Optimization of Liquid Sugar Production Process from Sago (Metroxylon spp.)

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Abstract. The level of sugar consumption will increase along with the population and income increasing. Sago can be used as an alternative raw material of sugar. This study was aimed to determine the characteristics of sago starch. The study were includes several stages: sago starch leaching, characterization and optimization of liquid sugar production from sago starch. Optimization of liquid sugar process was done by starch and water ratio (1: 4, 1: 5, 1: 6). The α-amylase and the amyloglucosidase were comprised of three levels concentration: 0.8 ml kg⁻¹, 1.0 ml kg⁻¹ and 1.2 ml kg⁻¹ starch. The result showed that physical characteristics of sago starch were fine powder, white colour, normal sago taste and normal sago flavor. The results also showed that sago starch did not contain lead, mercury and arsenic, with a total plate count of 4.5x10⁰ CFU/g and did not contain mold. The optimum condition of liquid sugar production was obtained by the starch water ratio 1: 4 with α-amylase 1.2 ml kg⁻¹ starch and amyloglukosidase 1.2 ml kg⁻¹ starch. The liquid sugar produced had physical characteristics of 60⁰ Brix, sweet taste, typical sweet aroma of sugar, and reddish-yellow color.

1. Introduction
Sugar is the most dominant sweetener, either as a part of household consumption or as a part of raw materials in food and beverage industries. The level of sugar consumption in Indonesia is still relatively low as compared to other countries, therefore it is expected that sugar consumption will continuously increase with the increased amount of population and people’s income. In order to meet demand for sugar, in particular that from food and beverage industries, a number of cane-sugar alternatives can be considered. Today, food and beverage industries tend to use glucose syrup. In Indonesia, raw material used to produce glucose syrup is starch, as it is abundantly available in both the amount and variety, such as tapioca, maize starch, tuber starch, and sago starch [1].

Sago starch has a great potential as a basic material to produce liquid sugar. Considering a high production of sago crop up to 20-40 Ha per year, utilization of sago into liquid sugar in addition to a completely staple food is expected to fulfil demand for sugar [2]. Sago starch can be processed into liquid sugar by hydrolyzing the starch using enzymes.

Production of liquid sugar from sago starch has been widely run using sago starch from different regions in Indonesia, such as West Java, Riau, North Celebes (Sulawesi), South Borneo (Kalimantan), and Papua (Irian Jaya) [3] and sago starch from South Sorong [4]. Research of liquid sugar from sago cultivated in South East Sulawesi had not been conducted, and therefore it was necessary to improve production of sago-starch liquid sugar in the province to reduce import of sugar. The objectives of the
present study were to determine optimum concentrations of starch and enzymes used in the production of liquid sugar from sago starch by enzymatic hydrolysis.

2. Materials and Methods
This study was conducted in Indonesian Center for Agricultural Postharvest Research and Development (ICAPRD), Bogor. Materials used included sago starch from Konawe, South East Sulawesi, α-amylase, amylglucosidase, and materials for chemical and biological analyses. Tools and equipment used include oven, analytical balance, filter cloth, stirrers, spoons, pH-meter (HI-2011), thermometer, refractometer, chromameter (Minolta 300), stoves, buckets, wooden stirrers, blender, spectrophotometer UV 6500, atomic absorption spectrophotometer AA-700, cuvettes, and glassware for analyses.

2.1. Research methods
Sago was cleaned and washed, before being dried at 40-50°C. Dried sago starch was tested for physical, chemical and microbiological characteristics. Subsequently, optimization of liquid sugar production was carried out using these following variables: (i) ratios between starch and water of 1:4, 1:5, and 1:6, (ii) α-amylase at three levels of concentration, e.g. 0.8 ml/kg starch; 1.0 ml/kg starch; and 1.2 ml/kg starch, (iii) amylglucosidase at three levels of concentration, e.g. 0.8 ml/kg starch; 1.0 ml/kg starch; and 1.2 ml/kg starch. Parameters of liquid sugar measured included color, total soluble solid, total sugar content, and pH. The best result from optimization was processed into liquid sugar and subjected to organoleptic, physical, chemical, and microbiology tests.

