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Research Article

The Process of Developing Gelatinization and Saccharification with Variations in Temperature and Period of Glucose Sago Material

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Abstract: Initial temperature variations carried out for the gelatinization stage and sampling every six hours during the saccharification process, which lasted 72 hours. The liquefaction process using the α-amylase enzyme and then proceeds to the saccharification process with the glucoamylase enzyme. The raw material used is sago flour, which has relatively high starch content. The method in this research 1) Sample preparation; 2) Gelatinization Process; 3) Liquefaction Process; 4) Saccharification Process; 5) Reducing Sugar Analysis; 6) Sweetness Level Analysis; 7) Dextrose Equivalent Analysis; 8) Statistical Analysis. The result for analysis obtained the average value of reducing sugar at the use of 121 degrees Celsius gelatinization temperature is 108.33 g/L higher than the value of reducing sugar at the use of 87 degrees Celsius gelatinization temperature is 94.56 g/L. The analysis obtained the average value of sweetness level at the use of 121 degrees Celsius gelatinization temperature is 23.22 degrees Brix higher than the value of sweetness level at the use of 87 degrees Celsius gelatinization temperature is 20.82 degrees Brix. The analysis obtained the average value of dextrose equivalent at the use of 121 degrees Celsius gelatinization temperature is 54,17 percent higher than the value of dextrose equivalent at the use of 87 degrees Celsius gelatinization temperature is 47.28 percent. The high potential of glucose syrup made from sago expected to motivate the development of home industries that use glucose syrup in various food productions.

Keywords: Alpha Amylase, Amylase Enzyme, Dextrose Equivalent, Glucoamylase Enzyme, Liquefaction.

1. Introduction

Glucose has used by the confectionery industry, beverages, and biscuits. It is based on several advantages of glucose syrup, that glucose can increase the smooth texture and depress the freezing point in ice cream, keep the freshness of cake for a long time and reduce cracking [1]. For sweets, glucose preferred because it prevents microbiological damage and improves texture [2].

The potential of sago production and area in Indonesia is tremendous, but only a small portion utilized. Indonesia has around 21 million hectares of potential land and allows for sago crops. Sago potential reaches 27 million tons per year. However, only about 300-500 thousand tons of sago starch used annually. One of the utilization of sago starch used as sago flour with a production of about 2.5 - 50 tons per hectare [3].

Compositionally, sago starch consists of 88 percent carbohydrate, 0.5 percent protein, minute amounts of fat. In terms of nutritional content, one hundred grams of dry sago starch contains 355 calories, including an average of 94 grams of carbohydrate, 0.2 grams of protein, 0.5 grams of dietary fiber, 10 mg of calcium, 1.2 mg of iron, and negligible amounts of fat, carotene, thiamine, and ascorbic acid [4].

Sago native starch contains 70-80 percent more amylopectin, while the amylose content is 15-30 percent [5]. The gelatinization onset temperature of starch from the upper part of the trunk is in the range of 65.3-68.2 degrees Celsius with a gelatinization conclusion

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temperature of 75-76 degrees Celsius. Starch from the lower part of the trunk has a lower gelatinization onset temperature and a higher gelatinization conclusion temperature [6]. Gelatinization temperature depends on the moisture content, degree of crystallinity within the granule, granule size, and amylose to amylopectin ratio [7].

Starch granules have very complex structures. The complexity built around variations in their composition (α-glucans, moisture, lipids, proteins, and phosphorylation), component structure, and variation between amorphous and crystalline regions. Amylose associated with large branches of amylopectin molecules comprises the amorphous region of granules, and amylopectin molecules with short branches comprise the crystalline region; therefore, a higher proportion of amylopectin in starch granules results in more excellent crystallinity [8].

Amylose is a long, straight, unbranched, and hydrophobic chain polymer with α- (1,4) -D-glucose bonds [9]. Amylose possesses the ability to form stable gels and films, into biodegradable plastic [10], food coatings, candy making, coatings on drugs [11]. The breakdown of the amylose chain depends on the temperature, the substrate's enzyme properties, and the length of the chain [5]. Amylopectin made up of chains of α-(1,4)-linked D-glucosyl units that interconnected through α-(1,6)-linkages. These components represent approximately 98–99 percent of the dry weight of starch [12].

