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Enzymatic Hydrolysis for Glucose-A Review

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Abstract— **Glucose syrup is a concentrated aqueous solution of various sugars like glucose, maltose and other nutritive saccharides obtained from the hydrolysis of edible starch. Cassava is the preferred starch source since it is a high source of carbohydrate while being organic and free of pesticides. The purpose of this paper is to review the information available on the enzymatic hydrolysis of cassava and investigate the best possible conditions and source of enzyme that would give a better yield of glucose syrup. The hydrolysis of cassava using different sources of amylase enzymes like rice, maize, sorghum and wheat have been explored and optimum conditions for germination and subsequent hydrolysis have been analysed. The effect of temperature, pH, germinating period, malting period etc. has been studied and different methods for obtaining the best possible yield has also been reviewed.**

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

I. Introduction

Cassava is one of the most essential crops in tropical regions [1]. Cassava is the newest addition to commercially available sweeteners and provides a fresh and guaranteed organic food ingredient source to industry processors. It does not cause air pollution or any environmental hazard and does not contribute to greenhouse effect problem [2]. Cassava starch can be used for the production of many types of sweeteners on enzymatic or acid hydrolysis by either acids or enzymes, or both. These sweeteners include maltose, glucose syrup, glucose and fructose [3]. Since cassava roots contain high starch content and low quantity of impurities such as protein and lipid, they are considered to be an excellent source of pure suitable starch for glucose syrup production.

In an attempt to expand existing markets for cassava, an existing Vietnamese technology for glucose syrup production was adapted to enable the production of glucose syrup from cassava flour. The process as carried out in Vietnam makes use of rice seedlings as amylase source to break down starch to produce sugars [4]. The hydrolysis of starch can be achieved by three methods, Acid, Acid-Enzyme or Enzyme-Enzyme hydrolysis. However, recently, acid hydrolysis has been replaced by enzymatic hydrolysis because it required the use of corrosion resistant materials, gave rise to high colour and salt ash and requires more energy for heating [5]. The Enzyme – Enzyme Hydrolysis is the most advanced industrial enzyme application in the food industry and is one of the most successful enzyme technology. Various grains may be used as a source of enzyme during the hydrolysis of starch to glucose [6]. The purpose of this paper is to investigate the hydrolysis of starch using various methods and to review the findings on the production of glucose.

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II . Enzymatic Hydrolysis Using Various Grains

A. Rice

Cecil (1995) has described a simple technology currently being used in cottage industries in Vietnam for producing maltose from cassava starch, using simple processing equipment and whole rice or maize seedlings as the source of enzymes. The existence of this low technology industry for making maltose from cassava starch has only recently been reported in the western press. In this process, paddy rice was germinated and allowed to sprout and this was added to a specific quantity of wet starch and water. Boiling for about 7 to 8 hours under specific conditions up to 111 degree celsius converts all the starch to maltose. The level of glucose in the product might be increased either by protecting the existing alpha amylase in the seedlings from thermal damage or by using a supplementary source of alpha amylase. It was found that most alpha amylases in rice work best below 50°C and are destroyed at 70°C [7].

Hammond and Ayernor (2000) used various types of cereal malts in the hydrolysis of starch and observed that rice malt gave the highest yield of sugars. The use of rice malt in combination with the enzyme amyloglucosidase in conversion of starch to sugars has also been investigated (Ayernor et al., 2002). Rice-malt extract was prepared and the combined effect of the extract and amyloglucosidase on yield of sugars was examined. The effect of rice malt extract on the rate of liquefaction and the effect of the enzyme on rate of saccharification was also studied. The sugar profiles were studied by High Performance Liquid Chromatographic method. It was found that the reaction rate of liquefaction increased with increasing enzyme concentration from 2% to 8% w/v and then began to level off up to 10%. Also, the reaction rate of saccharification process increased with increasing amyloglucosidase concentration. The highest yield of sugars resulted when 8% or 10% w/v rice-malt extract was combined with 300 amyloglucosidase unit/ml or