2.2. Research design
This research employed a Completely Random Design (CRD) and split-plot design. Each experiment was in duplicate, and therefore there were 54 units of experiment from 3 times of measurement in each parameter. Data obtained was analyzed using an F-test in four programs of SAS 9.1. Significance of variables was identified using Duncan Multiple Range Test (DMRT) at a level of 5%.

Figure 1. Flow chart Sago-starch liquid sugar production
3. Results and Discussion
Sago was starch obtained from water-mediated extraction process of sago palm pith. Starch of sago grown in Konawe was characterized physically, chemically, and microbiologically.

3.1. Physicochemical and microbiological characteristics of sago starch
Physical characteristics of sago starch included shape, color, taste and aroma. Sago starch was in the form of fine powder, white in color, a normal taste and aroma of sago. Chemical and microbiological characteristics of sago starch from Konawe, South East Sulawesi is presented in Table 1.

| No | Parameter       | Unit | Starch sago | Indonesian standard (SNI 3729 : 2008) |
|----|-----------------|------|-------------|-------------------------------------|
| 1  | Water           | %    | 7.21        | Max. 13                             |
| 2  | Ash             | %    | 0.11        | Max. 0.5                            |
| 3  | Fat             | %    | 0.56        | -                                   |
| 4  | Protein         | %    | 0.36        | -                                   |
| 5  | Carbohydrate    | %    | 91.76       | -                                   |
| 6  | Starch          | %    | 80.69       | Min. 65                             |
| 7  | Crude fiber     | %    | 0.37        | Max. 0.5                            |
| 8  | Pb              | ppm  | -           | Max. 1.00                           |
| 9  | Cu              | ppm  | 1.28        | Max. 10.0                           |
| 10 | Hg              | ppm  | -           | Max. 0.05                           |
| 11 | As              | ppm  | -           | Max. 0.50                           |
| 12 | Total Plate Count | colony/g | 4.5x10^1 | Max. 10^6                           |
| 13 | Mold            | colony/g | (-)      | Max. 10^4                           |

The sago starch showed moisture and ash content, met the requirement of SNI 3729:2008 [5]. Fat is a more effective source of body’s energy as compared to protein and carbohydrate. The sago starch had fat content of 0.56%. Furthermore, protein content of the starch was 0.36%. The carbohydrate content of sago starch was 91.76%. Carbohydrate has an important role in determining the characteristics of food materials, such as color, taste, aroma, and texture [6]. The sago in this experiment yielded starch content of 80.69% which met the requirement of SNI 3729:2008. Crude fiber content obtained from the starch was 0.37%, which met the requirement of SNI 3729:2008, no more than 0.5%. Sago starch only contains fiber in a very small amount, because the starch was obtained by extraction allowing elimination of some insoluble fiber with the dreg disposal and elimination of some soluble fiber with water drainage. Analysis of metals, including lead, copper, mercury, and arsen, was performed. Non-excessive amount of a heavy metal copper is essential to maintain the metabolism of the human body, while it becomes toxic when being consumed excessively. On the other hand, Pb, Hg, and As are non-essential heavy metals that have no functions for body, moreover, they become very dangerous and potentially cause toxicity [7]. Lead was not detected in the sago starch, and therefore meeting requirement of SNI 3729:2008. In addition to being toxic for nervous system, lead is hematologic and hemotoxic and alters kidney functions. Mobility of lead in the ground and plants tends to be slow. Normal content of this metal in plants ranges 0.5-3 ppm [8]. Copper analysis showed a content of 1.28 ppm, which met requirement of SNI 3729:2008. Copper prevents anemia through facilitating iron absorption, stimulating hemoglobin synthesis, and releasing iron storage from hepatic ferritin, in addition to being a part of ceruloplasmin enzyme [9]. Hg and As were not detected from the analysis, thus meeting requirement of SNI 3729:2008. Based on the microbial tests, the sago starch produced had met all requirements of SNI 3729:2008.
3.2. Optimization of sago-starch liquid sugar

Production of liquid sugar was done at starch-water ratios of 1:4, 1:5, and 1:6. Concentration of α-amylase and amylglucosidase used were 0.8 ml/kg, 1.0 ml/kg, and 1.2 ml/kg of starch. After 48-hour incubation, measurement of color, pH, total soluble solid, and total sugar content was performed.

