

## **Fructose separation from sorghum syrup by using HPLC approach: a review**

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### **Abstract**

Liquid sugar available today is usually the result of dissolving granulated sugar using hot water. Sorghum syrup can be obtained from the concentrate without involving crystallization, centrifugation, sieving and drying and dissolving processes. However, the sorghum syrup produced from the concentrate still containing complex sugar components such as sucrose, sugar, fructose and others. This review was examined the separation of fructose from sorghum syrup using the HPLC approach. Compared with artificial sweeteners or sugar derivative products such as dextrose, maltodextrin, sorbitol, saccharin, sucralose, and xylitol, sorghum syrup still has lower economic value. The manufacture of these sugar derivatives generally uses chemical processes such as chlorination of sucralose, hydrogenation of xylitol or enzymatic processes and fermentation of fructose. Chemical processes in general can pose a danger to consumer health, while enzymatic and biological processes require high operational costs and complex processes of enzyme and cell separation such corn fructose production. Chromatography is a technology for separating complex mixtures such as sorghum concentrates to obtain separate components, such as fructose syrup and byproducts. On a laboratory scale, sugar fractionation or fructose purification is generally carried out using High Performance Liquid Chromatography (HPLC) with the Carbowax ion exchange column as the stationary phase and ultrapure water as the mobile phase. The industrial scale fractionation in the food sector is still applied to palm oil processing. This is a great opportunity to conduct research related to the components of sorghum concentrates using chromatography column fractionation technology to obtain pure fructose with greater process efficiency and economics.

**Keywords:** fructose, liquid sugar, sorghum syrup, HPLC, fractionation process

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### **INTRODUCTION**

Sugar is a commodity that has an important role in the agricultural sector in the national economy. In general, sugar can be divided into two, namely natural and synthetic sugars. Natural sugar is sugar produced from plants containing sap such as sugarcane, sorghum, coconut, palm, palm, palm, dahlia, and stevia. Meanwhile, synthetic sugars are artificial sugars, for example aspartame and cyclamate (Rukmana, 2003). As for other types of sugar, namely sugar alcohols which have low calories when compared to other sugars. In the process of making sugar alcohol, several technologies such as chemical and biological are used. The shortcomings of the process of making sugar alcohol chemically can cause negative impacts for consumers due to the presence of chemical residues in the product. According to research by De et al. (2015), the process of making sucralose sugar involves chlorine in its manufacture, making dextrose sugar using the enzymatic hydrolysis process (Zhang et

al., 2018), and making xylitol sugar using the acid hydrogenation process under high pressure and at high temperature (Sharma, 2014). The manufacture of xylitol sugar takes a long time and requires energy and pure raw materials so that its operational costs are higher (Ambarsari et al., 2015).

With the development of culinary in Indonesia, both food and drink require sugar and usually in the form of sugar or liquid sugar. Most of the fast food culinary uses liquid sugar in the process of making their products. This is because liquid sugar dissolves faster than granulated sugar (Davidson, 2014). In addition, the process of making granulated sugar requires high production costs because of the water evaporation process which requires high latent calories (Rein, 2009).

Sugarcane is a source of sugar crops that are being developed in Indonesia. Another plant in Indonesia that can be developed as a source of sugar is sweet sorghum (*Sorghum bicolor* L). Currently, the average yield of sorghum seeds can reach 4-5 tonnes / ha and sorghum stems around 15 tonnes / ha, which so far are still considered as waste that is not fully utilized (Noerhartati and Tri, 2013). There is so much sweet sorghum that sweet sorghum has the potential to be used as a sugar-producing plant. When compared to sugarcane juice, the sugar content in sorghum sap tends to be greater, namely 16.8 °Brix, the total sugar is 142 g/L, while for sugarcane juice is 15.7 °Brix, the total sugar is 131 g/L (Andrezejewski, 2013). The fructose content in sorghum ranges from 0.3 - 0.7% (Taylor and Duodu, 2018).

