

Determination of Fructose Content in *Anacardium occidentale*

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Abstract

The increased production of fructose from over-ripe edible portion of *Anacardium occidentale* was carried out on a laboratory scale by acid hydrolysis method. The percentage of glucose and fructose in the edible portion of the fresh over-ripe apple of *Anacardium occidentale* are given as 2.1 and 5.2 respectively while the total available carbohydrate, water content and titratable acidity (ml. 10% NaOH $\equiv$ 100g apple) are 13.14, 89.54 and 72.21 respectively. The effect of temperature and acid concentration on the rate of formation of fructose and the optimal time for hydrolysis were determined. The optimum condition for acid hydrolysis was determined to be 70°C using 0.2 M acid concentration in 20 minutes.

Keywords: *Hydrolysis, over-ripe, Anacardium occidentale, fructose*

1. Introduction

The body responds to fructose in a different way than to glucose and sucrose. Fructose is more satiating and it is up to 1.8 times sweeter than sucrose, making it useful in foods and beverages for use in diabetic foods as it has very little effect on blood glucose and only a negligible effect on the secretion of insulin (Stravropoulos, 1985). Producing high fructose content syrup has become necessary since industrialists are in the battle of replacing sucrose with fructose. Plants in the families, compositae and amarydelledae have been found to contain high content of fructose in the form of inulin (Shallewberger and Birch, 1975; Bagaoutdinova *et al.*, 2001). Inulin has received a substantial attention as a source of fructose, but only a few procedures have been reported for the preparation of fructose syrup and crystals from it. These procedures were considered to be commercially unattractive either because of poor yield or/and nature of the final product obtained (Karsten *et al.*, 1991). Several extraction and measurement methods have been employed in the

determination of total sugar and starch contents in plant tissues. Depending on the type and source of tissue, total sugar and starch contents estimated from samples extracted with 80% hot ethanol were significantly greater than from samples extracted with a methanol: chloroform: water solution. The residual ethanol was observed not to interfere with the sugar and starch determination, rendering the removal of ethanol from samples unnecessary (Chow and Landhäusser, 2004). The use of phenol-sulfuric acid with a phenol concentration of 2% provided a relatively simple and reliable colorimetric method to quantify the total soluble-sugar concentration. Performing parallel sugar assays with and without phenol was seen to be more useful for accounting for the interfering effects of other substances present in plant tissue than using chloroform. For starch determination, an enzyme mixture of 1000U α -amylase and 5 U amyloglucosidase was shown to digest starch in plant tissue samples more rapidly and completely than previously recommended enzyme doses.

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Diluted sulfuric acid (0.005 N) was seen to be less suitable for starch digestion than enzymatic hydrolysis because the acid also broke down structural carbohydrates, resulting in over estimates of starch content. After the enzymatic digestion of starch, the glucose hydrolyzate obtained was measured with a peroxidase–glucose oxidase/*o*-dianisidine reagent; absorbance being read at 525 nm after the addition of sulfuric acid. (Chow and Landhäusser, 2004) Also high performance liquid chromatography (HPLC) with a universal evaporative light scattering detector has been used to determine free sugar and starch concentrations (Fateh *et al.*, 2007; Aberoumand and Deokule, 2009). The acid hydrolysis method is one of the many procedures used and has proved to be good for the production of fructose commercially (Booij *et al.*, 1993). This method and the use of spectrophotometer were adopted in this paper for determining the fructose content of *Anacardium occidentale* pulp. The plant is commonly known as cashew and a member of the flowering plant family anacardiaceae. The apple is kidney shaped and grows at the end of the pseudo fruit. Analysis of the cashew apple showed that it contains about 7–9% of reducing sugar (Ganter *et al.*, 1991).

In the present study, the fructose content and the increased production of fructose by acid hydrolysis of over-ripe apple of *Anacardium occidentale* was investigated. Also investigated were the effect of temperature and acid concentration on the rate of formation of fructose during hydrolysis, thereby establishing the optimum conditions for complete acid hydrolysis of the over-ripe *Anacardium occidentale* apples that grow in Nigeria. This also led to the determination of the percentage of glucose, total available carbohydrate, titratable acidity and water content of the over-ripe apple.