3.2.1. Color of liquid sugar

The color of the liquid sugar was assessed objectively using a chromameter by measuring its color values (\(^o_h\)). The ANOVA showed that variables of starch-water ratios, α-amylase concentrations, and amylglucosidase concentrations resulted in significant differences in color values (\(^o_h\)) at a confidence level of 95%. Moreover, Duncan post-hoc test showed that the sugar produced from starch-water ratio of 1:4 was significantly different from that produced from ratios of 1:5 and 1:6; however there was no significant difference between sugars produced from 1:5 and 1:6 ratios. There was a significant difference in color of sugar produced from α-amylase at 1.0 ml/kg starch and that from α-amylase at 0.8 ml/kg starch and 1.2 ml/kg starch, however sugar produced from α-amylase at 0.8 ml/kg starch and 1.2 ml/kg starch were not significantly different. There was no difference in color between sugars produced from amylglucosidase at three different concentrations.

Overall, the highest mean color (\(^o_h\)), 94.59\(^o_h\), in sago starch liquid sugar was obtained from starch-water ratio of 1:5 at α-amylase concentration of 0.8 ml/kg starch and amylglucosidase concentration of 1.2 ml/kg starch, showing a yellow color, while the overall lowest mean color (\(^o_h\)) was obtained from a ratio of 1:4 at α-amylase concentration at 1.0 ml/kg starch and amylglucosidase concentration of 1.2 ml/kg starch, showing a yellow color. All ratios of starch and water, concentrations of α-amylase, and concentrations of amylglucosidase resulted in a yellow color. Appearance of color was affected by a number of factors, such as heating process, presence of reducing sugars, and protein content. The protein content of the sago starch obtained from South East Sulawesi was 0.36%. A protein contained in starch reacts with reducing sugars through Maillard reaction, allowing the occurrence of non-enzymatic browning reaction. Color of liquid sugar was darker as the protein content was higher.

| Color (\(^o_h\)) | α-amylase concentrations | Amyloglucosidase concentrations |
|-----------------|--------------------------|--------------------------------|
|                 | 0.8 ml kg\(^{-1}\) starch | 1.0 ml kg\(^{-1}\) starch | 1.2 ml kg\(^{-1}\) starch |
| Starch-water ratio 1:4 | | | |
| 0.8 ml kg\(^{-1}\) starch | 84.25 \(a(A)\) | 82.59 \(a(A)\) | 87.52 \(a(A)\) |
| 1.0 ml kg\(^{-1}\) starch | 86.62 \(a(A)\) | 82.80 b\(A\) | 81.18 c\(B\) |
| 1.2 ml kg\(^{-1}\) starch | 84.12 \(a(A)\) | 83.58 \(a(A)\) | 82.42 \(a(A)\) |
| Starch-water ratio 1:5 | | | |
| 0.8 ml kg\(^{-1}\) starch | 92.87 \(a(A)\) | 93.52 \(a(A)\) | 94.59 \(a(A)\) |
| 1.0 ml kg\(^{-1}\) starch | 89.84 b\(B\) | 92.72 \(a(A)\) | 92.52 \(a(A)\) |
| 1.2 ml kg\(^{-1}\) starch | 93.72 \(a(A)\) | 94.44 \(a(A)\) | 94.15 \(a(A)\) |
| Starch-water ratio 1:6 | | | |
| 0.8 ml kg\(^{-1}\) starch | 92.67 \(a(A)\) | 92.57 \(a(AB)\) | 92.84 \(a(A)\) |
| 1.0 ml kg\(^{-1}\) starch | 91.56 \(a(A)\) | 91.78 \(b(B)\) | 91.74 \(a(A)\) |
| 1.2 ml kg\(^{-1}\) starch | 92.58 \(a(A)\) | 93.57 \(a(A)\) | 93.14 \(a(A)\) |

