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# SEED WASTE OF MANGO (*Mangifera indica*) AS RAW MATERIAL GLUCOSE SYRUP ALTERNATIVE SUBSTITUTE FOR SYNTHETIC SWEETENER

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**Abstract:** The search for alternative ingredients as natural sweeteners needs to be done. The sweeteners circulating in the market are synthetic sweeteners with no nutritional value and harm health. This research aims to produce a safe sweetener in the form of glucose syrup, which can be used as an alternative to artificial sweeteners through enzymatic reactions, and to find out the suitable reaction conditions to produce glucose syrup with the highest levels in several combinations of pH and temperature. Mango seed core is a source of carbohydrates that can be used as glucose syrup. The average starch content of 1940.1 grams of wet mango core is 10.09%. The starch was hydrolyzed with alpha-amylase and glucoamylase. It is biocatalysts through liquefaction stages (pH 5 and 6) and saccharification (temperature variables 55$^{oC}$and 60$^{oC}$).

**Keywords:** Mango Seed Core, Glucose Syrup, Starch Hydrolysis, Enzymatic Reaction.

## INTRODUCTION

Food is an integral part of human life. In terms of appearance and taste, additional substances (additives) are added to the food. These additives can function as sweeteners, colorants, flavor enhancers, or preservatives. However, many food manufacturers use these additives outside the provisions, especially synthetic additives.

One of the additives used in food is a sweetener. Manufactured foods or beverages generally use artificial sweeteners. Several synthetic food sweeteners on the market are sodium cyclamate, saccharin, sorbitol, and aspartame.

One of the most popular sweeteners is aspartame. Aspartame is an artificial sweetener with a sweetness level of 200 times sweeter than sugar (sucrose) and is commonly found in beverage and food products [1]. Aspartame is composed of amino acids so that the body will experience metabolism and amino acids in general. It is forbidden to consume aspartame for hereditary diseases related to mental weakness because it can cause brain damage and mental disability [2].

In Indonesia, according to the Regulation of the Minister of Health of the Republic of Indonesia Number: 208/MENKES/IV/1985 [3] concerning the requirements for the use of artificial sweetener food additives in food products, aspartame can be used safely and without problems, if it is in the allowed dose. For the food and beverage category, the maximum limit for the use of aspartame is 40 mg/kg BW [4]. In Bandung district found that from all samples studied, there were levels of aspartame, namely 68,834 mg/kg in powdered drink samples, and exceeded the maximum allowable use limit, which the ADI had set [5]. *Acceptable Daily Intake* which is 40 mg/kg BW.

Several studies mention the dangers of aspartame to health, resulting in increased serum urea and creatinine levels, both in ADI doses and doses exceeding the ADI [6], resulting in kidney disorders [7].

Considering the dangers posed by these synthetic sweeteners, it is necessary to have alternative sweeteners that can be applied to food as a substitute for synthetic sweeteners. So, one of the safe alternative sweeteners is glucose syrup.

## RESEARCH METHODS

### Sampling Techniques

Mango seed samples used to make glucose syrup in this study were mango seed waste of various varieties taken in a wet and fresh state and then washed thoroughly.

### Sample Preparation Samples

of mango seeds that have been cleaned and then peeled the skin of the mango seeds to reveal the core flesh of the seeds. Furthermore, the core pieces of seeds are collected, then soaked repeatedly until the water is evident.

### Making Flour The

Manufacturing flour from the core of mango seeds is carried out based on the method developed [8] with minor modifications. First, keep the core of the wet mango seeds crushed with a blender and then put it in clear water kneading. Then filtered and added sulfuric acid solution and then precipitated for some time. Then the mango seed core flour is deposited until it is white. Flour in the oven is dried until dry and pure white. The starch obtained is stored for the next stage.

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**Making glucose syrup**

Making glucose syrup from mango seed kernel flour is carried out based on the method developed by the Center for Postharvest Research and Development, Ministry of Agriculture, with slight adjustments.

