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***In Situ* Increased Reducing Sugar of Nang Lae Pineapple Juice**

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ABSTRACT

Nang Lae pineapple, (*Ananas comosus (L) Merr.*), has planted in Chiang Rai Province, could be used to prepare juice and achieved 35.5±2.0 mL per fresh fruit (100 g). The reducing sugar of six pineapple juice samples were determined using DNS method, which contained 42.22±14.23 mg/mL. Baker's yeast (5% w/v) was immobilized by entrapment method using 0.5% (w/v) sodium alginate. Yeast alginate entrapment bead was applied to pineapple juice and it could increase reducing sugar three times within 24 h by sucrose hydrolyzed enzyme. Activity of extracellular enzyme produced in 50 mM calcium chloride at 30 °C established 0.32 U/mL. Application of 5 and 10% (v/v) extracellular enzyme in pineapple juices could increase high content of reducing sugar within 20 min.

Keywords: Nang Lae pineapple, reducing sugar, sucrose hydrolyzed enzyme

INTRODUCTION

Pineapple (*Ananas comosus (L) Merr.*), the family Bromeliaceae, is a plant that grow well in the tropical countries. The main benefit of this plant is the fresh consumption and processing of various products (Morton and Miami, 1987). It is rich in many nutrients, including bioactive compounds, such as carbohydrates that provide energy, especially monosaccharides and disaccharide. The disaccharide sugar (sucrose, 5.9-12% by weight) is higher than monosaccharide including glucose (1.0-3.2%) and fructose (0.6-2.3%) (Radha and Mathew, 2007). The polysaccharide composes of soluble and insoluble fiber. Protease enzymes are important proteins in pineapple, that

helps digest protein in acidic conditions. It is also reported that bromelain is an anti-inflammatory agent. The major lipase found in pineapple is beta carotene. Vitamins contain riboflavin, vitamin C, vitamin A. Acids are citric acid and lactic acid, which is an important part of the blood vessels. There are also antioxidants in pineapple (Hossain et al., 2015).

Thailand has been cultivating pineapple for consumption by the popular varieties are 9 varieties: Pattawia, Intarachid, Kao, Phuket or Sawi, Naglae or Nam Peung, Trat Si Thong, Phu Lae, Huaimun and Phetchaburi. (Popluechai et al., 2007)

Nang Lae pineapple (Cayenne Group), other name is a Nam Peung pineapple, because the color of the texture and smell similar honey, planted in Nangla Sub-district, Mueang District, Chiang Rai Province. It have registered the Geographical Indication (49100013) since 2005. Ripe fruit is sweet between 16-20 Brix. It has sucrose higher than glucose and fructose approximately two times (Lu et al., 2014; Salunkhe and Kadam, 1995). The average acid content is quite low 0.30.

Sucrose hydrolyzed enzyme, β -fructofuranosidase (EC 3.2.1.26, other names such as sucrose and invertase), is an enzyme that catalyzes the hydrolysis of sucrose to glucose and fructose products. In nature, this enzyme can be found in microorganisms. Especially in yeast species, *Saccharomyces cerevisiae*, using in bakery can produces β -fructofuranosidase enzymes to convert sucrose to glucose and fructose (Shankar et al., 2013). Candy and syrup industries use this enzyme to sweeten the product by turning the molecule into a single molecule (Kulsherestha et al., 2013). Therefore, this research is interested to apply this enzyme produced from baker's yeast to change non reducing sugar (disaccharide) in Nang Lae pineapple juice to reducing sugar (monosaccharide).

MATERIALS AND METHODS

Materials

Fresh fruits of Nang Lae pineapples were obtained from pineapple farm in Nang Lae, Chaing Rai Province, Thailand. Perfect instant yeast for baking was purchased from supermarket in Chiang Rai Province. 3,5-Dinitrosalicylic acid was from Sigma-Aldrich Co. (USA). Sucrose, potassium sodium tartate and calcium chloride were bought from Ajax Finechem (Australia). Sodium hydroxide and acetic acid were from Loba Chemie PVT. Ltd. (India). Sodium alginate, food grade, was purchased from bakery shop, Chiang Mai Province, Thailand.

Pineapple juice preparation

Fresh Nang Lae pineapples were removed peel and cut to small pieces. Juice obtained by filtration with white cloth and used for further experiments.

Enzyme preparation

Cell immobilization by alginate entrapment method was modified from Meena and Raja (2004). Baker's yeast cells (2.5 g) were suspended in 0.5% (w/v) sodium alginate (50 mL) and stirred homogeneously. The suspension solution was drop in 0.2M calcium chloride (500 mL), stirring at a speed of 120 rpm. Gel beads were formed and continually stirred for 10 min. Free-yeast cells were removed by washing twice with 50mM calcium chloride (500 mL). Cell-alginate beads were transferred to 100 mL of 50mM calcium chloride and incubated at 30 °C for 24 h. Activity of β -fructofuranosidase was measured by sucrose hydrolysis.

