Enzymatic Hydrolysis of Cassava using wheat seedlings

Veena Ramachandran[1], Nisha Pujari[2], Tanmay Matey[3], Sunil Kulkarni[4]

***Abstract*— The hydrolysis of various starches such as sweet potato, corn, cassava etc. leads to the production of glucose syrup. Cassava is an organic starch that is high in carbohydrates, free from pesticides and is a fresh and guaranteed organic food ingredient source to industries. The glucose syrup from the enzymatic hydrolysis of cassava is healthy, has low color and neutral taste which makes it a preferred choice. The glucose content of the syrup is measured by its dextrose equivalent. In the present work, the enzymatic hydrolysis of cassava using wheat seedlings as the enzyme-source is investigated and the effects of various parameters on the quality of glucose syrup are studied. The optimum values of these parameters were also determined. It was observed that a decrease in pH, an increase in temperature up to a certain limit, and an increase in the time of hydrolysis leads to an increase in the glucose concentration of the syrup.**

***Index Terms*— cassava, glucose, amylase, hydrolysis.**

I. INTRODUCTION

Cassava (Manihot esculenta), is a perennial woody shrub which is mainly found in the tropical regions. It is used in food, paper and textile industries and for the production of alcohol [1]. Due to its low content of cellulose, high content of starch, rich organic nature and low ash content, cassava offers numerous advantages in comparison to other starch sources such as rice or maize. Glucose syrup is obtained by the hydrolysis of cassava starch by an acid or enzyme. Enzymatic hydrolysis has various advantages over acid hydrolysis in that the process is easy to control, effective even in mild ambient conditions, doesn't give rise to any by-products, and does not require very high temperatures. In this method, enzymes break the α-1, 4 and α -1, 6 molecular bonds of starch to more simplified smaller units of monomers. The rate of enzymatic hydrolysis depends on the molecular size and the structure of the substrates [2]. Enzymatic hydrolysis is achieved by an enzyme amylase which breaks the bonds between the starch molecules to form glucose. This enzyme is found abundantly in plants, animals, fungi etc.

Various techniques for the hydrolysis of cassava for glucose have been investigated in the past. The effects of different parameters such as temperature, pH, time of hydrolysis etc. have also been studied. Cecil described a simple technology for producing maltose from cassava starch using rice or maize seedlings as the source of enzymes [3]. The effects of rice malt extract on the rate of liquefaction and the yield of glucose was investigated by Hammond and Ayernor [4]. The physiochemical changes and diastatic activity during the germination of local paddy rice as an enzyme-source was studied by Ayernor and Ocloo [5]. The effect of variation of sorghum malt with cassava flour for glucose production was described by John-Dewole and Popoola [6]. Sorghum malt hydrolysis of cassava was further studied and the effects of process variables like temperature, pH and time of hydrolysis were described by Aderibigbe et al. [7].

The feasibility of the syrups derived from grains for industrial use was explored by Cadmus et al. [8]. Gas-liquid chromatography to determine the amount of isomaltose from unrefined glucoamylase preparations was also carried out. The kinetics of hydrolysis of cassava starch by the enzyme amyloglucosidase and effect of varying parameters on the hydrolysis was studied by Garba et al. [9]. The enzymatic hydrolysis of cassava starch by using enzyme Aspergillus niger isolated from groundnut seeds was investigated by Osarbie et al. [10]. The optimum temperature and pH for liquefaction and saccharification of starch for glucose production was further evaluated by Shadila binti Alias [11]. Isolation of α-amylase from wheat by heat-treating an extract followed by acetone fractionating, for the production of glucose was carried out by Krueger et al. [12]. The period of germination of maize for favourable amylase activity for the production of maltose syrup was studied by Ameko et al. [13].

II. AIM AND OBJECTIVE

The aim of the current study is to investigate the enzymatic hydrolysis of cassava for the production of glucose, using wheat seedlings as the source of enzyme. The effects of temperature and pH on the hydrolysis of cassava and the quality of glucose are also studied and optimum values of these parameters are reported. The parameters leading to the production of glucose syrup having the highest dextrose equivalence are considered to be the optimum conditions.

