The effect of alpha amylase enzyme on quality of sweet sorghum juice for chrystal sugar

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Abstract. Sweet sorghum juice (Sorghum bicolor L. Moench) has characteristics similar to sugar cane juice and potentially used for sugar substitutes that can support food security. Nevertheless the sweet sorghum juice contain starch which impede sorghum sugar crystallization. Therefore, research on the enzymatic process is needed to convert starch into reducing sugar. The experimental design used was the Factorial Randomized Design with the first factor was alpha amylase enzyme concentration (0, 20, 40, 60, 100, 120 μL/100 mL) and second factor was incubation time (0, 30, 60, 90 minute) at temperature 100 oC. The experiment was conducted on fresh sweet sorghum. The results showed that the addition of the alpha amylase enzyme increased the content of reducing sugar and decreased levels of starch. Elevating concentration of alpha amylase enzyme will increase the reducing sugar content in sweet sorghum juice. The optimum alpha amylase enzyme concentration to produce the highest total sugar was 80 μL/100 mL of sweet sorghum juice with the optimum incubation time was 90 minutes. The results of this study are expected to create a new sweetener for sugar substitution. From the economic prospective aspect, sorghum is a potential crop and can be relied upon to support the success of the food diversification program which further leads to the world food security.

Keywords: Sweet sorghum, enzyme, chrystal sugar

1. Introduction
Sweet sorghum (Sorghum bicolor (L.) Moench) juice similar with sugarcanethat may have potential as a source of sugar, such as the crystallized sugar. According to [1,2], the kind of sugar in the sorghum stalk, it can be divided into saccharin-type sweet sorghum and syrup-type sweet sorghum. Saccharin-type sweet sorghum, contains sucrose and can be used for refining crystal sugar. Syrup type sweet sorghumis used for producing syrup as it mainly contains glucose. The sugars content in sweet sorghum stalk juice mostly are sucrose and invert sugars such as glucose, fructose, maltose and xylose. Previous study reported that mannose, galactose and arabinose were not detected in sweet sorghum juice [1]. The syrup rich in vitamin C, calcium, proteins, riboflavin and nicotinic acid and was free of sulphur or any pesticide residues. The syrup being a table syrup, can be used in salad dressing, as a sweetener in baked goods or as ice-cream topping [3].
The crystallized sugar powder is easy to be used that causes the soluble sugars so practical in use, easy to be packed and distribution, and has a long shelf life because of low water content[4]. Sweet sorghum crystallized sugar (SSCS) can also be used for bread making, pancakes, salad, sweetening agent in a bakery and ice cream or pharmaceutical [5]. Sweet sorghum cultivars; Rio, CMSXS636, IESV91018LT, IESV93042SH and SPV1411 have potential in crystal raw sugar production because they have relatively higher sucrose concentration and AP greater than 75% [6].

Research on the hydrolysis process is needed to convert starch into reducing sugar. The hydrolysis process can be achieved by acid, acid-Enzyme ore nzyme hydrolysis methods. However, acid hydrolysis has been replaced by enzymatic hydrolysis because it required the use of corrosion resistant materials, gave rise to colour and salt ash and need more energy for processing[7]. Enzymatic hydrolysis is essential for the production of glucose syrups from starch because of the specificity of the linking bonds that characterize its physical and chemical properties. In addition the enzymatic method reduced the number of side reactions that accompany acid or alkali hydrolysis[8]. Therefore, the enzyme hydrolysis is the most advanced industrial enzyme application in the food industry and is one of the most successful enzyme technologies. Various grains may be used as a source of enzyme during the hydrolysis of starch to glucose [9].

The α-amylases (1,4-α-d-glucan-4-glucanohydrolase; EC 3.2.1.1) are ubiquitous enzymes, and they play a dominant role in carbohydrate metabolism. The α-amylases from bacteria and fungal sources have dominated applications in industrial sectors [10]. Bacillus subtilis, B. stearothermophilus, B. licheniformis and B. amylobacteri faciens are known to be good producers of α-amylase, and these have been widely used for commercial production of the enzyme for various applications. Large quantities of α-amylase were induced in sorghum when such grains were malted using traditional techniques [11]. Starch can be used to produce syrup containing fructose, glucose, or maltose, which is widely used in the food industry [12].

Sorghum starch hydrolysis using the alpha amylase enzyme has been studied by [13,14,15]. The optimum conditions for the glucose production from sorghum malt using α-amylase gelatinization at 80°C and liquefaction of amylase at a 90°C at pH 4[13]. The suspension should be brought to high temperatures (90-110°C) to initiate α-amylase for the starch kernel breakdown [16]. Other study found that, liquefaction under pressurized steam at 104°C was more effective compared to using water bath at 95 °C[17]. The increase in hydrolysis of starch has the correlation with the effect of high heat or temperature on the weaker areas on the starch granule, allowing the enzyme to break the granules of starch more extensively [18].