According to Ali et al. (2018), the process of making sorghum liquid sugar goes through several stages, namely extraction, filtration, centrifugation and evaporation. Liquid sugar is produced from several processes such as hydrolysis with the help of enzymes such as  $\alpha$ -amylase or glucoamylase (Ratna and Yulistiani, 2015), isomeration processes using glucose isomerase enzymes (BBPPP, 2006), and purification processes that will be purified with adsorbents. Then proceed with the evaporation process to obtain liquid sugar (Hull, 2010). The evaporated liquid sugar component contains a very complex content such as glucose, fructose, suctorsa, and minerals which are high enough to cause the sweetness level to be lower than sugar derivative products such as sucralose, sorbitol, and others. Sorghum liquid sugar is called vegan honey because sorghum is itself an 'anti-sugar' brigade. So that this liquid sorghum sugar can be used as a healthier substitute for sugar in the form of syrup.

Sorghum liquid sugar products have a low selling value because the production process requires a large amount of money, so liquid sorghum sugar is sold at a high price. Thus, to produce sorghum liquid sugar which has higher sweetness and a production process that does not require a lot of money, technological innovation is needed to be able to separate some of the minerals and impurities in the sugar content. One of the technologies that can be used is the fractionation technology where the technology can separate the fractions contained in a solution or suspension to obtain separate constituent components (Yuliasih et al., 2007).

Food processing using fractionation technology is usually used to separate palm oil, while research for the separation of sorghum has never been carried out. According to Stafford and Bliss (1973), to separate the fraction in sorghum, gel filtration column chromatography can be used, where the solution is applied to an agarose column measuring 1 x 40 cm. In addition, the fraction in sorghum can be separated by the CM-Sepaharose CL-6B ion exchange column chromatography method (Adefila et al., 2012). According to Guyon et al. (2013), HPLC-co-IRMS can be proven to separate fructose from other components in lemon juice without any purification process. Therefore an HPLC approach is needed to do fructose fractionation because

HPLC has been proven to be used to separate fructose components. From some existing literature, this technology still cannot be used on a large scale. Thus, scaling up is needed regarding sorghum fractionation using the liquid chromatography method so that it can be used by large industries. This review was examined the separation of fructose from sorghum syrup using the HPLC approach.

## **SORGHUM SYRUP**

Sugar is one of the basic ingredients consumed by the people of Indonesia Sugar is a simple carbohydrate because it dissolves quickly in water and is immediately absorbed by the body to be converted into energy (Lestari and Pramudhita, 2020). Various foods and drinks use ingredients from sugar to be used as sweeteners. The need for sugar is increasing by almost 95%, so sugar production is increasing. Sugar as sucrose can be obtained from sugar cane, palm sugar and sugar beets. Sugar is divided into three types, namely raw sugar, refined sugar and white sugar (Pujitiasih et al., 2014). There are other types of sugar besides the three types of sugar, namely liquid sugar / sugar syrup. In Indonesia, the development of liquid sugar potential is still a problem because it is still very minimal. The advantage of liquid sugar / sugar syrup is that the manufacturing process takes a shorter time when compared to printed sugar. In addition, liquid sugar is also very easily dissolved in water, unlike crystal sugar or printed sugar.

In general, liquid sugar brownish sorghum, has a sweet taste up to 6.50%, has a distinctive caramel aroma from the strong sap. According to Noerhartati and Rahayuningsih (2013), liquid sugar has a brix value of up to 60% (Table 1). Sorghum liquid sugar contains 61.84% total sugar. The total sugar consists of the amount of reducing and non-reducing sugars. Meanwhile, the average reduction sugar in liquid sugar sorghum reached 55.33%. In addition, sorghum liquid sugar has a viscosity of 70.00 mPa.s. The presence of high viscosity causes the value of the brix in liquid sugar sorghum to also be higher. Then the liquid sugar sorghum has a brix value of 67.50% (Noerhartati and Rahayuningsih, 2013).