Remark: the numbers followed by the same lowercase letters in the same column or the same capital letter on the same line indicate that they are not significantly different based on further DMRT tests at the level of 5%. Lowercase for α-amylase interactions, uppercase for amylglucosidase interactions.
3.2.2. Total soluble solids

ANOVA demonstrated that there was a significant difference in total soluble solids among liquid sugar produced from different starch-water ratios, α-amylase concentrations, and amylglucosidase concentrations at a confidence level of 95%. Duncan post-hoc test showed liquid sugar produced from starch-water ratios of 1:4, 1:5, and 1:6 were significantly different at α-amylase concentrations of 0.8, 1.0, and 1.2 ml/kg starch and amylglucosidase concentrations of 0.8, 1.0, and 1.2 ml/kg starch. Total soluble solids in starch-water ratio of 1:4 after 48-hour incubation was the lowest, 22.20ºBrix, at α-amylase concentration of 1.0 ml/kg starch and amylglucosidase concentration of 0.8 ml/kg starch, while the highest, 28.10º Brix, at both α-amylase and amylglucosidase concentrations of 1.2 ml/kg starch. The lowest total soluble solids, 18.40º Brix, from starch-water ratio of 1:5 was resulted from concentrations of α-amylase and amylglucosidase at 1.0 ml/kg starch, while the highest, 23.74º Brix, was obtained from concentrations of α-amylase and amylglucosidase at 1.0 ml/kg starch and 0.8 ml/kg starch, respectively. At starch-water ratio of 1:6, the lowest Brix was 15.90º Brix shown by two liquid sugar from both α-amylase and amylglucosidase concentrations of 0.8 ml/kg starch and from α-amylase and amylglucosidase concentrations of 0.8 ml/kg starch and 1.0 ml/kg starch, respectively, while the highest Brix, 18.20º Brix, was shown by liquid sugar from α-amylase and amylglucosidase concentrations of 0.8 ml/kg starch and 1.2 ml/kg starch, respectively.

More addition of water and lower concentration of starch reduced total soluble solids yielded as the amount of starch dissolved was lower. In this research, sago starch served as a substrate. Higher amount of substrate allows its more interaction with enzyme, and therefore more enzyme-substrate complexes are formed, resulting in higher reaction rates and more products yielded. This was noticed in liquid sugar from starch-water ratio of 1:4 which demonstrated the highest total soluble solids, 28.10º Brix, at both α-amylase and amylglucosidase concentrations of 1.2 ml/kg starch. On the other hand, liquid sugar from starch-water ratios of 1:5 and 1:6 underwent a decrease in total soluble solids. It occurred because sago starch was not completely hydrolyzed in the presence of excess water, and therefore reducing the amount of substrate. As a consequence, enzyme activity was not optimum, which led to lesser enzyme-substrate complexes formed, leading to less products formed from the reaction. Rate of enzyme-facilitated reactions is dependent on concentration of the enzyme. High concentration of enzyme and longer saccharification time result in an increased amount of products (reducing sugars) [10]. Saccharification time and an optimum temperature of 50ºC during production contribute to Brix value of liquid sugar [10] and [11].
Table 3. Mean TSS values of starch-sago liquid sago

|                      | Total soluble solids (°Brix) | 0.8 ml kg⁻¹ starch | 1.0 ml kg⁻¹ starch | 1.2 ml kg⁻¹ starch |
|----------------------|-----------------------------|--------------------|--------------------|--------------------|
| **Starch-water ratio 1:4** |                             |                    |                    |                    |
| 0.8 ml kg⁻¹starch    | 23.60 a(B)                  | 23.40 b(B)         | 23.00 c(C)         |                    |
| 1.0 ml kg⁻¹starch    | 22.20 b(C)                  | 23.10 a(B)         | 23.20 a(B)         |                    |
| 1.2 ml kg⁻¹starch    | 25.80 a(A)                  | 28.00 a(A)         | 28.10 a(A)         |                    |
| **Starch-water ratio 1:5** |                             |                    |                    |                    |
| 0.8 ml kg⁻¹starch    | 19.57 a(B)                  | 19.80 b(A)         | 20.80 a(B)         |                    |
| 1.0 ml kg⁻¹starch    | 23.47 a(A)                  | 18.40 b(C)         | 18.44 b(C)         |                    |
| 1.2 ml kg⁻¹starch    | 19.00 a(C)                  | 19.00 b(B)         | 21.40 a(A)         |                    |
| **Starch-water ratio 1:6** |                             |                    |                    |                    |
| 0.8 ml kg⁻¹starch    | 15.90 b(C)                  | 15.90 b(C)         | 18.20 a(A)         |                    |
| 1.0 ml kg⁻¹starch    | 16.90 a(A)                  | 17.00 a(A)         | 16.00 c(B)         |                    |
| 1.2 ml kg⁻¹starch    | 16.00 a(B)                  | 16.00 a(B)         | 16.00 a(B)         |                    |