III. MATERIAL AND METHOD

The process includes preparation of the wheat seedlings which would be the source of enzymes and the procedure for production of glucose. Good quality wheat seedlings were grown without the use of soil i.e. hydroponically.The enzymatic process consists of 3 main parts: gelatinization,

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Veena Ramachandran: Chemical Engg. Department,, Datta Meghe College of Engg., Airoli, Navi Mumbai, India,
Nisha Pujari: Chemical Engg. Department,, Datta Meghe College of Engg., Airoli, Navi Mumbai, India,
Tanmay Matey: Chemical Engg. Department,, Datta Meghe College of Engg., Airoli, Navi Mumbai, India,
Sunil Kulkarni:Assistant Professor,Chemical Engg. Department,, Datta Meghe College of Engg.,Airoli,Navi,MumbaiMaharashtra ,India ,Pin.400708,,Mobile:9833497367

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liquefaction and saccharification

A. Gelatinization

Two batches each consisting of 10.11g of crushed wet wheat seedlings (in the ratio of 1:3) and 57 ml of dry starch with 47 ml of water was taken in the shallow drums. To it 26 ml of warm water was added. All ingredients were mixed well till no lumps of starch were present. First batch was taken in a heating vessel. This mixture was stirred vigorously till the whiteness disappeared. An amount of 303 ml of water was kept to boil. 5 – 10 minutes later, the first batch of slurry was added to this boiled water and the whole mixture was brought to boil.

B. Liquefaction

Meanwhile, a second batch of slurry was prepared and after a few minutes the first batch of slurry was added to it. It was then mixed well during the addition of the boiling liquid. The resulting temperature must be close to 80°C. To increase the concentration of the mixture, another batch of slurry can be added by boiling the contents of the two batches and finally adding the third one. After this, the final mixture was covered and it was left to cool within the range 62°C – 68°C. It can take up to 2 hours to get the slurry cooled to this temperature.

C. Saccharification

In addition to this, 21g of crushed seedlings were added and stirred. This mixture was then left for about 4 hours. Further a starch test was done to check if any starch was still present in the mixture. If yes, more seedlings are to be added and it is left till all the starch disappears. If no starch is present, the mixture is briefly boiled and filtered immediately. This filtrate was concentrated to produce syrup. The filtrate was put back into the heating vessel and boiled. The water was boiled away steadily and the liquid became more concentrated; this caused a rapid change in its character. When the syrup became thick and concentrated enough, the heating vessel was removed from the heat and the resulting syrup was cooled. On cooling, it becomes very viscous. Thus, glucose syrup is produced using enzymatic process.

D. Tests

For the starch test, 140 ml of water was taken to which one drop of medicinal iodine was added. A drop of the filtered sample was added to this solution. If the colour of the solution changes to brick red, no starch is present, but if blueness occurs, it indicates that some starch is still left. In case the mixture turns blue, another batch of crushed seedlings is added to the mixture and this should be left for another 1-2 hours. The starch test in the present case showed that no starch was present in the syrup [14].

To calculate the dextrose equivalent, an amount of 3 g to 3.15 g of sample of reducing carbohydrates was diluted with water to 500 ml of solution. This solution was filled in a burette. 25 ml of cupri-tartaric solution was pipetted out and 18.5 ml of the sample solution from the burette was added to it and mixed well. The mixture was heated on a hot plate and left to boil for exactly 120 s. A few drops of methylene blue indicator was added to this and it was titrated with the sample solution (V_A) in the burette until the blue colour disappeared. The solution must be maintained at boiling throughout the titration process. The cupri-tartaric solution must be standardised using a 6.00 g/l solution of glucose R (V_o). Dextrose equivalent (DE) can be calculated from the equation:

$$DE = (300*V_o*100) / (V_A*M*D)$$

Where,
V_o = volume of glucose standard solution, in ml,
V_A = volume of sample solution, in ml,
M = weight of the sample, in g,
D = percentage of dry matter content in the substance