Starch is a primary raw material to produce glucose syrup, and it is widely used in the food and pharmaceutical industries [13]. It has reported that sago starch could be used as raw material for glucose syrup production [14]. The industrial processing of starch to sugars can be carried out either by acid or enzymatic hydrolysis. However, the use of enzymes is preferred to acid, once it produces high yields of desired products and less formation of undesired products such as toxics compounds [15].

Glucose syrup, according to Indonesia National Standardization, is defined as a vicious and clear liquid with the main component of glucose, which is obtained from the hydrolysis of starch by chemical or enzymatic process [16]. The industrial processing of starch to glucose, maltose, and dextrin involves gelatinization, liquefaction, and saccharification processes using the acid treatment, enzyme treatment, or a combination of acid and enzymes [13].

Starch gelatinization is the disruption of molecular orderliness within the starch granule. It results in granular swelling, crystallite melting, loss of birefringence, viscosity development, and solubilization [17]. Liquefaction is the process of melting starch gel to obtain lower viscosity by hydrolyzing starch into simpler molecules of oligosaccharides or dextrin through using the α-amylase enzyme [18], requires pH 6-7 at temperatures between 90-110 degrees Celsius [19]. In saccharification, liquid dextrin will further be hydrolyzed by a single enzyme (glucoamylase) or mixed enzyme (glucoamylase and pullulanase) commonly called dextrozyme to converted into glucose (Mithra and Padmaja, 2017) at temperatures of 55-66 degrees Celsius, pH 4-4.5 for 24-72 hours [20].

Amylases are enzyme that catalyzes the hydrolysis of starch into sugars. They are present in the saliva of humans and some other mammals, where it begins the chemical process of digestion. Amylases can be classified into different types; alpha, beta-amylase, and debranching enzyme. The α-amylases (EC 3.2.1.1) (alternative names: 1,4-α-D-glucan glucanohydrolase; glycogenase) act at random locations along the starch chain, α-amylase breaks down long-chain carbohydrates, yielding maltotriose and maltose from amylose, maltose, glucose and "limit dextrin" from amylopectin [21]. The optimum activity of α-amylase usually is at pH 4.8-6.5, and the temperature 60 degrees Celsius. However, the α-amylase enzyme derived from thermostable bacteria tends to be more stable at temperatures of 100-110 degrees Celsius [22].

Glucoamylase is one of the oldest and widely used biocatalysts in the food industry. The primary application of glucoamylase is the saccharification of partially processed starch/dextrin to glucose, which is an essential substrate for numerous fermentation processes and a range of food and beverage industries (Kumar and Satyanarayana, 2009). Similar to the β-amylase enzyme, glucoamylase can break down the structure of abundant complex polysaccharide into a small molecule. The advantage of this enzyme is beside it can break the α-1,4 glycoside bond, and it also breaks the α-1,6 glycoside bond. Therefore this enzyme can convert all starches into glucose. In general, this enzyme works at 45-60°C temperature with pH 4.5-5.0.

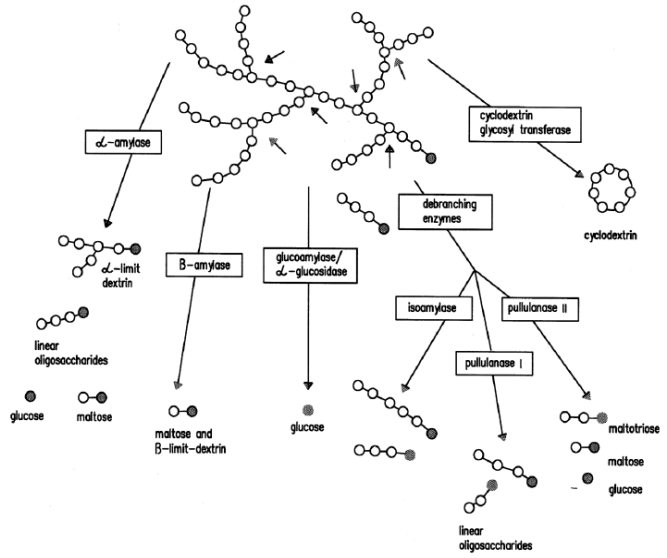

Figure 1. Glucoamylase Breaks Up the Structure of Polysaccharides into Small Molecules.