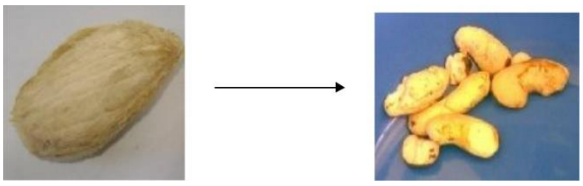
Mango seed core flour (starch) is dissolved in water to form a 30% starch solution. This starch has an initial pH of 4.0-4.2. The starch suspension was then adjusted for pH (pH 5 and 6) by adding NaOH. The rest whose pH has been adapted and then added with 0.1 ml of amylase enzyme. The suspension was liquefied by heating at 80°C-95°C for the optimum conditions for the alpha-amylase enzyme for 60 minutes. During this process, stirring is carried out using a magnetic stirrer. Every 30 minutes, the amylose content of the syrup was analyzed using the iodine test. The brown color of iodine means that all the amylose has been degraded to dextrin and the liquefaction process is complete.

The resulting dextrin solution was allowed to stand until the temperature dropped to 60°C. The pH of the solution after liquefaction ranged from 5.0 to 6.0. The dextrin solution has then adjusted the pH between 4.0-4.5 for the optimum condition of the amyloglucosidase enzyme by adding HCl. The dextrin solution was added with the enzyme glucoamylase as much as 0.1% by weight of starch. Then the saccharification process was carried out by keeping the temperature at 60°C for 48 hours using a water bath shaker. The glucose syrup solution produced in the saccharification process is added with activated carbon as much as 2% dry weight of starch for the purification process, namely by heating this syrup solution at a temperature of 80°C for 10 minutes. After purification using activated carbon, the glucose syrup solution was filtered, and then the reducing sugar content was tested using the Nelson-Somyogi method. After that, the concentration is carried out using *a vacuum rotary evaporator* where the concentration-time varies depending on the desired syrup solids content.

## RESULTS AND DISCUSSION

**Sample preparation (Mango Seed Core)**

The mango seeds used in this study were taken directly from Taruna Jaya Restaurant in Ampenan, West Lombok Regency. Mango seed samples that have been taken from the sampling location are cleaned and then peeled the skin of the mango seeds to reveal the core flesh of the seeds. Before being processed into starch flour, the core of mango seeds is first washed with water to minimize impurities such as soil and sap. The cores have been washed into pieces and soaked repeatedly until the water is clear to remove the sap present in the sample, which is feared to interfere with the hydrolysis process. The mango seeds' core is collected until it becomes quite a lot. The sample will be continued into the process of making starch. This study obtained that the mango seeds are clean, as shown in Figure 1.



(a) (b)
Figure 1. (a). Mango seeds, (b) Core Mango seeds

**Making Flour Starch**

Starch is a polysaccharide found in plants in the form of granules. Many starch granules are stored in the stems, roots, tubers, seeds, and fruit [9]. The manufacture of starch starts by crushing the core of the wet mango seed. It aims to damage the cells so that the starch granules are quickly released in the following process to separate the starch from other impurities easily.

Then the starch slurry obtained is followed by a filtering process. Filtering is carried out to remove starch granules from the cells and separate them from other components that are not soluble in water. The filtered solution is continued to a precipitation process by being left for 5-8 hours to separate pure starch from other parts such as dregs. According to [10], the deposition rate is determined by the size of the starch granules, the acidity of the soaking water, the protein content involved, plus other colloidal substances. The deposition of granules generally lasts for 24 hours and will produce a sediment thickness of about 30 cm.

The residue (wet starch) was then dried, using a low temperature of 40°C to keep the sample from being damaged in its composition. Drying evaporates the moisture content to obtain a completely dry starch. The moisture content will affect the stability during storage. High water content can cause the starch to be less stable, so starch can be damaged. This dry starch is ground using a mortar and directly sieved to get a fine starch powder. It was done to facilitate the starch hydrolysis process. The color of the starch obtained is slightly brownish, as shown in Figure 2. The results of the starch manufacturing process are presented in Table 1.

Figure 2. Starch Powder

Table 1. Percentage of Core Starch of Mango Seeds

| Repetition 1 | Weight of Mango Seed Core | Weight of Starch | Percentage of Starch |
|---|---|---|---|
| 1 | 699.22 | 80.45 | 11.51% |
| 2 | 490 | 88.35 | 71.7 |
| 3 | 750.79 | 58.10 | 61% |

From the results shown in Table 1, the percentage of starch from the core of mango fruit seeds obtained was 7.27%-11.51%.