2. Materials and Method

Sample Collection

Fresh over-ripe and unbroken *Anacardium occidentale* apples were collected from a farm in Ogbomoso, Nigeria and taken to the laboratory, washed and the water drained off. A sample was taken to the Department of Pure and Applied Biology, Ladoke Akintola University of Techno-

logy, Ogbomoso, Nigeria for identification by Prof. Osudina.

Reagents

Anthrone powder, sodium hydroxide, ethanol, sulphuric acid, phenolphthalein, alkaline copper tartrate, sodium hydroxide, arsenomolybdate reagent, citric acid, resorcinol reagent, hydrochloric acid, alkaline copper tartrate, sodium carbonate, iodine solution, (Sigma chemicals, London), all these chemicals were used as supplied.

Spectrophotometer

The spectrophotometric analysis was carried out on a HELIOSα v4. 60 UV–Visible spectrophotometer.

Preparation of the Fruit and Extraction

500g mixture of small and large fruits was prepared as for eating, and the proportion of waste was determined. The edible portion was then cut up finely. Water content was determined in duplicate samples of 50g by drying to constant weight at 100°C. Duplicate portions of 100g were extracted with about 200 mL of cold 95 % ethanol for 48 hours. The tissue was then extracted with hot 80 % ethanol in a Soxhlet apparatus for about 16 hours, and the ethanol from the combined extracts was evaporated off using a rotary evaporator. The residue was up to 200 mL in a graduated flask (Solution A). Free acid was determined on 20 mL of this extract by titration with 0.1M NaOH. Phenolphthalein was used as indicator.

Preparation of Anthrone Solution

0.2g of anthrone powder was dissolved in 100 mL concentrated sulphuric acid. The solution was then mixed properly and kept for at least 12 hours. The resulting solution was used as anthrone reagent.

Preparation of Different Molar Solutions of H₂SO₄

0.98, 1.96, 3.92, 5.88 and 7.84g of concentrated H₂SO₄ were put into different standard flasks and made up to mark with distilled water in order to obtain 0.1, 0.2, 0.4, 0.6 and 0.8 M solutions respectively.

Preparation of Different Molar Solutions of NaOH

1.6, 3.2, 4.8, 6.4 and 8.0g of NaOH pellets were weighed and dissolved with distilled water in a 100 mL standard flask and made up to the mark with distilled water in order to obtain 0.4, 0.8, 1.2, 1.6 and 2.0 M solutions respectively.

Hydrolysis of Solution A

The hydrolysis was done on a laboratory scale and the procedures used were similar to general hydrolysis method.

50 mL each of the solution A was pipetted into five different 500 mL flasks and 200 mL of each of the prepared standard solutions of H_2SO_4 introduced into each flask and mixed properly. The mixture was heated to a maximum temperature of $110^\circ C$. As the temperature was increasing 1 mL of the hydrolysate was pipetted into test tubes at different temperatures (35, 50, 70, 90 and $110^\circ C$) and neutralized with 10% NaOH. The reaction mixture was then placed in an ice bath to cool rapidly. The neutralized solution was diluted using 18 mL of distilled water. Then 1 mL of the diluted solution was pipetted into a test tube and 2 mL of anthrone reagent was added for quantitative analysis of the fructose formed.

Effect of Temperature on the Rate of Formation of Fructose

The acid hydrolysis was carried out at temperatures between $35^\circ C$ and $110^\circ C$. The fructose formed was determined by quantitative analysis of 1 mL of the hydrolysate using 2 mL of anthrone reagent.

Effect of Acid Concentration on the Rate of Fructose Formation

The effect of acid on the formation of fructose during hydrolysis was carried out using the five different concentration of H_2SO_4 acid prepared and the results were analyzed. Fructose concentration versus hydrolysis time and fructose concentration versus acid concentration were plotted; and the optimum acid concentration and hydrolysis time for the hydrolysis were determined from the plots.

Determination of Reducing Sugars using Nelson-Somogyi Method.