The sweet sorghum for crystal sugar production must have a high content of sucrose in the stalks and low levels of starch which impede sorghum sugar crystallization [19,20]. To achieve this goal, the starch present in the sorghum sap should be first hydrolyzed into its reducing sugars. Therefore, the aim of this research was to study the effect of alpha amylase enzyme on quality of sweet sorghum juice for crystal sugar.

2. Materials and Methods

2.1. Materials

The raw material used is sweet sorghum stalk for producing sweet sorghum juice obtained from Yogyakarta, Indonesia. The enzyme used is a thermostable α-amylase enzyme [13] derived from Bacillus licheniformis[11,21,22] under the trade name Termamy[14, 24]. There agents used were Pb-acetate, (NH4)2HPO4 10% solution, Luff-Schoorl reagent, 25% H2SO4, 5% starch, Sodium thiosulfate 0.1N, 20% KI solution, 25% Hydrochloric acid (HCl) solution, and 50% Sodium Hydroxide solution (NaOH) solution, 0.1% phenolphthalein, 37% HCl, 3,5-Dinitroso cyclic acid (DNSA) solution, activated carbon, glucose, and distilled water.
2.2. Enzymatic process of sweet sorghum juice
The sweet sorghum stalk was cleaned and then extracted by series of mills [24]. The juice coming out of milling section was first screened and heated up to 100°C [25,26]. A total of 100 mL of boiled sorghum sauce were taken and put into a glass bottle and various quantity of α-amylase enzyme was added. The concentration of α-amylase enzyme and incubation time were varied on the basis of statistical experimental design[14]. The experimental design used was the Factorial Randomized Design with the first factor was alpha amylase enzyme concentration (0, 20, 40, 60, 80, 100, 120 μL/100 mL) and second factor was incubation time (0, 30, 60, 90 minute) at temperature 100°C.

2.3. Analysis of sweet sorghum juice
Sweet sorghum juice after the enzymatic process were analyzed for reducing sugar and total sugar by using Luff-Schoorl method [26,27], for starch using DNS method (Bintang 2010), for viscosity using a brookfield programmable DV-III rheometer [28] for brix using refractometer [29,30] and for pH using pH meter [27,31].

3. Results and Discussion

3.1. Reducing sugar content in sweet sorghum juice
The fresh sweet sorghum juicereducing sugars is given in Figure 1. The addition of the alpha amylase enzyme increased the content of reducing sugar and decreased levels of starch. Increased concentration of alpha amylase enzyme will increase the reducing sugar content in sweet sorghum juice. The optimum alpha amylase enzyme concentration to produce the highest total sugar was 80 μL/100 mL of sweet sorghum juice with the optimum incubation time was 90 minutes. The results of this study are in accordance with those reported by [14, 15]. The amount of α-amylase in hydrolysis process of sorghum strach gave positive effect to the response on dextrose equivalent (reducing sugar expressed as glucose). On the other hand, the liquefaction time has slightly negative effect on the amount of dextrose equivalent. As the liquefaction time increases, the amount of dextrose equivalent decreases [15]. This is because the longer exposure of the enzyme to high temperatures, which are needed for gelatinization of the starch granules and for achieving a good susceptibility to enzyme action, could lead to slight enzyme deactivation [32]. The best reducing sugar production by hydrolysis using α-Amylase of manihot esculenta was observed when temperature of 74°C, pH of 5.5 and incubation time of 90 minute [14]. Liquefaction of 25% (w/v) sorghum slurry was completed in 60 minute with B. licheniformis α-amylase (BLA) concentration of 0.08% (v/w) of flour in the absence of CaCl2 supplementation [33]. However [17] mentioned that the optimum α-amylase enzyme concentration was 0.15% (v/w) for 60 minute of liquefaction time.

In order to get the carbon and energy stored in starch, α-amylase enzyme used to break down the polymer to smaller sugar units, which is eventually converted to the individual basic glucose units [21,22,23]. The bacterial alpha-amylase randomly attacks only the alpha-1,4 bonds [34]. The enzyme mode of action depend on the starch source and enzyme system. The enzymatic hydrolysis percentages increased when the granules were incubated with alpha-amylase and that the granule susceptibility was affected by granule size and concentration of the hydrolysis products [34,35]. Reducing sugar production was strongly affected by the variation of variables on alpha amylase and glucoamylase hydrolysis of cassava starch [14].
3.2. **Total sugar content in sweet sorghum juice**

The total sugar of fresh sweet sorghum juice is given in Figure 2. The total sugar content of fresh sugar sorghum juice is in the range of 3.87 to 20.88%. The enzyme concentration of 80 μL / 100 mL of sweet sorghum juice is the optimum concentration of enzyme to produce the highest total sugar. At incubation time 90 minutes, the total sugar produced tends to be more than the other incubation time.