Remark: the numbers followed by the same lowercase letters in the same column or the same capital letter on the same line indicate that they are not significantly different based on further DMRT tests at the level of 5%. Lowercase for α-amylase interactions, uppercase for amyloglucosidase interactions.

3.2.3. Total sugar

It was noticed that starch-water ratios, α-amylase concentrations, and amyloglucosidase concentrations resulted in a significant difference in total sugar of liquid sugar produced at a confidence level of 95%. Furthermore, Duncan post-hoc test showed that total sugar was significantly different among liquid sugar from starch-water ratios of 1:4, 1:5, and 1:6. Total sugar of liquid sugar from α-amylase concentration of 0.8 ml/kg starch was significantly different from that from α-amylase concentrations of 1.0 ml/kg starch and 1.2 ml/kg starch, however there was no significant difference in total sugar between liquid sugar prepared from α-amylase concentrations of 1.0 ml/kg starch and 1.2 ml/kg starch. Total sugar of liquid sugar from amyloglucosidase concentration of 1.2 ml/kg starch was significantly different from that from amyloglucosidase concentrations of 0.8 ml/kg starch and 1.0 ml/kg starch, however there was no significant difference in total sugar between liquid sugar prepared from amyloglucosidase concentrations of 0.8 ml/kg starch and 1.0 ml/kg starch.

Mean total sugar of sago-starch liquid sugar from starch-water ratio of 1:4 was the lowest, 17.79%, at α-amylase concentration of 1.0 ml/kg starch and amyloglucosidase concentration of 0.8 ml/kg starch, while it was the highest, 27.45%, at both α-amylase and amyloglucosidase concentrations of 1.2 ml/kg starch.

At starch-water ratio of 1:5, mean total sugar of sago-starch liquid sugar was the lowest, 16.26 %, at both α-amylase and amyloglucosidase concentrations of 1.0 ml/kg starch, while it was the highest, 22.99%, at α-amylase concentration of 1.0 ml/kg starch and amyloglucosidase concentration of 0.8 ml/kg starch. At starch-water ratio of 1:6, mean total sugar of sago-starch liquid sugar was the lowest, 9.42%, at α-amylase concentration of 1.2 ml/kg starch and amyloglucosidase concentration of 0.8 ml/kg starch, while it was the highest, 16.57%, at α-amylase concentration of 1.0 ml/kg starch and amyloglucosidase concentration of 0.8 ml/kg starch. Rate of enzyme-facilitated reactions is dependent on concentration of the enzyme. At a certain concentration of substrate, reaction rate is higher with rising concentration of enzyme [12].
Table 4. Mean total sugar values of starch-sago liquid sago