0.2 mL of solution A was pipetted into a test tube and made up to 2 mL with distilled water. 100 mg

of standard glucose was dissolved in 100 mL of distilled water to serve as stock for the standard. Then 10 mL of the stock solution was diluted with distilled water to 100 mL to serve as the working standard solution. Then 0.2, 0.4, 0.6, 0.8 and 1 mL of the working standard solution were pipetted into different test tubes and made up to 2 mL with distilled water. 2 mL of distilled water was pipetted into a separate test tube and used as the blank. Then 1 mL of alkaline copper tartrate reagent was added into each test tube and the test tubes placed in a boiling water bath for 10 minutes. The test tubes were removed from the water bath and cooled then 1 mL of arsenomolybdate reagent was added to all the test tubes. The volume of each test tube was made up to 10 mL with distilled water. After 10 minutes the test tubes were placed in a spectrophotometer and the absorbance reading taken at a wavelength of 620 nm. The amount of reducing sugar present in the sample was calculated from the standard curve plotted.

Determination of Total Carbohydrate

100 mL of solution A was placed in a boiling tube and hydrolyzed with 2.5M HCl in a boiling water bath for 3 hours. It was cooled to room temperature and neutralized with solid Na_2CO_3 until there was no more effervescence. The volume was made up to 100 mL and centrifuged. 0.5 mL and 1 mL aliquots were taken from the supernatant for analysis. Then 0.0, 0.2, 0.4, 0.6, 0.8 and 1 mL portions of the working standard solution were pipetted into different test tubes and made up to 1 mL with distilled water. Then 4 mL of anthrone reagent was added to all the test tubes and heated for 8 minutes in a boiling water bath. It was cooled rapidly and placed in a spectrophotometer. The absorbance reading was obtained at a wavelength of 630 nm. A calibration curve was obtained by plotting concentration of the standard against absorbance from which the amount of carbohydrate content of the sample was calculated (Sadasivam and Manickam, 2004).

Determination of Fructose Content

2 mL of solution A was added to 1 mL of resorcinol reagent and 7 mL of dilute HCl. Then from the working standard 0.0, 0.2, 0.4, 0.6, 0.8 and 1 mL were pipetted into different test tubes and made up to 2 mL with distilled water. Then 1 mL of resorcinol reagent and 7 mL of dilute HCl were added to each of the test tube. All the test

tubes were heated in a water bath at a temperature of 80°C for 10 minutes. The test tubes were removed from the water bath, cooled and placed in a spectrophotometer. The absorbance reading was taken at a wavelength of 520 nm within 30 minutes. A standard graph was plotted from which the amount of fructose content of the sample was calculated (Sadasivam and Manickam, 2004).

3. Results and Discussion

The percentage of glucose, fructose and sucrose in the edible portion of the fresh over-ripe apple are given as 3.1, 5.2 and 3.3 respectively while the total available carbohydrate, water content and titratable acidity (10% NaOH $\equiv$ 100g apple) are 13.14, 79.54 and 72.21 respectively. These were obtained using the spectrophotometric technique. The value of fructose obtained using the hydrolysis method was found to be lower than that obtained via the spectrophotometric method (4.6%).

The fructose content was observed to be higher than glucose. The carbohydrate contents of the over-ripe apple were too low because of the destruction of fructose during hydrolysis. This is shown to be the case due to the fact that on boiling 2% solution of fructose with hydrochloric acid (5% by vol.), about 28% of the fructose present was destroyed during the first hour of hydrolysis and almost twice that amount after 2 hours. Glucose was almost unchanged by this treatment.

Effect of Temperature on Fructose Formation

In this study, we investigated the effect of low, moderate, medium and high temperatures on acid hydrolysis of the over-ripe *Anacardium occidentale* apple extract. The variation of the percentage increase in concentration of fructose with hydrolysis time at different temperatures is shown in figure 1. At 35°C, the maximum percentage increase in concentration of fructose was 20.08% during the first 15 minutes of the hydrolysis and as the time of hydrolysis increased, a sharp decrease was observed at a concentration of 17.58% which finally dropped to 10.20%. The low percentage concentration of fructose obtained with increasing time of hydrolysis can be attributed to the formation of hydroxymethyl furfural (HMF) as a by-product. At 50°C, the maximum percentage increase in concentration of fructose (21.74%) was observed at 15 minutes which could be a result