The process of making sorghum liquid sugar goes through several stages, namely extraction, filtration and centrifugation (Ali et al., 2018). The first step is extracting sorghum stem sap using the milling technique. Before the sorghum stalks are ground, the sorghum stalks must be stored for 1-2 days so that they are wilted. After the sorghum stalk is stored, it is continued with a crusher process, so that the gross sap and sorghum stem dregs are obtained. The next step is the 1st stage of the filtration process, so that the 1st net sap is obtained (Noerhartati and Rahayuningsih, 2013). According to Noerhartati (2012), the extraction of liquid sugar sorghum sticks is carried out by adding 1000 rpm lime water and 1000 rpm flocculant addition. Langlah then carried out the deposition process and continued with the second stage filtering process to separate the sap from impurities (blotong). After that, it is followed by the second net evaporation process until liquid sugar is produced.

According to Noerhartati and Rahayuningsih (2013), the stalk of sorghum will affect the color of the liquid sugar produced. The color change that tends to brown in liquid sugar is due to a non-enzymatic browning reaction, namely caramelization and mailard reactions during the heating process. The yield of sorghum stem liquid sugar is calculated based on the ratio between the weight of liquid sugar after the extraction process with the weight of raw material (sorghum stem juice) according to the treatment multiplied by a percent. The yield of sorghum liquid sugar is very high due to the grinding / squeezing process. The faster the process of milling / squeezing the

sorghum stalks, the higher the yield produced (Putrianti et al., 2016). In addition, the existence of a very high brix value will affect the yield produced. The higher the brix value, the higher the yield produced (Dewi et al., 2014).

Table 1. Characteristics of Sorghum Liquid Sugar.

| Parameter      | White sorghum syrup | Red sorghum syrup |
|----------------|---------------------|-------------------|
| Color          | Light brown         | Dark brown        |
| Yield (%)      | 43.4                | 44.6              |
| TSS(Brix)      | 63.3                | 67.7              |
| pH             | 6.8                 | 6.9               |
| Glucose (ppm)  | 32.125              | 31.075            |
| Fructose (ppm) | 147.541             | 290.398           |
| Sucrose (ppm)  | 153.459             | 302,455           |

Source: Noerhartati and Rahayuningsih (2013)

Sorghum liquid sugar is usually called sorghum nectar, this sugar has a thick honey-like shape. Sorghum liquid sugar is called vegan honey because sorghum is itself an 'anti-sugar' brigade. So that this liquid sorghum sugar can be used as a healthier substitute for sugar in the form of syrup. In addition, liquid sugar can be said to be vegan honey because sorghum is high in fiber which can help the digestive system function properly. In addition, sorghum is low-glycemic, so it is suitable for consumption by people with diabetes. Sorghum liquid sugar can also prevent cancer, is high in antioxidants, a source of vitamins and gluten free (Suarni, 2012).

Table 2. Nutritional Content of Sorghum Liquid Sugar

| Component               | Per 100 g sorghum syrup |
|-------------------------|-------------------------|
| Total fat (gr)          | 0                       |
| Protein (gr)            | 0                       |
| Total carbohydrate (gr) | 75                      |
| Cholesterol (gr)        | 8                       |
| Sodium (mg)             | 10                      |
| Phosphorus (mg)         | 5                       |
| Calcium (mg)            | 100                     |
| Magnesium (mg)          |                         |

Source: Ratnavathi et al. (2016)

The nutritional content of sorghum liquid sugar contains quite high minerals such as 8 mg of sodium, 10 mg of phosphorus, 5 mg of calcium, and 100 mg of magnesium (Table 2). The presence of a high enough mineral content can affect the sweetness of sorghum liquid sugar (Ratnavathi et al., 2016). The presence of minerals in sorghum liquid sugar causes the sweetness level of this sugar to be lower than sugar derivative products such as sucralose, sorbitol, and others. Even though the need for liquid liquid sugar is quite high, the selling value of liquid sugar is still low when compared to sugar derivative products. The process of making sugar derivatives can use several technologies such as chemical and biological. The shortcomings of the chemical process of making sugar derivatives can have a negative impact on consumers due to the presence of chemical residues in the product (Ambarsari et al., 2015). In addition, sorghum liquid sugar does not contain total fat, protein, and

cholesterol, so this liquid sorghum sugar can be used as a healthier sugar substitute in the form of syrup (Suarni, 2012).