In this study, initial temperature variations carried out for the gelatinization stage and sampling every 6 hours

during the saccharification process, which lasted 72 hours. The liquefaction process using the α-amylase enzyme and then proceeds to the saccharification process with the glucoamylase enzyme. The raw material used is sago flour, which has relatively high starch content. The high potential of glucose syrup made from sago expected to motivate the development of home industries that use glucose syrup in various food productions. Moreover, this product can be used as an alternative for sugar in the Papua region in the future.

2. Research Methods

2.1. Sample preparation

Sago flour obtained from North Luwu regency, South Sulawesi province, Indonesia. Sago flour that dried first using a blower. Then its moisture content was determined by drying 5 g of sample in an air-oven at 105 degrees Celsius to constant weight. This starch sample contained 11,2 percent moisture.

2.2. Gelatinization Process

Sago flour made in suspension by adding distilled water. CaCl2 cofactor added, and the pH of suspension adjusted to 6.0-6.5 by adding acid or base solution. The suspension heated according to gelatinization treatment temperatures were 87 degrees Celsius and 121 degrees Celsius. Alpha-amylase enzyme added, and when the temperature has reached the temperature treatment, the temperature maintained for 15 minutes.

2.3. Liquefaction Process

Suspension temperature lowered to 80 degrees Celsius and the suspension added by the alpha-amylase enzyme. The stirring process carried out for 90 minutes. At this stage, the results obtained were maltodextrin.

2.4. Saccharification Process

After 90 minutes of the liquefaction process, the suspension temperature cooled until 60 degrees Celsius and pH set to 4.5 for the saccharification stage. Glucoamylase enzyme added into the suspension and stirred for 5 minutes. The suspension inserted into the Erlenmeyer 250 ml to incubated in a water bath shaker for 72 hours. Sampling was done in every 6 hours.

2.5. Reducing Sugar Analysis

The DNS method is a colorimetric technique that consists of a redox reaction between the 3,5-dinitro salicylic acid and the reducing sugars present in the sample. The reagent is a solution formed by the following compounds: 3, 5-Dinitrosalicylic acid (2-hydroxy-3,5-dinitrobenzoic acid), which acts as an oxidant, Rochelle salt (sodium potassium tartrate), which prevents the dissolution of oxygen in the reagent and sodium hydroxide to provide the medium required for the redox reaction to occur [23].

2.6. Sweetness Level Analysis

The sweetness level tested using a hand-refractometer. The sample was pipetted using a dropper and dripped on the surface of the hand-refractometer, the number seen on the device [24], [25]. Results of sugar content usually expressed as total soluble stable or reliable soluble content; however, reliable soluble content appears to be the most widely reported terminology [26]. °Brix or percentage (%), as the unit of refractometric measurement for TSS or SSC, is equivalent and interchangeable [27].