of the breakdown of sucrose. It then declined to a concentration of 17.85% after another 15 minutes. At this stage the temperature affected the fructose formation. Thus thermal degradation affected the fructose concentration, but it gradually picked up again after 25 minutes. At 70°C, for the first 15 minutes there was a steady rise of fructose formation to a maximum percentage increase in concentration of 21.97%. The hydrolysis condition favored the formation of fructose but further hydrolysis caused a gradual decrease in the fructose concentration to a minimum of 18.19% and finally to 17.76%. At this stage the action of heat on fructose formation was more pronounced. Thermal degradation reduced the fructose formed but more was formed as the hydrolysis progressed. At 90°C, there was a gradual increase in the concentration of fructose to a maximum of 20%. After 15 minutes, it declined to a minimum of 17.58%. At 105°C, instead of an increase, a decrease was observed but after 15 minutes there was a tendency to increase but the fructose formed was destroyed again by thermal degradation.

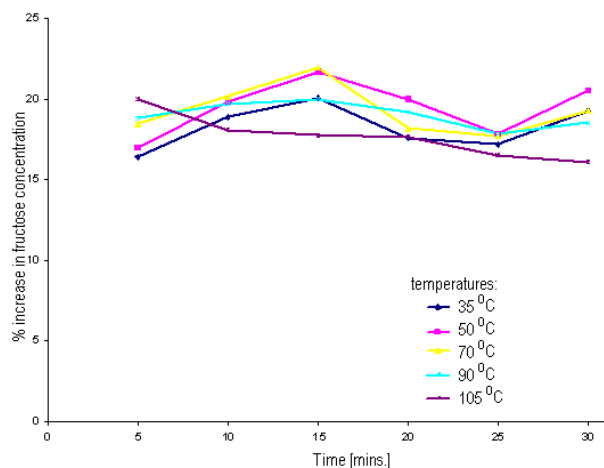


Figure 1: Percentage increase in fructose concentration at different temperatures

Effect of Acid Concentration on Fructose Formation

With acid concentration of 0.1M, an initial increase in fructose concentration of 16.48% was obtained, followed by a gradual increase in fructose formation to a maximum of 20% within 30 minutes of hydrolysis. With 0.2M acid concentration the percentage increase in fructose concentration at the start of the hydrolysis was observed to be the highest at 21.97%, 20.08% after

5 minutes and increased to a maximum value of 21.97% within 20 minutes, then a decline to a minimum of 18.83%. With 0.4M, there was an initial fructose concentration increase of 17.58%. It then rose to a maximum value of 20% after 15 minutes but later declined to a minimum of 18.11%. It was observed that most of the fructose formed was destroyed. At 0.6M acid concentration, there was a gradual increase in the fructose concentration to 20.55% after 25 minutes. The fructose formed was destroyed partially to a minimum of 16.52%. At 0.8M acid concentration, there was an initial increase in fructose concentration to 19.31% but after 15 minutes there was a gradual decline to a minimum of 18.83%. These effects are shown in figure 2.

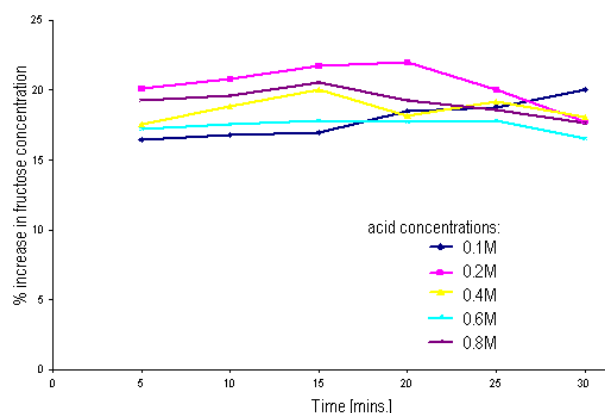


Figure 2: Percentage increase in fructose concentration with increase in time at different acid concentration

Determination of Optimum Conditions

At low acid concentration, the fructose concentration was observed to be low. This could be attributed to the formation of more of the by-product, hydroxymethylfurfural during the breaking down of the carbohydrate. At high concentration, the formation rate was observed to be rapid but the acid degradative effect was very pronounced thus destroying the fructose formed. Fructose was best produced at moderate acid concentration of 0.2 M producing the highest percentage yield of 21.97% at a maximum time of 20 minutes (figure 3). It was also observed that the optimum hydrolysis temperature is 70°C since the highest percentage increase in fructose concentration (21.97%) was obtained at this temperature (figure 4).

