Effect of Sodium hydroxide Steeped of Low-grade Maize on Enzyme Hydrolysis

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DOI:

ABSTRACT

Effect of NaOH on low-grade maize hydrolysis by enzyme was studied. The low-grade maize was steeped in 0.5%w/v NaOH at 60 °C for 17 h before using in liquefaction. The α-amylase was used in liquefaction to hydrolyze polysaccharides into oligosaccharide and maltose. In the liquefaction process, the α-amylase concentrations (12, 24, 36, 48, 60, 72, 84, 96 and 108 unit/g of maize) at 6 h were studied. The highest of maltose contents at 6 h of control and NaOH steeped maize were 85,631.11 mg/l and 82,191.67 mg/l when used α-amylase concentration of 96 and 72 U/g, respectively. With NaOH steeping, the reducing sugar was higher than control maize, meanwhile it spent the low concentration of enzyme for hydrolysis. Using glucoamylase in saccharification with liquefied hydrolysate obtained from 72 U/g α-amylase, this was applied to hydrolyze maltose into glucose. The glucoamylase concentration (50, 75, 100, 125, 150, 175 and 200 unit/g of maize) and digestion time (every 12 hours for 60 hours) were performed to

produce the highest glucose amount. The highest glucose content at 48 h of NaOH steeped maize was 103,276.67 mg/l that observed in glucoamylase concentration of 125 U/g.

Keywords: low-grade maize, sodium hydroxide, enzyme hydrolysis, liquefaction, saccharification

INTRODUCTION

Starch is the major carbohydrate of maize, averages about 70-72% starch (dry basis) of the kernel. Normal maize starch consists of about 75 %wt branched amylopectin and about 25 %wt amylose, that is linear or slightly branched (Spier et al., 2012). There are also some proteins and lipids in maize starch granules (Abiose and Ikujenlola, 2014). Maize starch is enormous quantity which provides an almost unlimited raw material supply to produce many kinds of starch production. The common food ingredient from maize starch, used in thickening sauces or soups, and in making corn syrup and other sugars (Jeffrey and Maria, 2014). The low-grade maize is the most of the broken kernels and consist of the other components of maize that cause to low cost.

Alkali treatment is widely used in the process of many traditional foods (Cai et al., 2014). Especially, sodium hydroxide (NaOH) can also aid in the extraction of starch by soaking raw materials in alkali solution, it can be easy to separate starch from the binding protein, simplify the starch extraction process, and improve the purity of starch products (Karim et al., 2008; Han and Hamaker, 2002; Ragheb et al., 1995). Moreover, alkali treatment is a simple and effective method of regulating the crystal structure and processing properties of starch and can be applied to the development of starchy food under various digestibility and starch carriers (Han and Lim, 2004; Qiao et al., 2016; Tamaddon and Kazemi, 2017). Each method has advantages and disadvantages, the thermo alkaline pretreatment changes drastically the appearance in some area of kernels (protein, lipids and its principal component, starch) (Palacios-Fonseca et al., 2013). Therefore alkaline pretreatment starch process that resulting in high reducing sugar yield after enzyme hydrolysis. However, alkali pretreatment should be adjusting the

optimal pH with acid before enzymes hydrolysis process. The resulting in a high cost of chemical using both of alkaline and acid.

Glucose is an important primary chemical or intermediate that can be transformed into products of medium (fructose or gluconic acid) to high added value (penicillin). Industrial manufacturing of dextrose hydrolysates involves two successive steps: liquefaction, carried out after, or together with gelatinization by the action of α-amylase; and saccharification, to induce further transformation of maltodextrins into glucose by glucoamylase (Govindasamy et al., 1997). The objective of this study was to know the effect of NaOH on enzyme hydrolysis of low-grade maize. Enzyme concentration and reducing sugar were investigated in this research.

MATERIAL AND METHODS

Materials

Low-grade maize grain was obtained from Phattananikom Kaset Company (Phrea, Thailand). Maize grains were ground by hammer mill (ASAKO, Thailand) and sieved to give around 2 mm particle size. The low-grade maize sample that was used in this investigation approximately composed of 84.47% starch, 8.70% protein, 2.96% fat or oil, 3.08% fiber, 1.45% ash and 2.46% moisture.