**Making Syrup (Hydrolysis of Starch)**

In this study, glucose syrup was manufactured using enzymes by hydrolysis of starch using enzymes. Hydrolysis using enzymes has many advantages compared to using acid. The cutting of starch chains by enzymes is more regular and specific than the results of cutting starch chains by acid [11]. In addition, acid hydrolysis is easier to carry out, cheaper. Still, it has drawbacks compared to enzymatic hydrolysis, namely the emergence of unwanted colors and tastes, so that it can reduce product quality [12]. A small amount, the purification step (removing ash), and color formation can be kept to a minimum [13]. The enzymes used in the starch hydrolysis process are amylase and glucoamylase enzymes.

There are two stages in converting the starch in the starch hydrolysis process, namely the liquefaction and saccharification stages. Liquefaction is a partial starch hydrolysis process characterized by a decrease in viscosity, while saccharification is a further process of hydrolysis to produce glucose [12]. This liquefaction process aims to completely dissolve starch, prevent isomerization of reducing groups from glucose, and facilitate the work of enzymes. Alpha-amylase was used to hydrolyze starch [14]. The starch liquefaction process is carried out by combining two processes, namely gelatinization, and dextrinization. To a certain extent, it prevents retrogradation in other processes followed by the addition of alpha-amylase enzymes.

The material used in the manufacture of glucose syrup at this liquefaction stage is starch from the core of mango seeds with a suspension of 30% of the weight of the starch by dissolving the core starch of mango seeds with distilled water by stirring so that the starch dissolves into the distilled water to form a starch suspension. The optimum concentration of starch that had undergone pre-hydrolysis with -amylase enzyme was about 30-35% by weight of the material [15]. If the starch solution is too concentrated, it will be difficult to be appropriately suspended so that the starch particles are deposited during the gelatinization process.

The starch suspension was then adjusted to pH 5-6 by adding 0.5 N NaOH solution and 0.5 N HCl. It was aimed at increasing the activity of the alpha-amylase enzyme. Then the alpha-amylase enzyme was given to speed up the hydrolysis reaction. This enzyme will break down the core polysaccharide of mango seed starch into the form of maltodextrin. The mechanism of alpha-amylase is the degradation of amylose into maltose and maltotriose [16].

Starch → dextrin + maltose + maltotriose + glucose

Suspension is then continued to the heating process until it reaches a temperature of 95oC by stirring for ± 60 minutes to get the maximum initial hydrolysis results. This temperature was chosen because it is the optimum temperature for the work of the alpha-amylase enzyme. Where the liquefaction temperature is too high, it will cause enzyme damage, but if it is too low, it will result in imperfect gelatinization [17]. In the liquefaction process, the things that need to be considered are substrate concentration, use of stable enzymes at high temperatures, temperature regulation, pH regulation and stirring, and direct and continuous heating [18-20]. Adjusting the pH of the solution can be used NaOH and HCl.

The results showed that the starch suspension was initially cloudy white during the liquefaction process and slowly cleared up with a yellowish color. Then there is an increase in viscosity when heating at temperatures up to 85oC, or a gelatinization process occurs. Then at temperatures above 95oC, there is a decrease in density (more watery than before). The change in the starch solution is usually followed by swelling of the starch granules. Because the number of hydroxyl groups in starch molecules is very large, the ability to absorb water is very large. Starch and water are heated to break the hydrogen bonds contained in the starch polymer chain so that it has the effect of weakening the starch polymer chain bonds in starch granules. When the polymer bonds in the starch granules begin to weaken, the granules will absorb water, and swelling occurs in the starch granules, and that's when the alpha-amylase enzyme acts as a catalyst to break the -1,4 glycosidic bonds present in starch [16].