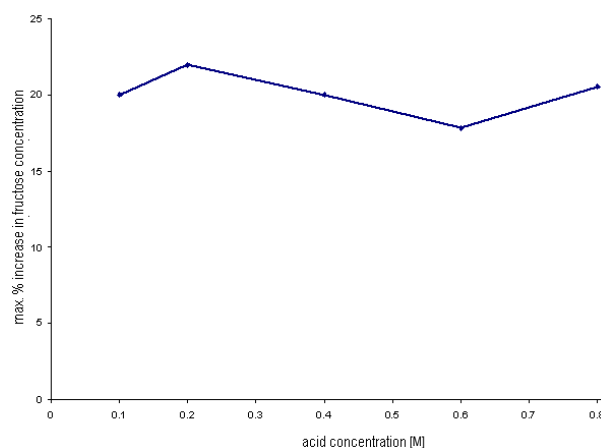


Figure 3: Determination of optimum acid concentration

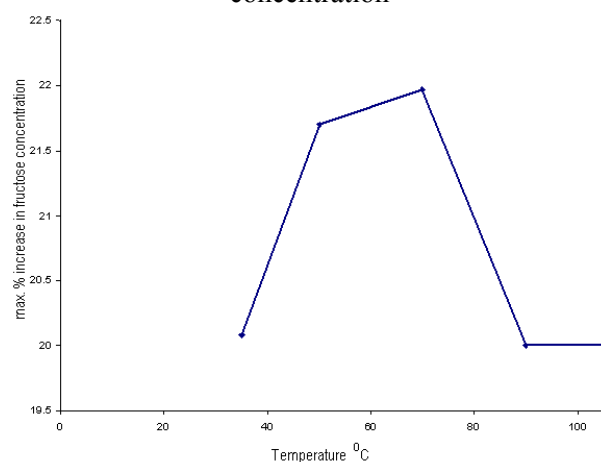


Figure 4: Determination of optimal temperature for hydrolysis

Sugars are known to be signaling molecules to regulate the growth and development of unicellular and multicellular organisms (Hanover and White, 1993). Fructose as a sugar is classified by some researchers as an excellent source of carbohydrate while others have reported its poor utilization or its outright toxic effect (Cho and Yoo, 2011). This research work showed that the fructose content of *Anacardium occidentale* is higher than that in apricot, banana, peach, pineapple and plum but lower than that found in apples, grapes and pears.

Anacardium occidentale is eaten generously in Nigeria when in season. Due to the high fructose content there is the tendency of accumulation of excess fructose in the body system of the regular eaters. Studies have shown that fructose is not completely absorbed in the small intestine but the excess is transported to the large intestine where

fermentation occurs thereby generating hydrogen which is transported to the lungs. Also the presence of fructose in the large intestine causes the colonic flora to produce carbon dioxide, organic acids, etc (Skoog and Bharucha, 2004) which cause gastrointestinal symptoms such as bloating, diarrhea, flatulence and gastrointestinal pains (Beyer et al., 2005). Over-consumption of fructose has been described as being responsible for insulin resistance and obesity (Elliott et al., 2002; Lustig, 2006; Isganaitis and Lustig, 2005), elevated LDL cholesterol and triglycerides leading to metabolic syndrome (Basciano et al., 2005). It has also been found to increase adiposity (Jürgens et al., 2005).

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4. Conclusion

The glucose, fructose and total carbohydrate contents of the over-ripe apple were determined. The effects of increase in temperature, acid concentration and the best hydrolysis time were also determined. By using acid concentration of 0.2 M the fructose content of the fresh over-ripe apple was increased by 21.97% within a time limit of 20 minutes. People who have gastrointestinal disorder should be discouraged in consuming much of *Anacardium occidentale* apple due to the high fructose content.

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