NaOH treatment

Low-grade maize sample was treated by steeping in 0.5% w/v NaOH aqueous solution. Maize sample (25 g dry basis) was accurately weight and added 150 ml of NaOH solution in ratio of 1:6, maize to NaOH solution with continuous agitation at 60°C for 17 h. The control experiment was used distilled water instead of NaOH. After steeping in NaOH solution, the starch slurry was neutralized with 1 M HCl to pH 6-6.5. Control and NaOH treated maize slurry were gelatinized by autoclaving at 121°C and 15 psi pressure for 15 min.

Liquefaction

The gelatinized maize starch from control or NaOH steeped maize was liquefied by adding α-amylase enzyme. The experimental condition was various α-amylase concentrations of 12, 24, 36, 48, 60, 72, 84, 96 and 108 unit/g of maize. The maize slurry was continuously shaken to obtain uniform dispersion. The digestion was hold in a constant temperature water bath at

75°C. The liquefied hydrolysate was collected at 6 hours. The liquefied hydrolysate from each experiment was centrifuged at 3000 rpm for 10 min. The reducing sugar of supernatant was analyzed by using the dinitrosalicylic acid; DNS method (Miller, 1959). The absorbance was measured at 540 nm by using a UV/visible spectrophotometer (HP 8453, Hewlett Packard, Germany). Maltose was used as a standard. Each analysis was performed in triplication.

Saccharification

The hydrolysate of NaOH from liquefaction was again hydrolyzed by the aqueous solution of glucoamylase with various concentrations (50, 75, 100, 125, 150, 175 and 200 unit/g of maize). The saccharified hydrolysate was kept in every 12 hours for 60 hours. The maize hydrolysate in this process were digested at 55°C in a water bath for various periods while being continuously shaken at about 150 rpm. The supernatant of hydrolysate was analyzed for reducing sugar as a glucose content by using DNS method.

Dextrose equivalent (DE) from liquefaction and saccharification was calculated as following:

$$DE = (\text{g reducing sugar}) / (\text{g dry solid weight}) \times 100\%.$$

The residue yield from hydrolysate was determined as

$$\% \text{ yield} = (\text{dry weight of hydrolysate residue, g}) / (\text{dry weight of low-grade maize, g}) \times 100\%.$$

Statistical analysis

Statistical analysis was done for some selected results. ANOVA and Tukey's-b were performed as multiple range tests to compare means at $p<0.05$ significant level by using the software (SPSS Statistic 17.0).

RESULTS

Liquefaction

In liquefaction process, in Figure 1, the α-amylase enzyme concentration (12, 24, 36, 48, 60, 72, 84, 96 and 108 unit/g of maize) at 6 hours were studied. The content of maltose was investigated because α-amylase is enzyme that catalyze the hydrolysis of the internal α-1, 4-glycosidic linkages in starch, converting starch into low-molecular-weight products. The maize starch was high efficiency digestion that showed in a high content of maltose

as possible. The maltose contents were calculated in term of DE values. DE values of control and NaOH steeped maize were increased when α-amylase increased. Mostly, the DE value of NaOH steeped maize was higher than control maize. The α-amylase concentrations of 72 and 96 U/g were presented the highest of DE values for NaOH and control, respectively. In these concentrations, the highest of maltose contents were showed about 82,191.67 and 85,631.11 mg/l for NaOH and control, respectively.

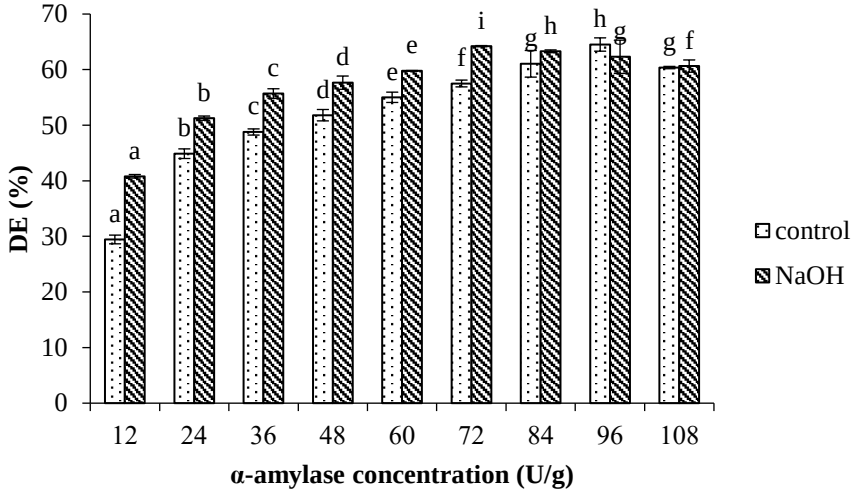

Figure 1. DE value of control and NaOH steeped maize in liquefaction.
abcd represent significant differences ($p<0.05$)