200 amyloglucosidase unit/ml at 60 degrees at a pH of 4.5. The syrups produced by the rice-malt extract had glucose, maltose and other sugars. This may be due to the various enzymes in rice malt like amylases, dextrinases and amyloglucosidase. Thus, the combination of rice-malt and amyloglucosidase could increase the yield of sugar to DE 80 from cassava starch [8].Ahure and Ariahu (2013) evaluated the quality of glucose syrup from cassava hydrolysis by rice malt crude enzymes extract. Four syrups using 5% rice malt-95% cassava flour, 10% rice malt-90%cassava flour, 15% rice malt-85% cassava flour and 20% rice malt-80% cassava flour were produced. The physiochemical and sensory properties like viscosity, brix, sugar content, refractive index, colour, taste etc. were evaluated. The brix and refractive indices values showed that the limiting factor in the hydrolysis of cassava starch is the substrate concentration. However, there is no significant change in the four samples in terms of taste. The use of crude enzyme extract reduced the viscosity which is advantageous to flow and pumping requirements [9].

Ayernor and Ocloo investigated the physicochemical changes and diastatic activity associated with germinating of local paddy rice as a source of enzymes in starch conversion. They did this by germinating paddy rice for 0, 5, 7 and 9 days period at 32°C. Physical and chemical analysis, viscosity determination as well as determination of diastatic activities were carried out using standard methods. The malting loss was highest in 9-day malt i.e. about 59.75%. Germination significantly ($P<0.05$) affected both the thousand grains weight as well as the starch content of the paddy rice grains. Analysis of the data obtained showed that thousand grains weight correlated with starch content of the grain. The sacccharification time decreased as malting time increased. The effect of germination on viscosity of malt paste at temperatures of 75 and 36°C was studied. It was found that for both temperatures, viscosity decreased during malting. These physical and chemical changes observed were due to the increase in the activity of hydrolytic enzymes present in the grain [10].

B. Sorghum

John-Dewole and Popoola studied effect of variation of the Sorghum Malt with Cassava Flour for the production of glucose syrup. This research contains study about production of glucose syrup using different weight ratio of sorghum malt and cassava flour and study different parameters of the syrups produced. For germination process well cleaned and sorted Sorghum grains were used, it soaked in clean water for 24 hours and after every 8h water was changed. On the basis of weight ratio of sorghum malt and cassava flour, five different samples of glucose syrup were produced and named as GA, GB, GC, GD and GE respectively.

Significant different were shown by each sample in dextrose content, moisture content acid content and pH in result. Different weight ratio of sorghum malt shows variation in the chemical parameters. The range of moisture content was from 5.33 – 8.26%, ash content 0.010 – 0.040%, the dextrose equivalent 37.00 – 39.33%, the acid content 0.02 – 0.09%, the pH 5.4 – 5.8, and the brix value 82 – 86%. Except brix value there were significant difference observed between parameters. Values of chemical parameters of all samples were within the glucose syrup specifications set by the Standard Organisation of Nigeria (SON). The only weight ratio of sorghum malt and cassava flour acceptable for all the parameters is 1:4. Hence, a weight ratio of sorghum malt and cassava flour to produce adequate amount of glucose syrup having 1:4 ratio and under the specification of SON [11].

Ayoola et al. used sorghum malt and cassava having an aim to compare the amount of fermentable glucose produced by hydrolysis and observing the effects of pH and temperature. Enzymatic hydrolysis is most important for the production of glucose syrups from starch. They have studied two-step enzymatic hydrolysis of cassava and sorghum malt using α-amylase. Gelatinized process occurs at 80ºC and then liquefaction of amylase at a 90ºC carried with saccharification after cooling it till temperature of 60ºC. The effects of temperature and pH on saccharification process were studied. Results showed that the favorable temperature for the conversion of starch to fermentable glucose was at 60ºC. Hence it was concluded that optimum conditions for the production of glucose from both Cassava and Sorghum malt for glucose syrup production are obtained at pH of 4 and saccharification temperature of 60º. As compared to pH 5, 6 and 7 the favourable pH was 4 because of higher yield percentage. Therefore sorghum malt could be considered as a raw material for the production of glucose syrup [12].