2.7. Research Process

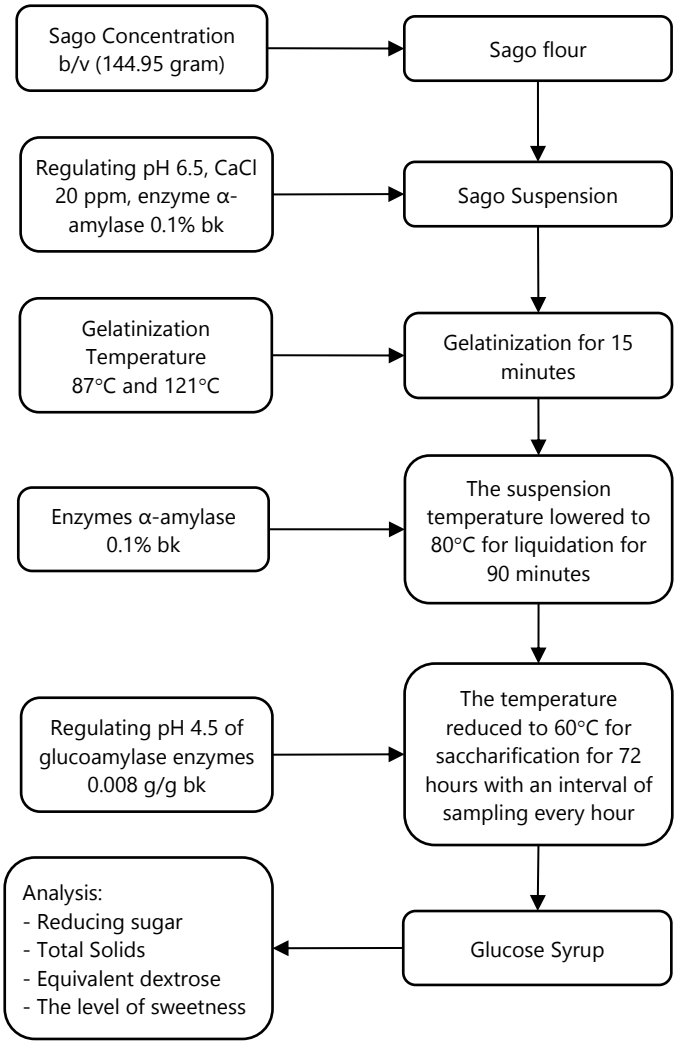

Figure 2. Research Flowchart

2.8. Dextrose Equivalent Analysis

Dextrose equivalent (DE) is related to reducing sugar levels and expressed in percent. Dextrose equivalent is the total reducing sugars expressed as dextrose and calculated as a percentage of overall dry matter [28]. Dextrose

equivalent (DE) value calculated using the following equation:

$$DE = \frac{Reducing\ Sugar\ Content}{Total\ Soluble\ Solid} \times 100\% \quad (1)$$

Dextrose Equivalent (DE) is a quantity that represents the total value of a starch reducing agent or starch modification product in percent units. DE is related to the degree of polymerization (DP). The degree of polymerization states the number of monomer units in one molecule. The monomer unit in starch is glucose, so maltose has a Degree of polymerization two and Dextrose Equivalent 50 [29].

3. Results and Discussions

3.1. Reducing Sugar Level

Reducing sugar is all sugars that could reduce due to the presence of free aldehyde or ketone groups so that it can reduce an electron acceptor compound [30]. Examples of sugars include reducing sugars are glucose, mannose, fructose, lactose, and maltose [31]. The acquisition of reducing sugars on gelatinization temperature variations tends to increase the use of both at 87ºC temperature and 121ºC temperature. The analysis obtained the average value of reducing sugar at the use of 121ºC gelatinization temperature is 108.33 g/L higher than the value of reducing sugar at the use of 87ºC gelatinization temperature is 94.56 g/L.

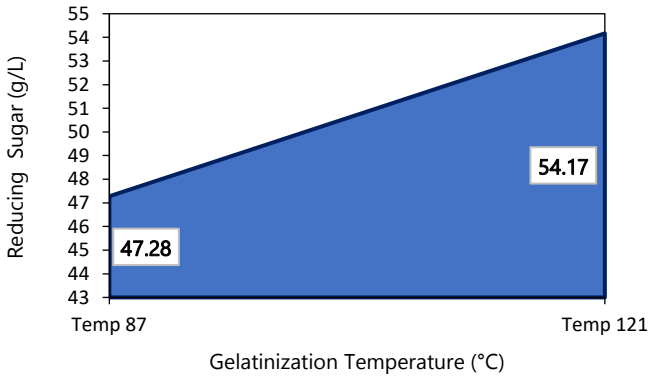

Figure 3. Reducing Sugar Level

When aqueous starch suspensions heated above the gelatinization temperature, irreversible swelling of the granules occurs along with a concomitant change of structural order, but granules still maintain their identity. These changes included granule swelling due to absorption of moisture in the amorphous regions of the granule, leaching of small molecular weight polymers including amylose, loss of the crystalline order and the consequent loss of birefringence, leaching of more abundant molecular weight polymers from the granule including fragments of amylopectin and, finally, starch solubilization [32].

