M 2018 Integrated production of biodiesel and bioethanol from sweet potato *Renew. Energy* **124** 114–20

[8] He C R, Huang C L, Lai Y C and Li S Y 2017 The utilization of sweet potato vines as carbon sources for fermenting bio-butanol *J. Taiwan Inst. Chem. Eng.* **79** 7–13

[9] Ginting E, Utomo J S and Yulifianti R 2015 Potensi Ubijalar Ungu sebagai Pangan Fungsional *Iptek Tanam. Pangan* **6**

[10] Pontoh J and Low N H 1995 Glucose syrup production from Indonesian palm and cassava starch *Food Res. Int.* **28** 379–85

[11] H.C.S. de Whalley 1964 *ICUMSA Method of Sugar Analysis* (Amsterdam)

[12] Hildreth A . and Brown G B 1942 Modification of Lane-Eynon Method for sugar determination *J. AOAC Int.* **25** 775–8

[13] Snow P and O’Dea K 1981 Factors affecting the rate of hydrolysis of starch in food *Am. J. Clin. Nutr.* **34** 2721–7

[14] Bjorck I, Granfeldt Y, Liljeberg H, Tovar J and Asp N 2018 Food properties affecting the digestion and absorption of carbohydrates *Am. J. Clin. Nutr.* **59** 699–705

[15] Yankov D, Dobreva E, Beschkov V and Emanuilova E 1986 Study of optimum conditions and kinetics of starch hydrolysis by means of thermostable α-amylase *Enzyme Microb. Technol.* **8** 665–7

[16] Lin H J, Xian L, Zhang Q J, Luo X M, Xu Q S, Yang Q, Duan C J, Liu J L, Tang J L and Feng J X 2011 Production of raw cassava starch-degrading enzyme by Penicillium and its use in conversion of raw cassava flour to ethanol *J. Ind. Microbiol. Biotechnol.* **38** 733–42