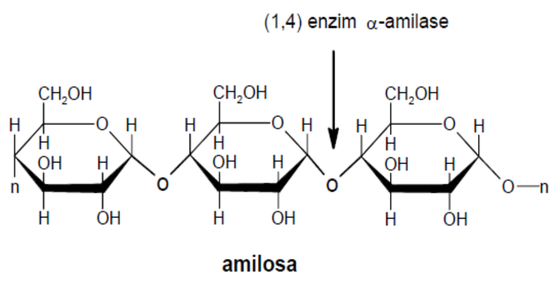



Figure 4. The mechanism reaction of the alpha-amylase enzyme is to degrade the -1,4 glycosidic bond amylose

Meanwhile, the decrease in viscosity occurred as a result of the action of the alpha-amylase

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enzyme breaking the -1,4 glycosidic bond when swelling of the starch granules occurred. As a result of breaking the bond, the granules become broken and result in the release of liquid from a gel from starch, making the suspension's viscosity decrease. The mechanism reaction of the alpha-amylase enzyme is to degrade the -1,4 glycosidic bond amylose (figure4).

Then the liquefaction stage ends with an iodine test on the suspension. To determine whether amylose is still present in this stage, marked by a brownish-red color appearing or a reddish blue color disappearing when a small amount of the solution is dropped with a little iodine. The following reaction occurs:

**KI (s) + $H_2O$ (aq) → $K^+$ (aq) + $I^-$ (aq)**
**$I^-$ (aq) + $I_2$ (s) → $I_3^-$ (aq)**
**$I_3^-$ (aq) + amylose (aq) → amylose-triiodide (blue-red precipitate)**

Then the suspension was added with 0.5 N HCl to pH 3.5. It is intended to turn off the work of the enzyme so as not to affect the suspension in storage for further hydrolysis.

In the advanced stage (saccharification), glucoamylase enzymes are used to attack the -1,6 glycosidic bonds in amylopectin. Glucoamylase is added in enzymatic hydrolysis. Converting starch into glucose is produced more because glucoamylase can break the bonds in starch that the addition has not broken of the alpha-amylase enzyme. The hydrolysis product made with glucoamylase has a sweeter taste than the hydrolysis product using hydrochloric acid. Besides that, glucoamylase can prevent side reactions because the enzyme catalyst is particular [14].

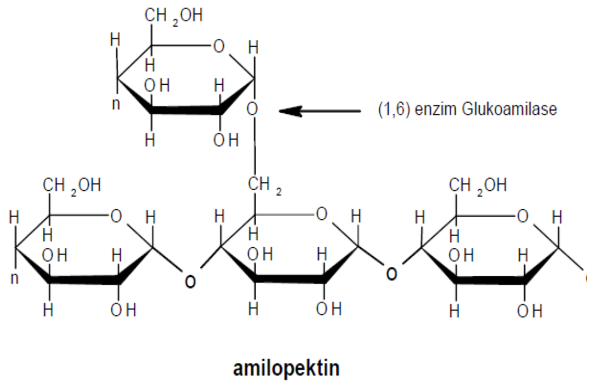


Figure 5. The mechanism reaction of the alpha-amylase enzyme is to degrade the -1,6 glycosidic bond amylose

Before adding glucoamylase, the suspension was conditioned to optimum pH (4.5) of glucoamylase by 0.5 N NaOH. The suspension of the enzyme was hydrolyzed with a water bath shaker for 48 hours at a temperature of 550-60oC. It was done because the optimum conditions for the activity and stability of the glucoamylase enzyme were pH 4, temperature 40-60oC, and reaction time around 48-96 hours [15]. The mechanism of action of the glucoamylase enzyme to degrade amylopectin by breaking -1,6 glycosidic bonds (figure 5).

From the study, the viscosity of the solution (glucose syrup) decreased compared to the density when starting the process. The color of the glucose syrup obtained is slightly cloudy due to impurities from the side reaction during hydrolysis. Then bleaching agent, namely activated carbon, removes impurities during the hydrolysis process remaining in the syrup. The addition of activated carbon also aims to stop the enzyme activity. The syrup did not hydrolyze the remaining starch. The residue filtered on the filter paper is in the form of brownish gels, which are thought to be mineral substances and other impurity residues in the hydrolysis process.

The glucose syrup obtained is transparent yellow to cloudy brown. The following is a picture of glucose syrup obtained from the hydrolysis of the core starch of mango seeds.

Figure 6. Glucose Syrup from Mango Seed

## CONCLUSION

Mango seed can be used as a source of glucose syrup using the enzymatic hydrolysis method. The right reaction conditions to produce glucose syrup with the highest levels were found in liquefaction pH six and saccharification temperature of 60$^o$C.

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