Pomeranz [31] states, in this process, amylose molecules are released into the water phase, which covers the granules, so the structure of starch granules becomes more open, and more water enters the granules, causing the granules to swell and increase in volume. The water molecule then forms a hydrogen bond with the sugar hydroxyl group of the amylose and amylopectin molecules. On the outside of the granule, the amount of free water decreases, while the amount of amylose released increases. Amylose molecules tend to leave granules because their structures are shorter and quickly dissolve.

As a result of gelatinization, alpha-amylase and glucoamylase enzyme become easier to hydrolyze the structure of amylose and amylopectin. Each hydrolyzed sugar chain has one reducing sugar group so that the more starch hydrolyzed into medium-chain sugar, the higher the amount of reducing sugar. The value of reducing sugars produced also increases with the length of time of saccharification. The table below shows the value of reducing sugars in glucose syrup increases as the increase of saccharification time, where it starts from 0 hours (control), continues to increase until it reaches 72-hour reaction time. Increasing in reducing sugars value occurs as the increase of saccharification time caused by increased contact between sago starch (substrate) and the enzymes used.

Table 1. Variation of Gelatinization Reducing Sugar.

Saccharification Time	Temperature	
	87°C	121°C
0 hour (control)	77.91	89.18
6 hours	83.59	95.10
12 hours	86.38	97.42
18 hours	87.98	100.22
24 hours	90.22	102.30
30 hours	93.42	105.54
36 hours	97.18	107.83
42 hours	94.46	110.71
48 hours	98.14	114.07
54 hours	100.78	117.11
60 hours	102.70	120.47
66 hours	106.55	121.90
72 hours	109.91	126.54
Average	**94.56**	**108.34**

Hydrolysis using α-amylase and the glucoamylase together produce reducing sugars such as glucose, maltose, dextrin, maltose, maltotriose, and maltotetraose. The longer the time used in the saccharification process, the more contact that occurs between the enzymes against sago starch so that the breaking of α-1.4 glycosidic bonds and α-1.6 glycosidic in amylose and amylopectin in sago

starch is higher. That is because the longer the reaction process, the more optimal the enzyme activity furthermore, as the reaction time increases, the more hydrolyzed starches and the increase in enzyme activity to break glycosidic bonds into simple sugars, which causes the amount of reducing sugars to increase [33].

3.2. Sweetness Level

The level of sweetness is one parameter of how much simple sugars formed in a product or food ingredient [34]. The sweetness level of glucose syrup measured using a hand refractometer. The working principle of the tool is to measure the sweetness of glucose syrup based on the number of dissolved solids that dominate in a food [27], [35]. The acquisition of sweetness level on gelatinization temperature variations tends to increase the use of both at 87 degrees Celsius temperature and 121 degrees Celsius temperature. The analysis obtained the average value of sweetness level at the use of 121 degrees Celsius gelatinization temperature is 23,22°brix higher than the value of sweetness level at the use of 87°C gelatinization temperature is 20,82°brix.

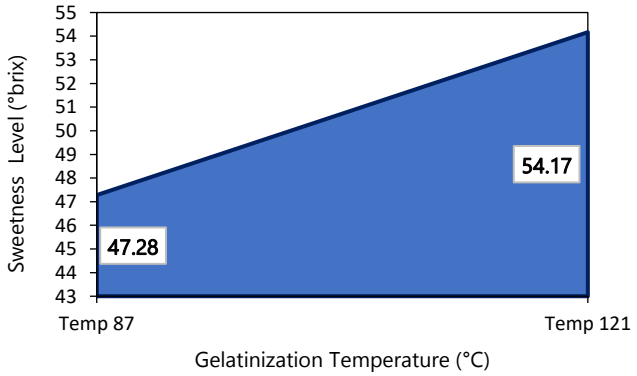

Figure 4. Sweetness Level

The sweetness level obtained from the total dissolved solids. The total dissolved solids of an ingredient include reducing sugars, non-reducing sugars, organic acids, pectin, salts, and proteins which are very influential on °brix [36]. It is explained that the components measured as total dissolved solids are sucrose, reducing sugars, organic acids, and proteins [37]. Besides, the increase in total solids during the gelatinization process can be due to a large number of small particles from cutting starch chains, especially in the amylopectin fraction in the crystalline region during the gelatinization process, where the particles become dissolved solids in suspension.