| Starch-water ratio | α-amylase concentrations | Amyloglucosidase concentrations | Total sugar (%) |
|--------------------|--------------------------|---------------------------------|-----------------|
| 1:4                | 0.8 ml kg\(^{-1}\) starch | 21.98 \(^{a(b)(A)}\)          | 23.71 \(^{a(A)}\) | 20.94 \(^{b(C)}\) |
|                    | 1.0 ml kg\(^{-1}\) starch | 17.79 \(^{b(B)}\)            | 18.80 \(^{b(B)}\) | 23.03 \(^{a(B)}\) |
|                    | 1.2 ml kg\(^{-1}\) starch | 21.55 \(^{b(A)}\)            | 23.10 \(^{b(A)}\) | 27.45 \(^{a(A)}\) |
| 1:5                | 0.8 ml kg\(^{-1}\) starch | 18.78 \(^{b(B)}\)            | 18.46 \(^{b(A)}\) | 21.73 \(^{a(A)}\) |
|                    | 1.0 ml kg\(^{-1}\) starch | 22.99 \(^{a(A)}\)            | 16.26 \(^{b(A)}\) | 16.32 \(^{b(C)}\) |
|                    | 1.2 ml kg\(^{-1}\) starch | 17.08 \(^{a(C)}\)            | 16.29 \(^{a(A)}\) | 18.93 \(^{a(B)}\) |
| 1:6                | 0.8 ml kg\(^{-1}\) starch | 15.32 \(^{a(B)}\)            | 14.81 \(^{a(A)}\) | 16.56 \(^{a(A)}\) |
|                    | 1.0 ml kg\(^{-1}\) starch | 16.57 \(^{a(A)}\)            | 15.18 \(^{b(A)}\) | 11.81 \(^{b(C)}\) |
|                    | 1.2 ml kg\(^{-1}\) starch | 9.42 \(^{b(C)}\)             | 12.16 \(^{a(B)}\) | 13.20 \(^{a(B)}\) |

Remark: the numbers followed by the same lowercase letters in the same column or the same capital letter on the same line indicate that they are not significantly different based on further DMRT tests at the level of 5%. Lowercase for α-amylase interactions, uppercase for amyloglucosidase interactions.

Reaction rate is linear with the amount of products yielded and with decreased amount of substrate. In this case, the yielded product was liquid sugar. Total soluble solids and total sugar measured were different, which might be due to the fact that not all portions of sago starch were hydrolyzed completely by enzymes, allowing some starch deposit identified as soluble solids.

Total sugar and total soluble solids become two parameters of liquid sugar production from sago starch. Experiment with starch-water ratio of 1:4 at both α-amylase and amyloglucosidase concentrations of 1.2 ml/kg starch was an optimum formulation to produce liquid sugar from starch of sago obtained from South East Sulawesi for yielding the highest total sugar of 27.45% and total soluble solids of 28.10\(^{o}\)Brix.

3.2.4. pH

It was noticed that starch-water ratios, α-amylase concentrations, and amyloglucosidase concentrations resulted in significant difference in pH of liquid sugar produced at a confidence level of 95%. Furthermore, Duncan post-hoc test showed that there was a significant difference in pH value between liquid sugar prepared from starch-water ratios of 1:4 and 1:5, however that from starch-water ratios of 1:4 and 1:5 were not significantly different from that prepared from ratio of 1:6. pH value of liquid sugar prepared from α-amylase concentration of 0.8 ml/kg starch was significantly different from that prepared from 1.0 ml/kg starch concentration, however liquid sugar from both α-amylase concentrations were not significantly different from that prepared from 1.2 ml/kg starch. Concentrations of amyloglucosidase did not show significant difference in pH value. Mean pH value of sago-starch liquid sugar from starch-water ratio of 1:4 was the lowest, 4.43, at α-amylase concentration of 0.8 ml/kg starch and amyloglucosidase concentration of 1.2 ml/kg starch, while it was the highest, 5.13, at both α-amylase and amyloglucosidase concentrations of 1.2 ml/kg starch.

At starch-water ratio of 1:5, mean pH value of sago-starch liquid sugar was the lowest, 3.99, at α-amylase and amyloglucosidase concentrations of 0.8 ml/kg starch and 1.2 ml/kg starch, respectively, while it was the highest, 4.71, at α-amylase concentration of 1.0 ml/kg starch and amyloglucosidase concentration of 0.8 ml/kg starch. At starch-water ratio of 1:6, mean pH value of sago-starch liquid sugar was the lowest, 4.08, at α-amylase concentration of 1.2 ml/kg starch and amyloglucosidase concentration of 1.0 ml/kg starch, while it was the highest, 5.10, at both α-amylase and amyloglucosidase concentrations of 1.0 ml/kg starch.
Table 5. Mean pH values of starch-sago liquid sugar