Saccharification

The liquefied hydrolysate from NaOH treatment which shown the highest DE value at α-amylase concentration of 72 U/g for 6 h was continuously hydrolyzed in saccharification. In saccharification, the highest of reducing sugar was determined by variables of glucoamylase enzyme concentration (50, 75, 100, 125, 150, 175 and 200 U/g) and digestion time duration (0, 12, 24, 36, 48 and 60 h). The amount of reducing sugar that expressed as glucose was observed because the glucoamylase is enzyme that hydrolyzed of glucose polymer and maltose into glucose. The liquefied starch was digested into more glucose syrup as shown in Figure 2. The amount of glucose was increased when the glucoamylase concentration and digestion

time increased. NaOH steeped maize hydrolysate was hydrolyzed by using glucoamylase in concentration from 50 to 125 U/g at 0 h that resulted in increasing the amount of glucose from 57,261.90 to 103,276.67 mg/l. However, the excessive glucoamylase concentration and digestion time resulted in the glucose content was decreased, such as glucoamylase concentration more than 125 U/g and at the time 48 h. Thus, the highest glucose content (103,276.67 mg/l) was shown in glucoamylase concentration of 125 U/g for 48 h.

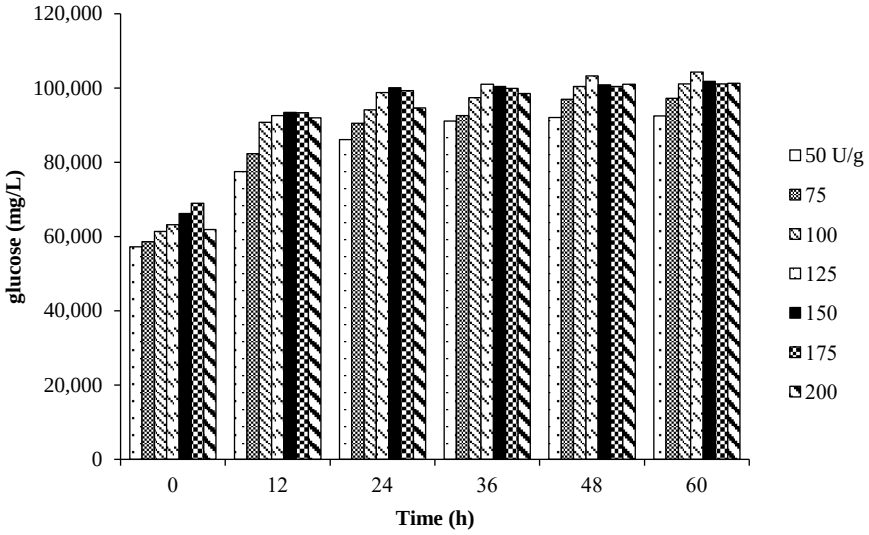

Figure 2. Glucose contents of NaOH steeped maize in saccharification**.**

Figure 3, the DE value of saccharified hydrolysate at 48 h slightly increased when glucoamylase concentration increased until 125U/g. In concentration of glucoamylase higher than 125U/g, this resulted in decreasing of DE value. Moreover, it is certain that DE value of maize hydrolysate of saccharification was higher than that of liquefaction because maltose and/or other glucose polymers were continuously digested into glucose in sacharification. The highest DE values of hydrolysate from liquefaction (72U/g α-amylase) and saccharification (125U/g glucoamylase) were showed 64.22 and 84.82%, respectively.

The residue yield percentages of NaOH steeped maize in liquefaction and saccharification were 21.78 and 21.46% which had no difference.