The higher the temperature and the longer the cooking, the higher of total sugar obtained because the higher the temperature and the longer the cooking process, the evaporation process of free water in the product will be higher. If evaporation higher, the water content decreases so that the total percentage of sugar increases. Furthermore, the higher the hydrolysis temperature, the higher the glucose levels obtained. Hydrolysis using high temperatures causes the starch to expand and break so that the long chain of glucose units from amylose and amylopectin becomes shorter and subsequently breaks into glucose units.

The value of the sweetness level obtained increases with the increase in saccharification time. The table below shows the value of sweetness level in glucose syrup increases as the increase of saccharification time, where starts from 0 hours (control), continues to increase until it reaches 72-hour reaction time.

Table 2. Variation of Gelatinization Sweetness.

Saccharification Time	Temperature	
	87°C	121°C
0 hour (control)	18.10	20.60
6 hours	20.50	21.90
12 hours	20.00	22.30
18 hours	20.20	22.60
24 hours	20.40	22.70
30 hours	20.70	23.00
36 hours	21.10	23.50
42 hours	20.70	23.60
48 hours	21.20	23.80
54 hours	21.40	24.10
60 hours	21.80	24.30
66 hours	22.10	24.60
72 hours	22.40	24.80
Average	**20.82**	**23.22**

This increase is due to the longer time used in the saccharification process, the more contact that occurs between enzymes against sago starch so that the α-1,4 glycosidic and α-1,6 glycosidic bonds in amylose and amylopectin in sago starch are higher. That is because the longer the reaction process, the more optimal the enzyme activity because the longer the interaction between enzymes and substrate causes more glucose to form. The increase in glucose acquisition produced is due to the longer hydrolysis; the chance of collision between water molecules and starch molecules will be longer so that it will produce more glucose. The longer the reaction time, the enzyme work will also be more optimum.

3.3. Dextrose Equivalent Level

The extent of starch hydrolysis generally expressed in terms of the dextrose equivalent (DE), a quantity that indicates the number of dextrose molecules released from the hydrolysis of starch. Glucose has a DE value of 100, while starch has a DE of zero. Dextrose equivalent is related to reducing sugar levels and expressed in percent. Acquisition of dextrose equivalent on gelatinization temperature variations tends to increase the use of both at 87°C temperature and 121°C temperature. The analysis obtained the average value of dextrose equivalent at the

use of 121ºC gelatinization temperature is 54,17% higher than the value of dextrose equivalent at the use of 87ºC gelatinization temperature is 47,28%. Dextrose equivalent value increases with increasing reducing sugar levels. Thermal treatment can damage and melt the amorphous and crystalline parts of starch so that amylose and amylopectin can diffuse out of the granules. When amylose and amylopectin diffuse, enzymes can more easily hydrolyze to produce oligosaccharides and dextrin with relatively small molecules.

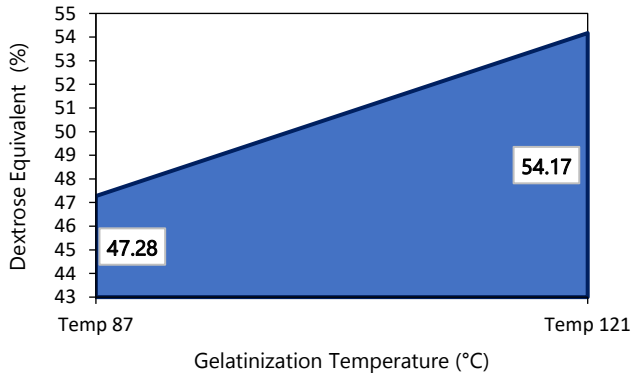

Figure 5. Dextrose Equivalent Level

Each hydrolyzed sugar chain has one reducing sugar group so that the more starch hydrolyzed into pure chain sugar, the higher the amount of reducing sugar. This indirectly increases the equivalent dextrose value. So that the higher the gelatinization temperature used, the value of dextrose equivalent is also higher. The dextrose equivalent value obtained increases with the increase in saccharification time. The table below shows the value of dextrose equivalent in glucose syrup increases as the increase of saccharification time, where starts from 0 hours (control), continues to increase until it reaches 72-hour reaction time.