## FRUCTOSE

Fructose is ketohexose which is often referred to as invert sugar. This is because fructose is produced from hydrolysis or the breakdown of sucrose into two parts, namely glucose and fructose. The molecular formula for fructose is  $C_6H_{12}O_6$  and has a molar mass of  $180.156 \text{ g}\cdot\text{mol}^{-1}$  (Vaclavik, 2014). The crystals in fructose have a density of  $1.55 \text{ g / ml}$ . In addition, fructose is very soluble in water with a solubility of  $4000 \text{ g / L}$  at  $25^\circ\text{C}$  (Siegel & Kathy, 2015). Fructose is very water soluble because fructose has five hydroxyl groups that can form hydrogen bonds with water molecules (Figure 1) (Gedde and Hedenqvist, 2019).

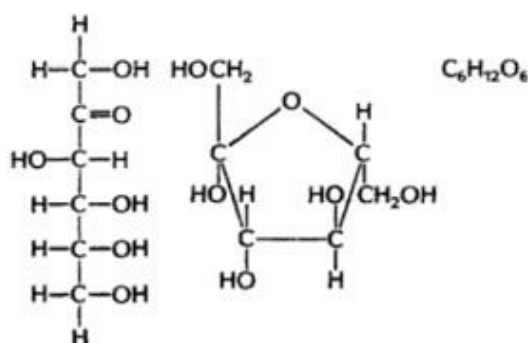


Figure 1. Fructose Molecular Structure (James et al., 2006).

The physical properties of fructose itself are found in many raw sugarcane and fruits. The crystalline form of fructose is a needle crystal. In addition, fructose is colder soluble than glucose. One of the carbohydrate sugars that can dissolve in alcohol and ether is fructose so that fructose is different from all carbohydrate sugars (Caballero et al., 2015), and fructose is soluble in glycerin and acetone so it is different from glucose (Panda, 2011). Fructose has a sweet taste, is white, and has no smell (Tappy, 2018). While the chemical properties of fructose are that it decomposes when heated to a temperature of  $160\text{-}170^\circ\text{C}$ , when one water molecule is released it will turn brown. On further heating with temperatures over  $190 - 220^\circ\text{C}$ , the brown color will change to black caramel and eventually completely turn into carbon dioxide. Fructose requires more acid for inversion. On prolonged heating, the rotational power of fructose changes without obvious decomposition. Fructose is more easily broken down than glucose with the same acid concentration and at the same temperature (Caballero et al., 2015).

According to Madan (2013), the structure of fructose is defined as follows: (1) elemental analysis and determination of the molecular weight of fructose show that fructose has the molecular formula  $C_6H_{12}O_6$ . (2) Fructose will perform reduction with the help of  $\text{Na / Hg / Water}$  to produce Sorbitol. After that, the reduction was carried out again with the help of  $\text{HI}$  and red phosphorus to produce a mixture of n-hexane and 2-iodohexane. This reaction shows that the six carbon atoms in fructose are in a straight chain (Figure 2).