IV. RESULTS AND DISCUSSIONS

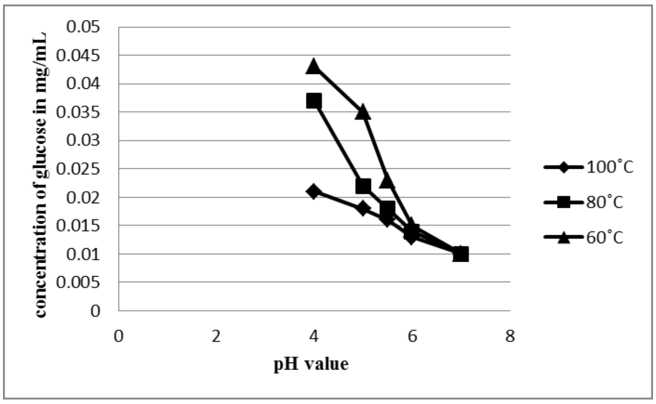

Figure 1: Concentration of glucose mg/ml vs pH value

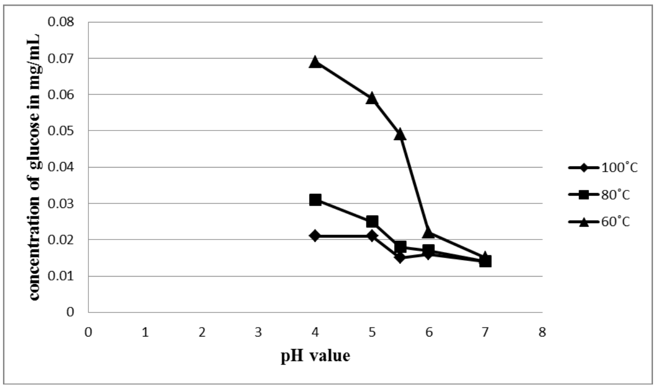

Figure 2: Concentration of glucose mg/ml vs pH value

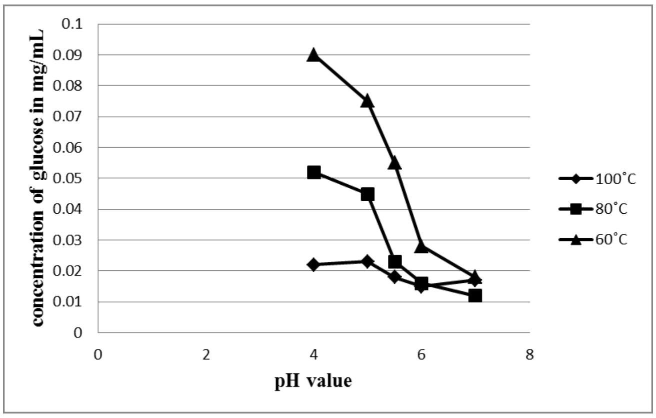

Figure 3: Concentration of glucose mg/ml vs pH value

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Figure 1 describes the conditions of glucose syrup during enzymatic hydrolysis for a period of 30 min, from which it could be stated that concentration of glucose syrup was high at a pH of 4 at 60˚C.Figures 1, 2 and 3 show that, the concentration of glucose syrup decreases with an increase in pH. Maximum glucose concentration can be found at a pH of 4-5. It is also observed that the concentration of glucose syrup is highest at a temperature of 60˚C and pH 4 for a time period of 2 hours. It can be seen that the temperature, pH and the time of hydrolysis play an important role on the production of glucose syrup by the process of enzymatic hydrolysis.

V. CONCLUSION

The results of the present investigation show that cassava starch can successfully be hydrolyzed by enzymes to form glucose. Using wheat seedlings as the source of enzymes, the highest concentration of glucose was found at 60°C after 2 hours of operation, at a pH of 4. It was found that as the time of hydrolysis increases, the concentration of glucose increases, while it decreases with an increase in pH. Other seedlings such as rice, maize, sorghum etc. can also be used as a source of the enzyme amylase. There is no undesirable byproduct formed and the yield of glucose from enzymatic hydrolysis is said to be greater than that from acid hydrolysis. Thus, this is a simple process that can be employed in order to make glucose syrup from cassava starch.

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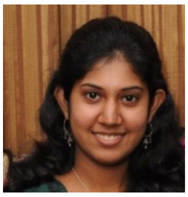
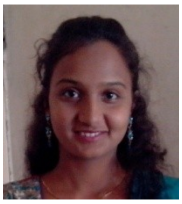
Author Profiles



Ms. Veena Ramachandran is doing her B.E. in chemical engineering from Datta Meghe college of Engineering, Airoli, Navi Mumbai, India. She performed her final year project on the enzymatic hydrolysis of cassava starch to produce glucose. She also published a review paper on the same. She has published 3 international review papers.



Ms. Nisha Pujari is doing her B.E. in chemical engineering from Datta Meghe college of Engineering, Airoli, Navi Mumbai, India. She performed her final year project on the enzymatic hydrolysis of cassava starch to produce glucose. She also published a review paper on the same.

Mr. Tanmay Matey is doing his B.E. in chemical engineering from Datta Meghe college of Engineering, Airoli, Navi Mumbai, India. He performed his final year project on the enzymatic hydrolysis of cassava starch to produce glucose. He also published a review paper on the same.

Mr. Sunil J. Kulkarni has completed his Masters in Chemical Engineering from Tatyasaheb Kore Institute of Engineering and Technology, Warananagar. He is working as Assistant Professor in Chemical Engineering Department of Datta Meghe College of Engineering, Airoli, Navi Mumbai,

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India. He has published 10 international review and research papers and presented 15 research papers in international conferences. His area of research includes adsorption, heat transfer augmentation, environmental engineering. He is member of many professional bodies such as ISTE(Indian Society of Technical Education). He is on the reviewer board of many international journals and reviewed many international papers.

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