Enzyme activity assay

The activity of the enzyme was modified from Shankar *et al.* (2013) and Arruda and Vitolo (1999) by mixing 100mM sodium acetate buffer, pH 4.5 (1.0 ml), enzyme solution (0.5 mL) and water (1.5 mL). Then incubated at 35°C for 5 min. The reaction was started by adding 1% (w/v) sucrose solution (1.0 mL) and incubated the mixture at the same temperature for 10 minutes. DNS solution (2.0 mL) was poured to stop reaction, boiled for 10 minutes and added 40% (w/v) sodium potassium tartrate (0.5 mL). The absorbance of solution was measured at 540 nm. The activity of the enzyme was determined by comparing with the glucose standard. One unit of enzyme is defined as enzyme produced the product that equivalent 1 μ mol of glucose per min at 35°C, pH 4.5.

Reducing sugar assay

DNS method was used to determine reducing sugar (Miller, 1959). The mixture of sample (0.5 mL), 100mM sodium acetate buffer, pH 4.5 (1.0 ml), water (1.5 mL) and DNS solution (2.0 mL) was boiled for 5 min. Then added 0.5 mL of 40% (w/v) sodium potassium tartrate and cooled down the mixture to room temperature. Reducing sugar was determined by measuring the mixture at 540 nm.

Increasing reducing sugar in pineapple juice

Nang Lae pineapple juice was mixed with enzyme solution to give final enzyme concentration of 5% and 10% (v/v) for 100 mL, respectively. The

mixtures were incubated at 35 °C. Reducing sugar was observed during 24 h by DNS method.

RESULTS AND DISCUSSION

Fresh fruit of Nang Lae pineapple was peeled and squeezed through white cloth. The juice has been 35.5±2.0 mL per fresh fruit (100 g). The Reducing sugar from 6 samples were determined and found that juices contained reducing sugar 19.82-62.82 mg/mL (42.22±14.23 mg/mL). The result showed varieties of reducing sugar concentration. It could explained that it effect came from a harvest times, soil and climate (Bartholomew et al., 2003)

The changing of reducing sugar in pineapple juice (100 mL) by yeast alginate entrapment bead was observed during 14 days and reducing sugar profiles was showed in figure 1. Reducing sugar increased rapidly during 24 h and producing high yield at this times. It can increase reducing sugar with 3.8 times (75.40 mg/mL) of original juice (19.82 mg/mL). This phenomenon could explain that yeast can produce hydrolyzed enzyme which catalyzed sucrose to monosaccharide (Shankar *et al.*, 2013 & Kulsherestha *et al.*, 2013). At times, 24 to 48 h, reducing sugar decreased rapidly and remained 0.7 times (13.72 mg/mL) of original juice, because yeast leaded to fermentation state which change monosaccharide to alcohol (Walker & Stewart, 2016). In the same way, when times increased reducing sugar in juice decreased. At the date of fourteen, reducing sugar in pineapple juice remained 5.3% of original juice and when determined activity of enzyme in this juice which used sucrose as substrate. It was found that juice has been establish 2.0 U/mL of enzyme activity. All of this results could guide to prepare pineapple juice contain high content of reducing sugar by applying yeast-alginate bead in pineapple juice and used time less than 24 h.

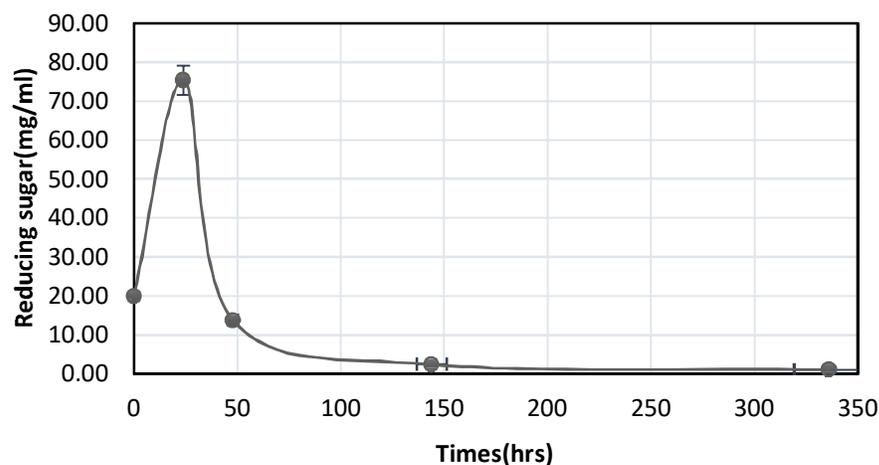


Figure 1. Reducing sugar profiles of Nang Lae pineapple juice containing yeast-alginate beads.