Table 3. Variation of Gelatinization Dextrose Equivalent (DE).

Saccharification Time	Temperature	
	87°C	121°C
0 hour (control)	38.95	44.59
6 hours	41.79	47.55
12 hours	43.19	48.71
18 hours	43.99	50.11
24 hours	45.11	51.15
30 hours	46.71	52.77
36 hours	48.59	53.91
42 hours	47.23	55.35
48 hours	49.07	57.03
54 hours	50.39	58.55
60 hours	51.35	60.23
66 hours	53.27	60.95
72 hours	54.95	63.27
Average	**47.28**	**54.17**

The value of reducing sugars influences the equivalent dextrose value, so the longer the hydrolysis process takes place, the higher the contact between the enzyme and sago starch so that the hydrolysis results in the form of pure chain sugar increasingly. Each hydrolyzed sugar chain has one reducing sugar group so that the more starch hydrolyzed into pure chain sugar, the value of reducing sugar is also higher. The relationship between the value of reducing sugars with dextrose equivalent value that the longer the reaction process took place, the more starch that hydrolyzed by enzymes to produce dextrin. Much dextrin increases DE from the resulting dextrin products [38]. Furthermore, dextrose equivalent (DE) states that the total reducing sugar content calculated as dextrose (glucose), so the longer the reaction takes place, the more starch that can be hydrolyzed into pure compounds, in this case, is dextrose (glucose), causing DE value increased [39], [40].

The food and beverage industry currently a tendency to use glucose syrup. This based on several advantages of glucose syrup compared to sucrose, such as glucose syrup does not crystallize like sucrose when cooking at high temperatures, the crystal core not formed until the glucose syrup solution reaches 75% saturation [41]. The raw material for making glucose syrup is starch, for example, tapioca, sago, cornflour, tuber starch. One of the tubers starch that has excellent potential to develop into glucose syrup is starch from arrowroot, which contains 15% to 30% starch. Glucose syrup, or often also called liquid sugar, contains D-glucose, maltose, and D-glucose polymers made through starch hydrolysis. The process of glucose syrup starch hydrolysis can use an enzyme catalyst, acid, or a combination of both [42].

Lehninger [43] added that the high substrate concentration with enzyme activity is increasing. Eventually, the boundary point will be reached, and after this point is reached/exceeded, the activity will only increase so small as the substrate concentration increases while increasing the pH with a fixed dose of the enzyme in the saccharification stage, the enzyme activity, namely the ionic nature of the carboxyl group and amino acids, changes in conformation and catalytic function to further hydrolyze the α-1.4 glycosidic linkages in the pieces of dextrin to glucose which calculated as DE values.

Forgaty [44] that the glucoamylase enzyme is capable of hydrolyzing the cutting of glucoside bonds from the non-reducing end of the starch polymer. The glucoamylase enzyme is an echoenzyme that produces β-glucose from a non-reducing terminal chain in amylose, amylopectin by hydrolyzing the ά-1,4-glycosidic bond in sequence [45], [46]. Therefore, the enzyme can convert all starch into glucose. Increasing the concentration of starch and the pH of saccharification of reducing sugars contained in glucose syrup increases, due to high substrate

concentrations, the starch hydrolyzed by enzymes is more excellent, and there is a tendency to increase in pH the glucose levels increase, where during incubation for 18 hours there is further degradation by the glucoamylase enzyme [47]. Under these conditions, an estimated change in the activity of the enzyme is due to an increase in saccharification pH, which results in the ionization of the active group of enzymes that play a role in binding the substrate and transforming into products.

4. Conclusions

The advantage of starch hydrolysis with acids and enzymes is that it is easy to get the primary raw materials, a more straightforward process than using acids, in the use of fewer enzymes, equipment is not complicated, so it does not require much work, we get more precise and cleaner glucose syrups. While the weakness of the hydrolysis of starch with acids and enzymes is that the enzymes used still imported, so the price is relatively high, and the use of acid catalysts can cause corrosion to the tool even though the use of acid catalysts has reduced. The need for ongoing research so that glucose can found in a variety of food materials and can process and used for the food industry effectively.

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