Aderibigbe et al. investigated the sorghum malt hydrolysis of cassava starch. The effects of process variables like temperature, pH and time were studied for hydrolysis of cassava flour to glucose syrup. Using the experimental data a polynomial regression was developed. Their experiments resulted that the production of reducing sugar was depends on the variation of variables on hydrolysis of sorghum malt. The response surface methodology is a modeling technique used to study the relationship between controllable factors and observations. Gelatinization is an important process for producimg sugars from starch which increases the sensitivity of the starch to amylolysis and the temperature of starch depends on its biological source. The gelatinization temperature range of 68 to 77 ºC was observed while analyzing sorghum starch. Therefore the extraction of the endogenous enzymes of sorghum malt prior to gelatinization is necessary and this was performed in order to make maximum possible quantity of enzyme. In this experiment a mash ratio 1:1 and 2:3 was used. It was observed that the reducing sugar concentration was more in 1st case while the latter gave higher wort extract mostly because it had more starch. On hydrolysis larger amount of dextrins were produced. A study using full factorial design showed that maximum production of reducing sugar can be achieved only at time of 1.5 h. Time has the most prominent effect on the reducing sugar production. Hence, at time 1.5 h, pH 5.5 and 74 ºC with malt to cassava flour ratio 1:1, the highest amount of reducing sugar was produced and the maximum reducing sugar production at most favourable condition was 80.34 g/l representing 22.95% conversion or 22.95 dextrose equivalent (DE) [13].

C. *Wheat*

Kruger et al. worked to isolate alpha-amylase from wheat so that future work with this enzyme could be possible. The amount of α-amylase present in wheat is more during germination hence malted wheat was used for isolation process. They isolated wheat alpha-amylase from malted Canadian hard red spring by heat-treating an extract at 70 degrees celsius. This was followed by acetone fractionating, complexing with glycogen and finally by ion-exchange chromatography. Ion exchange chromatography and acetone fractionation was used for analysing α-amylase percentage. It was observed that the overall recovery of alpha-amylase activity is 27%. This indicated that there are three major and one minor enzyme component in malted wheat [14].

D. Maize

Ameko et al. studied the activity of amylase using starch as substrate. The method is simple in use for determining the period of germination for favourable amylase activity in extracts of maize malt crude for production of maltose syrup. The timing for the decolourisation of standard acid permanganate solution obtained by the released glucose showed the activity amylase enzyme. The period of germinaton for optimum activity of amylase in extracts of maize malt crude from batches of Obaatanpa-Pokuase, Obaatanpa-Fumesua, Obaatanpa-MOFA, Mamaba and Golden Jubilee local maize were determined to have germinative capacities greater than or equal to 90% . Peak periods of amylase activity occurred on 4th and 9th day of germination for Obaatanpa-Pokuase, and 5th and 10th day for the other varieties. The rate of decolourisation in 2nd case was 1.51 – 2.01 faster than with Obaatanpa-MOFA having the fastest decolourisation rate. Extracts of Obaatanpa-MOFA malt crude was used to process fresh Cassava to brown maltose syrup with pH 4.60 – 5.30, 0.45% sulphate ash, DE 40%, and 33.4% water content. Yield of syrup was 23.59% (mL/g wet starch).Increasing glucose concentrations resulted in decreasing decolourisation times and this was seen in the standard plot of the decolourisation of an acidified permanganate solution by standard glucose solutions [15].