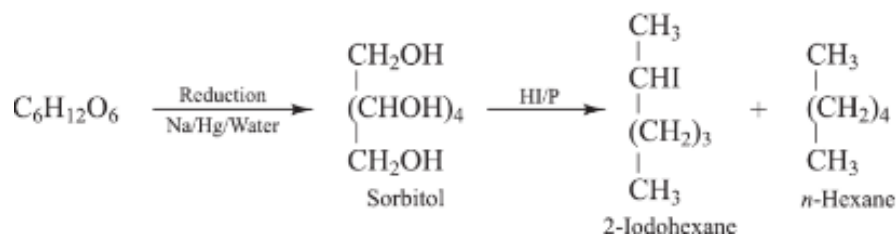


Figure 2. Fructose reaction Madan (2013).

(3) Fructose reacts with hydroxylamine, hydrogen cyanide, and phenyl hydrazine. This indicates the presence of a carbonyl group in the fructose molecule, (4) The addition of bromine water is then carried out, but no reaction occurs. This will rule out the possibility of the presence of the  $-\text{CHO}$  group, (5) Furthermore, the oxidation with nitric acid is carried out to produce glycolic acid and tartaric acid which contain a smaller number of carbon atoms than fructose (Figure 3). This indicates that the ketone group is in position 2. It is at this point that the molecule will break apart.

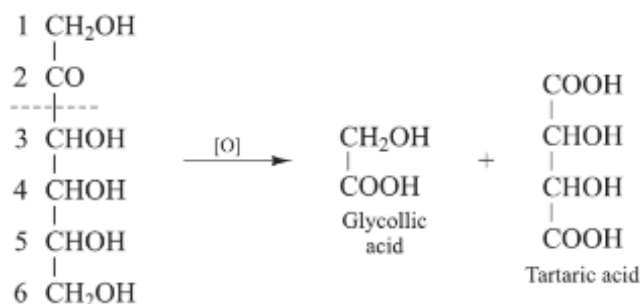


Figure 3. Fructose oxidation Madan (2013).

Fructose is a monosaccharide found in many types of plants. In plants, fructose is found in trees, flowers, vegetables and berries which can be in the form of monosaccharides and as a component of sucrose (Prahastuti, 2011). Fructose is a simple sugar which is among the sweetest natural sugars. Pure fructose has a very sweet taste, is white, is crystalline solid and is very easily dissolved in water. Fructose has a high sweetness up to 1.5 times higher than sugar. The digestion process for fructose takes longer and is absorbed more slowly, so it is considered a healthier sweetener. Fructose needs to be converted first into glucose in the liver for energy. This sugar is found in fruits, mapple syrup and honey (Partic, 2014). According to Arif (2020), the fructose content in sweet sorghum is 10 percent. The fructose content in sorghum ranges from 0.3 - 0.7% (Taylor and Duodu, 2018).

## LIQUID CHROMATOGRAPHY

Chromatography is one of the methods used to separate solutes by a dynamic differential migration process in a system consisting of two phases, namely the diem phase and the mobile phase (Rizalina et al., 2018). One of these phases will move continuously in a particular direction. In addition, in this phase there are substances that will show differences in mobility, this is due to differences in adsorption, solubility, partition, molecular size, vapor pressure and ionic charge density. So that each substance can be identified by analytical methods (Rubiyanto, 2017). The mobile phase in chromatography is in the form of gas or liquid, while the stationary phase is

in the form of a liquid or solid. The mobile phase acts as a solute carrier through the medium until it is separated from other solutes. Meanwhile, the mobile phase has a function as an adsorbent such as an adsorbent for activated aluminum, silica gel and ion exchange resin. In addition, the stationary phase also dissolves solutes, causing partition between the stationary phase and the mobile phase (Dong, 2006).