Yeast-alginate beads was incubated in 50mM calcium chloride at 30 °C and extracellular enzyme produced by yeast was determined activity of enzyme using sucrose as substrate for 24 h. The results showed that extracellular enzyme could produce within 1 hour and activity of enzyme was accumulated to 32 U/100 mL at 24 h which increase 6.4 times when compared with activity at first hours (Figure 2). Thus baker's yeast could produce sucrose hydrolyzed enzyme with 12.8 U/g of dried yeast at 24 h.

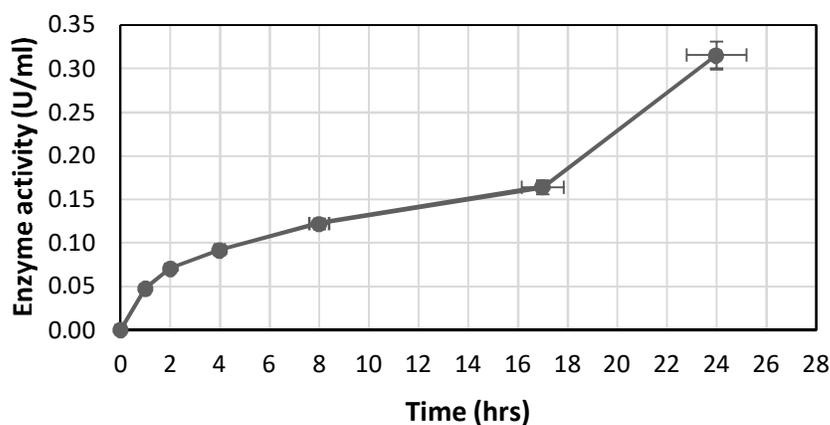


Figure 2. Sucrose hydrolyzed enzyme profile of yeast- alginate immobilization in 50mM calcium chloride at 30 °C.

The bead was prepared by entrapment of yeast (5%) with sodium alginate (0.5%). Extracellular enzyme (0.32 U/mL) from bead in 50 mM calcium chloride could be achieved at 30 °C for 24 h and was applied to pineapple juices with various concentrations. The result has been found that enzyme solution (5%) in pineapple juice could hydrolyze sucrose to produce reducing sugar at 10 and 20 min included 1.1 and 1.3 of original pineapple juice, respectively and reducing sugar accumulated to maximum yields about 50 mg/mL after 20 min, whereas pineapple juice contained enzyme (10%) could increase reducing sugar higher than 5% of enzyme within 7.5% and 3.5% at 10 and 20 min, respectively. Therefore, both of enzyme concentration in pineapple juice could increase reducing sugar in pineapple juice.

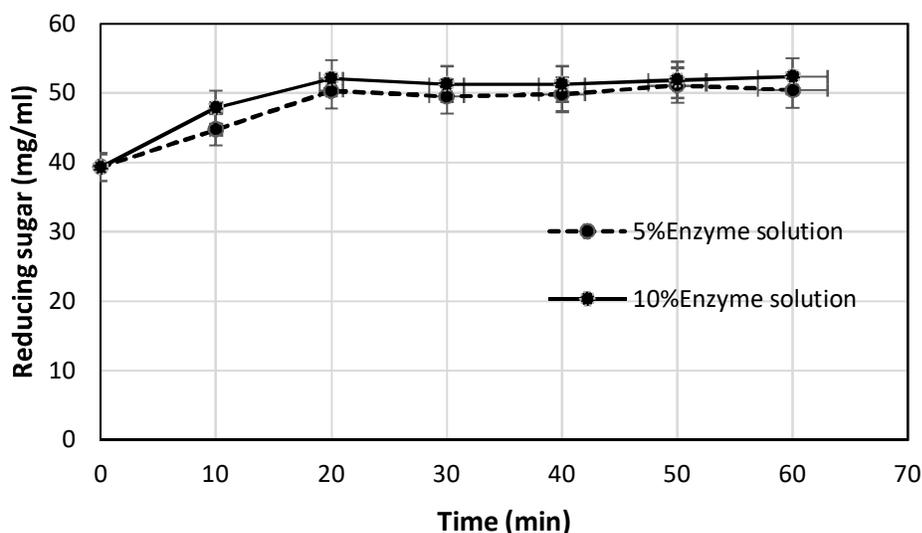


Figure 3. Effect of enzyme concentration on Nang Lae pineapple juice at 35 °C

CONCLUSION

Nang Lae pineapple has a high contents of sugar. It could be change sugar profiles by increasing monosacharide interm of reducing sugar from nonreducing sugar of disaccharide. Two procedures for increasing reducing sugar in pineapple juice could be done. First procedure, immerse yeast-alginate bead directly to pineapples juice and control time for 24 h at 30 °C. Second procedure, use extracellular enzyme produce from yeast- alginate bead and applied to pineapples juice for 20 min at 35 °C.

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