| α-amylase concentrations | Amyloglucosidase concentrations (pH) |
|--------------------------|-------------------------------------|
| Starch-water ratio 1:4   | 0.8 ml kg⁻¹ starch                   |
|                          | 1.0 ml kg⁻¹ starch                   |
|                          | 1.2 ml kg⁻¹ starch                   |
| 0.8 ml kg⁻¹ starch       | 4.57 a(A)                            |
| 1.0 ml kg⁻¹ starch       | 4.70 a(A)                            |
| 1.2 ml kg⁻¹ starch       | 4.43 a(A)                            |
| Starch-water ratio 1:5   | 0.8 ml kg⁻¹ starch                   |
|                          | 1.0 ml kg⁻¹ starch                   |
|                          | 1.2 ml kg⁻¹ starch                   |
| 0.8 ml kg⁻¹ starch       | 4.70 a(A)                            |
| 1.0 ml kg⁻¹ starch       | 4.07 b(A)                            |
| 1.2 ml kg⁻¹ starch       | 3.99 b(A)                            |
| Starch-water ratio 1:6   | 0.8 ml kg⁻¹ starch                   |
|                          | 1.0 ml kg⁻¹ starch                   |
|                          | 1.2 ml kg⁻¹ starch                   |
| 0.8 ml kg⁻¹ starch       | 4.37 a(A)                            |
| 1.0 ml kg⁻¹ starch       | 4.68 a(A)                            |
| 1.2 ml kg⁻¹ starch       | 4.45 a(A)                            |

Remark: the numbers followed by the same lowercase letters in the same column or the same capital letter on the same line indicate that they are not significantly different based on further DMRT tests at the level of 5%. Lowercase for α-amylase interactions, uppercase for amyloglucosidase interactions.

In normal condition, α-amylase has an optimum range of pH at 5-7. Moreover, according to previous studies conducted by [13] and [14] optimum pH of α-amylase is 5.2 and that of amyloglucosidase is 4.5. pH measurement of liquid sugar in this study was carried out after 48-hour incubation, reporting values ranging 3.95-5.13, which were not in accordance with a pH optimum of enzyme mentioned. This occurred as during 48 hours of incubation, the sugar had undergone fermentation which led to formation of more acidic pH rather than optimum pH of enzyme. Low or high pH may impact on protein denaturation which causes reduction in enzyme activity. pH that gives the highest reaction rate of enzyme is called as the optimum pH. This optimum pH was obtained by determining the amount of sugar formed [12]. pH value impacts of process of sugar formation as enzyme activity is affected by pH, and pH value suitable to enzyme activity accelerates the process of starch hydrolysis. Suitable pH to starch hydrolysis of sago obtained from South East Sulawesi ranges 3.99-5.13. In this research what is the optimum pH for sugar formed.

3.3. The best quality of liquid sugar

The quality of liquid sugar was determined by a number of factors, such as color, aroma, taste, and other factors such as chemical and microbiological characteristics. Quality is essential to meet expectation of consumers and producers. The best quality of liquid sugar from the starch of sago obtained from South East Sulawesi (at starch-water ratio of 1:4, α-amylase concentration of 1.2 ml/kg starch, and amyloglucosidase concentration of 1.2 ml/kg starch) is presented in Table 6. Organoleptic evaluation performed on the selected liquid sugar was aimed to evaluate taste and aroma of the product. In terms of aroma, 47% panelist perceived that the liquid sugar did not contain aroma while the rest, 53%, perceived that the liquid sugar contained aroma. In terms of taste, 7% panelist perceived that the liquid sugar had a rather sweet taste, 50% panelist perceived that it had a sweet taste, and 43% of panelist perceived that it has a very sweet taste. It can be concluded that the liquid sugar prepared from starch-water ratio of 1:4, α-amylase concentration of 1.2 ml/kg starch, and amyloglucosidase concentration of 1.2 ml/kg starch contained a sweet taste, which was in accordance with SNI 01-2978992 requirement [15]. The sweet taste was related to the length of saccharification time, which resulted in increased amounts of products (reducing sugars) [10] and high concentrations...
of enzyme used which resulted in high content of glucose, giving a sweet taste to the liquid sugar produced. Production optimization of liquid sugar using both concentrations of α-amylase and amyloglucosidase of 1.2 ml/kg starch resulted in viscosity level of 60°Brix. The viscosity level was selected in order that the sugar did not harden, did not show an overly dark color, and was easy to analyze. This occurred as total soluble solids which was expressed in °Brix in the liquid sugar remained low, 60°Brix, suggesting a high content of moisture.