Liquid chromatography is a widely accepted separation method for the analysis and purification of certain compounds in a sample in a number of fields, including: in the food, pharmaceutical, environmental and biotechnology industries. Usually this liquid chromatography method is used to separate large amounts of compounds. Liquid chromatography separation method based on stationary phase and mobile phase (Susanti and Dachriyanus, 2018). Liquid chromatography has advantages in analysis accuracy and high sensitivity and is suitable for separating nonvolatile compounds that are not resistant to heating (Skoog et al., 2017). In addition, the liquid chromatography method is able to separate molecules from a mixture which is very easy to use. The material to be analyzed using liquid chromatography can be avoided for decomposition or damage to the material being analyzed. As well as this liquid chromatography method using a column which is the heart of the chromatography. The success or failure of an analysis depends on the selection of the appropriate experimental column and conditions. For columns from this chromatography it can be reused (Rubiyanto, 2017).

Liquid chromatography has now become the method of choice for most types of sugar analysis, from determining common sugars in foods to identifying more complex, high-sensitivity samples (Canovas et al., 2006). Compounds separated by liquid chromatography have the same mechanism as other types of chromatography, namely by binding to the difference between the forces between molecules in the sample and their mobile and stationary phases (Skoog et al., 2018). The mobile phase in the form of a liquid will carry the compound to flow through the stationary phase so that interactions will occur in the form of adsorption of the compound by solids in the column. The speed at which a component moves depends on how long it is held by the adsorbent solid in the column. So that to get a perfect separation it is necessary to select the stationary phase and the mobile phase appropriately. In determining the mobile phase and stationary phase, it must be seen from their polarity and solubility (Rubiyanto, 2017).

According to Canovas et al. (2006), in determining the components of monosaccharides and disaccharides, HPLC methods can be used with various different columns, namely the Aminopropyl - Silica column detection RI and Anion Exchange Chromatography with Pulsed-Amperometric Detection. If using an Aminopropyl - Silica column, RI detection must meet the conditions of the HPLC such as a 250 mm x 4.6 mm column, a mobile phase in the form of acetonitrile with a flow rate of 1.8 ml / minute, and an injection volume of 10 to 20  $\mu$ l. Whereas for Anion Exchange Chromatography with Pulsed-Amperometric Detection, the column is Carboxylic measuring 250 x 4 mm, the mobile phase is NaOH with a flow rate of 1.0ml / minute, and injection volume of 20 to 50  $\mu$ l.

Another study conducted by Dira (1995) states that the separation of monosaccharide and disaccharide components in honey or liquid sugar can be done using the KCKT method  $\mu$  Bondapak-NH<sub>2</sub> column and acetonitrile: water mixture eluent (75: 25). The flow rate was determined at 0.6 mL / minute using a UV detector. The drawback of this study is that the effect of column temperature on the separation of each component of honey is not seen

According to Ratnayani et al. (2008), determination of glucose and fructose levels in honey or liquid sugar was carried out by diluting the sample to a volume of 50 mL and then centrifuging for 30 minutes. The sample was filtered with 0.45  $\mu$ m filter paper. Then the sample was injected as much as 20  $\mu$ L into the chromatograph and the system was made with the best separation conditions, all components were left separate. The advantage of this research is that the eluent used is deionized water, besides being cheap, the eluent is non-toxic. In addition, the eluent has a polarity that matches carbohydrate so that the separation between glucose and fructose can produce good resolution. As for the detector used is the refractive index detector, this is because the detector is suitable to be used to separate the carbohydrate components.

Factors that affect the separation results in liquid chromatography are the composition of the mobile phase, the rate of water, and the presence or absence of acid addition (Amin et al., 2016). According to Rosyidiati (2019), there is an increase in flow rate in the mobile phase it will sharpen the peak shape, increase selectivity, reduce the value of the tailings and can reduce pump pressure. Meanwhile, if there is a decrease in flow rate it can increase the resolution value and slow down the elution time.

## CONCLUSIONS

Sorghum syrup could be used as the raw material of fructose manufacturing. Fructose production from this sorghum syrup was not chemical or enzymatic process but physical process by using liquid chromatography. HPLC was evaluated as the effective process to separate and analyze fructose content of sorghum syrup. Therefore, liquid chromatography could be used as the fractionation process of sorghum syrup to get pure fructose by using the HPLC approach.

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