Oluwole and Kosoko studied the production and development of high protein and energy density beverage from malted yellow maize and white sorghum. For a period of 48 hours and 72 hours the cereals were subjected to malting and was milled into flour. It resulted in significant ($p \leq 0.05$) increase in crude protein. This showed that the biochemical reactions occurring during malting is because of the breakdown of protein compounds into peptides and amino acids which also affects the protein among other molecules in the germinating grains. Malt extract was obtained by reconstituting the flour in water. The result concluded that the sensory qualities and proximate parameters of the developed beverages were majorly affected by the change in malting period. It was observed that the developed beverage's crude protein was between 12.30% to 17.80%, fat content was from 2.63% to 10.13%, ash content between 1.25% and 5.30% while the energy content was from 135.40 and 166.52kcal. The sample from 72 hours maize malt based on the overall sensory acceptability was the most preferred sample. [16]

E. Others

Pontoh et al. (Indonesia) explained about the hydrolysis of cassava starch in Indonesia to produce glucose syrup. Glucose syrup from cassava starch contains High Fructose Syrup (HFS) and it is produced by hydrolysis of starch. Materials for the process are obtained from local commercial plants in Indonesia. Hydrolysis contains liquefaction and saccharification process in which the pH is adjusted between 5-7 and dextrose level around 12.5-15.0. The process samples were analysed by HPLC. The liquefaction time for cassava to reach DE level 12-15 was 30 min. The conversion of starch to glucose is maximum after saccharification and it is around 95-96% after 48-72 hours. Julius & Nicholas concluded that this could be used for glucose syrup production [17].

Cadmus et al. explored the feasibility of the syrups derived from grains for industrial use and to determine the amount of isomaltose from unrefined glucoamylase preparations. Starch in ground samples of corn, wheat, and sorghum was converted enzymatically to glucose with a combination of alpha-amylase and glucoamylase. Gas-liquid chromatography was used to determine isomaltose in syrups. It was found that the rate of starch liquefaction is related to the amount of enzyme added. The activities of glucoamylase and alpha-amylase rapidly increase during the initial 4-5 days, after which it decreases. The glucose syrups produced by enzymatic hydrolysis of the various grains were substituted for crystalline glucose in many fermentations. The low yields with wheat syrup were most probably due to the high concentration of soluble nitrogen in this product. Thus when substrates containing less nitrogen content is desired, corn, corn flour or sorghum can be used [18].

In their paper, Pradistsuwana et al. tried to apply an ultra-filtration system for increasing the reducing sugar production from the enzymatic hydrolyzation of cassava pulp. The cassava pulp was hydrolysed with hydrochloric acid and the effects of pH and temperature on enzyme activity was studied. To improve the yield of hydrolyzation, ultrafiltration was applied. Highest enzyme activity for α-Amylase was at 50°C with a reducing sugar production of 2.0 mg/ml. The highest amount of reducing sugar was at 60°C for glucoamylase which was 9 mg/ml. The 0.5:0.5 ratio of the enzymes α-amylase and glucoamylase, gave the highest reducing sugar from hydrolyzation at 9.88 mg/ml. It was observed that hydrolyzation with ultra-filtration produced the reducing sugar at a rate 20% higher than the system without ultrafiltration [19].

Robertson et al. studied native or raw starch digestion for an energy efficient biorefining of grains. Better molecular disassembly and depolymerisation of starch to glucose are key to reducing energy use in the bioconversion of glucose to chemicals and fuels. In ethanol production, these biorefining steps use 10-20% of the total energy content of the fuel ethanol. Lowering the starch-to glucose processing temperature to that of the fermentation temperature would be an energy-conserving alternative. This is known as cold hydrolysis and this is a step toward a "green" method for the production of fuel ethanol. Intrinisic activity, inhibition, thermal stability, and pH stability are important to consider during the selection of the best raw-starch digesting enzyme. Thus, new processing approaches in simultaneous liquefaction, saccharification, and fermentation may help to minimize or eliminate contamination issues [20].