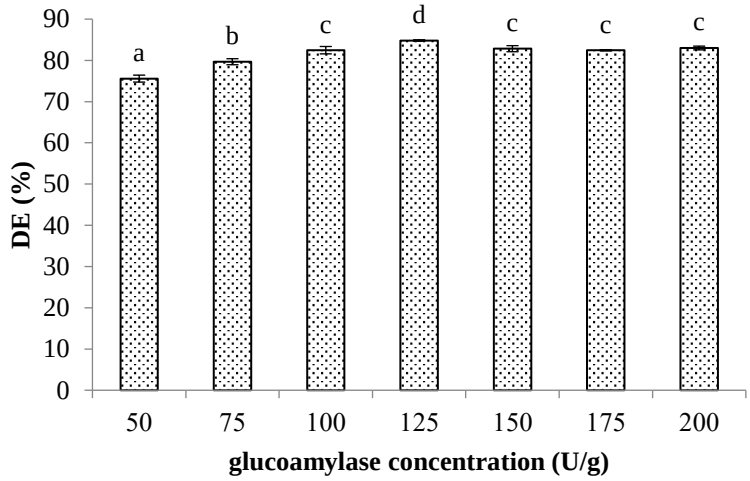

Figure 3. DE values of NaOH steeped maize in saccharification at 48 h. abcd represent significant differences ($p<0.05$)

DISCUSSION

In the liquefaction, the DE value of NaOH steeped maize was higher than control in the same α-amylase concentration of 72 U/g at 6 h. For saccharification, the highest glucose content at 48 h of NaOH steeped maize was 103,276.67 mg/l that observed in glucoamylase concentration of 125 U/g. From this result compare with control maize without NaOH in glucoamylase concentration of 125 U/g for 48 h, the amount of glucose was 90,466.67 mg/l. From the results found that at the same concentration of glucoamylse, NaOH steeped maize shown significantly higher the glucose content than control maize. Therefore both of liquefaction and saccharification, the maize that steeped in NaOH was digested into reducing sugar which higher content than control maize. From the results, the degree of hydrolysis of maize increased after NaOH treatment. The maize was submitted on NaOH, resulted in enzyme susceptibility on starch hydrolysation. According to Spier et al., 2012, the percentage of hydrolysis of the alkaline treatment on maize starch was increased with increasing concentration of NaOH reagent from 26.5% in native starch to 29.7% in the starch treated with 0.18% NaOH. Wang and Wang, 2003 reported that NaOH treated maize caused loosening of

association of protein and lipid on the starch granules surface. The removal of protein from starch granules, there were a lot of empty surfaces for the enzyme to diffuse and adsorb. Moreover, NaOH treatment seemed to degrade the surface of the starch granules even before hydrolysis. The surface treatment caused an enlargement of pores and channel on the surface of maize starch. Therefore, the enzyme was able to penetrate deeper and easier into the starch granules because of the pre-treatment with NaOH. Furthermore, NaOH affected the surface of starch with a possible dissociation of double helix favoring the enzyme attack.

The partially maize starch was digested into maltose in liquefaction and the glucoamylase was continuously hydrolyzed maltose into glucose in saccharification. From Figure 1 and 3, the DE values of NaOH steeped maize in saccharification were higher than liquefaction because the starch was hydrolyzed in 2 steps. According to Mistry and Eckhoff, 1992, the activity of glucoamylase was enhanced in the case of alkaline starch or that the maltose produced from alkali starch was converted into glucose at a faster rate than that from commercial starch.

CONCLUSION

Enzymes hydrolysis in liquefaction, the low-grade maize that steeped in NaOH solution was shown higher reducing sugar content than control maize. The results conclude that NaOH effected to improve and support the hydrolysis of enzymatic in liquefaction. NaOH treatment maize has advantages in terms of reduce the concentration of enzymes as well as increased starch digestion into more reducing sugar compare to control maize. The degree of hydrolysis of starch was enhanced by pre-treating the starch with NaOH.

ACKNOWLEDGEMENTS

This work was financially supported by Research and Researcher for industry (RRi), Thailand Research Fund (TRF), Phathananikom Kaset Co., Ltd (Thailand) (Grant No. PHD59I0015) and Graduate School of Chiang Mai University. This research work was partially supported by Department of Industrial Chemistry, Faculty of Science, Chiang Mai University, Environmental Science Research Center, Faculty of Science, Chiang Mai

University and Center of Excellence in Materials Science and Technology, Faculty of Science, Chiang Mai University.

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