![Figure 1](image1.png)

**Figure 1.** The reducing sugar content of fresh sweet sorghum juice at the concentration of enzyme 0(♦), 40(■), 80(▲), and 120(×) (µl/100 mL) with incubation time 0, 30, 60 and 90 minutes.

![Figure 2](image2.png)

**Figure 2.** The total sugar content of fresh sweet sorghum syrup at the concentration of enzyme 0(♦), 40(■), 80(▲), and 120(×) (µl/100 mL) with incubation time 0, 30, 60 and 90 minutes.

The decrease in total sugar content can also be caused by the formation of other molecules such as ethanol, lactic acid, acetate, and pyruvate. Total sugar content in sweet sorghum juice may decrease after being stored for two weeks[38]. Levels of sucrose produced after storage for two weeks decreased and formed ethanol and organic acids, which formed by the presence of bacteria.

3.3 Starch content in sweet sorghum juice

Average starch content in fresh sweet sorghum tends to decrease with increasing concentration of enzyme amylase. The difference of fresh sweet sorghum starch content and after storage is caused by the hydrolysis process during storage. The results of this study fit the statement that the process of...
starch hydrolysis runs slowly [36]. In addition, the presence of amylopectin content in the sorghum starch causes amylopectin to be difficult to hydrolyze [20].

3.4. Brix value of sweet sorghum juice
The brix of fresh sweet sorghum juice is given in Figure 3. The Brix range in different treatment of hydrolysis is 16.00 (concentration enzyme 0µl/100mL with incubation time 30 minute) to 24.2 (concentration enzyme 120µl/100mL with incubation time 30 and 60 minute). This suggests that the higher the amylase concentration the brix sweet sorghum juice increases. The content of sugar in the juice of sweet sorghum varies in different varieties [40]. The Brix range in different varieties of sweet sorghum is 14.32 - 22.85% ([39]. The good quality syrup could be made when the raw juice was at least have the brix of 14 degrees. The final brix of prepared syrup generally 70-75 degrees (corresponding to syrup temperatures of approximately 106ºC) and a minimum shelf-life of 6 to 9 months[41]. Thus sweet sorghum juice in this study can be used as an excellent raw material.

3.5. pH of sweet sorghum juice
The fresh sweet sorghum juice pH is given in Figure 4. The pH range of fresh sweet sorghum in different treatment of hydrolysis is 5.85 (concentration enzyme 0µl/100mL with incubation time 90 min) to 6.67 (concentration enzyme 40µl/100 mL with incubation time 90 minute). The soluble starch hydrolysis by purified α-amylase was worked out at pH ranging from 4.0 to 9.0 at 60ºC[42]. The optimum enzyme activity was found in pH range of 6.5 to 7.5 with maximum activity at pH 7.0. The higher activity of α-amylase from Bacillus licheniformis mutant in pH range from 4.0 to 8.0[43]. Juice can be made into crystal sugar if it has a pH between 6.00 to 6.50 [31]. In this study, fresh sugary sorghum juice was eligible for use as a raw material for making crystal sugar with concentration enzyme treatment 40µl/100mL with incubation time 90 minute.
3.6. Viscosity of sweet sorghum juice

The viscosity of fresh sweet sorghum juice is given in Figure 5. The pH range of fresh sweet sorghum in different treatment of hydrolysis is 4.00 (concentration enzyme 0μl/100mL with incubation time 0 min) to 5.60 (concentration enzyme 120μl/100 mL with incubation time 60 minute). The addition of alpha amylase enzyme concentration will decrease the viscosity of hydrolyzate[44], but the viscosity of hidrolyzates depends on the kind of starch. Viscosity is influenced by the solute contained in it. The higher the viscosity of a liquid the more the dissolved substance in it [45].

4. Conclusion

The addition of the alpha amylase enzyme increased the content of reducingsugar and decreased levels of starch. Increased concentration of alpha amylase enzyme will increase the reducing sugar content in sweet sorghum juice. The optimum alpha amylase enzyme concentration to produce the highest total sugar was 80 μL/100 mL of sweet sorghum juice with the optimum incubation time was 90 minutes. This study are expected to create a new sweetener for sugar substitution.

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