Table 6. The best treatment quality of liquid sugar

| Parameter          | Unit       | Liquid sugar | Indonesian standard (SNI 01-2978992) |
|--------------------|------------|--------------|--------------------------------------|
| aroma              | -          | Sugar aroma  | No aroma                             |
| taste              | -          | Sweet        | Sweet                                |
| color              | -          | Yellow-red   | No color                             |
| Water              | % b/b      | 35.26        | Max. 20                              |
| Ash                | % b/b      | 0.06         | Max. 1                               |
| Reducing sugar     | % b/b      | 50.46        | Min 30                               |
| Metal contamination: | ppm       |              | Max. 1                               |
| - Pb               | ppm        | -            | Max. 1                               |
| - Cu               | ppm        | 1.24         | Max. 10                              |
| - Zn               | ppm        | 3.59         | Max. 25                              |
| - As               | ppm        | -            | Max. 0.5                             |
| Microbial contamination: | colony/g | (-)          | Max. 5 x 10^2                        |
| - Total plate count| colony/g   | (-)          | Max. 50                              |
| - Mold             | colony/g   | (-)          | Max. 50                              |
| - Yeast            | colony/g   | (-)          | Max. 50                              |

Reducing sugars produced were 50.46%, meeting SNI 01-2978992 which requires glucose-syrup to have reducing sugars at least 30%. This reducing-sugars content was associated with length of saccharification time and concentrations of enzymes used to allow microorganisms to break down α,4 and α,6 bonds in dextrin and oligosaccharides molecules.

The water content of liquid sugar was 35.26%, the result did not meet the standard of glucose syrup or liquid sugar, which was a maximum of 20%. This occurs because the total dissolved solids (TPT) expressed in °Brix in liquid sugar which was still low at 60 °Brix so that there was still a large amount of water content. The large amount of free water or water in sugar can help microbial growth activities and chemical activity in liquid sugar which can affect the durability of the shelf life of liquid sugar products.

The liquid sugar did not contain lead, while it had copper at 1.24 ppm, in accordance with SNI 01-2978992 requirement (less than 10 ppm). The zinc content of the liquid sugar was 3.59 ppm, which also met SNI 01-2978992 requirement (less than 25 ppm). There was no arsen detected in the liquid sugar. Microbiological analysis reported negative results for two parameters tested, e.g. total plate count (TPC) and total mold/yeast count, which was in accordance with SNI 01-2978992 requirement of glucose-syrup quality. TPC or total bacteria was not associated with indicator microorganisms as it used plate count agar (PCA) culture medium, which is non-selective medium generally used for growth of all bacteria, either good or pathogen. Sensitivity of food materials microbial spoilage depends on composition and condition of raw materials after processing [6].

4. Conclusion

Starch of sago obtained from Konawe, South East Sulawesi appeared as a white fine powder with a typical sago taste and aroma; contained moisture of 7.21%, ash of 0.11%, protein of 0.36%, crude fiber of 0.37%, carbohydrate of 91.76%, starch of 80.69%, copper of 1.28 ppm, no lead, no mercury, no arsen; and showed total plate count of 4.5x10^1 colonies/g and no detection of molds. The optimum condition in production of liquid sugar from starch of sago obtained from South East Sulawesi was resulted from
starch-water ratio of 1:4 at both α-amylase and amyloglucosidase concentrations of 1.2 mL/kg starch. The liquid sugar produced had physical characteristics of 60° Brix, sweet taste, typical sweet aroma of sugar, and reddish-yellow color. Moreover, it showed 35.26% moisture, 0.06% ash, 50.46% reducing sugar, 1.24 ppm copper, 3.59 zinc, and no contents of lead and arsen. Microbial analysis results demonstrated that total plate count and mold count of the sugar met the required standards.

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