Garba et al. studied the kinetics of the hydrolysis of cassava starch by the enzyme amyloglucosidase. In this process, dried cassava flour was hydrolysed using this enzyme and the effects of varying temperature, pH and substrate concentration were studied to determine suitable kinetic parameters for the production of glucose syrup. Based on their research, the optimum temperature for amyloglucosidase activity in the production of glucose syrup was 60 degree celsius, with a pH of 5.5 and the optimum substrate concentration was 3wt%. The Thus increase in temperature past the optimum temperature tends to reduce reaction velocity by reducing enzyme activity. Using this information, a reaction path for the production of glucose syrup from starch hydrolysis was established [21].

Despite being a rich source of starch, root crops such as cassava and sweet potato have not been widely exploited for the production of syrups like high fructose syrup (HFS), which is a popular sweetener for the food and beverage industries. The factors that contribute to the cost of production of HFS are the cost and labour-intensive steps that are involved in the production of starch, different processing temperatures and pH for the enzyme reactions, poor extractability of starch, etc. In order to overcome the cost associated with the preparation of starch, the feasibility of using native cassava/sweet potato flours and their blends with rice flour and wheat flour, as the raw material for HFS production was studied. The slurry from cassava-rice flour blends after saccharification contained 70-72 g reducing sugars/100 g, which was higher than that released from native cassava flour (~69%). Blends of sweet potato with rice or wheat yielded saccharified mash with lower content of reducing sugars (60-66%) as compared to that obtained by using cassava. Although the percentage conversion to fructose after isomerization was similar for cassava/sweet potato or their blends with cereal flours (42-43%), fructose yield was higher in native cassava flour and cassava-rice blends (28-29 g/100 g) than the other flour blends [22].

Osaribie et al. studied the enzymatic hydrolysis of cassava starch (Mannihot esculenta) to glucose was studied; considering various process parameters such as temperature, pH and substrate concentration. The source of enzyme amylase used was Aspergillus niger isolated from deteriorated groundnut seeds . It was observed that glucose concentration increases progressively with increase in age of the fungi. Thus, the most viable period of the A. niger cell is between four and five days probably corresponding to the accelerated growth phase of the cells. It was also found that the highest glucose concentration wasobtained between pH 4 and 5. The best temperature for the cell was between 50 to 58°C at which maximum glucose concentration was obtained. Furthermore, the results obtained, show that at a fixed cell concentration, the rate of hydrolysis increases proportionally to substrate concentration at low levels and gradually become constant at high substrate concentration, which is in agreement with Michealis-Menten kinetic model for enzyme catalyzed reactions [23].

Soto et al. studied the influence of starch source in the required hydrolysis time for the production of maltodextrins. The main sources of starch for maltodextrins industrial production are corn, potatoes and rice, but they can also be obtained from a variety of materials such as tapioca, wheat, sorghum, etc., which depend on the availability and price of the raw materials in each country. Depending on the differences in chemical composition and structure of the starch, the enzymatic hydrolysis time required to obtain desired maltodextrin with DE will be different for each type of starch. The main aim of this research was to determine how it affects the composition of the starch source and to determin the required enzymatic hydrolysis time for the production of maltodextrins with different DE in a batch reactor. It was observed that there were no statistically significant differences ($p < 0.05$) in moisture content, carbohydrates and total starch between the different starches, but they were differences in the content of the rest of its components like ash, proteins, lipids, amylose, etc. The temperature at which the starch enzymatic hydrolysis is performed is selected from the specifications of the supplier of the enzyme. Hence, the starch chemical composition and its lipids, proteins, phosphorus and amylose content have great influence on the starch susceptibility to its enzymatic hydrolysis, as well as its physicochemical and functional properties [24].

Van der Maarel et al. studied the properties of the starch-converting enzymes of the amylase family. There are two types of glucose polymers present in starch: (i) amylose and (ii) amylopectin. Amylose is a linear polymer which consists of up to 6000 glucose units with 1-4 glycosidic bonds. Most of the enzymes that convert starch belong to the -amylase family or family 13 glycosyl hydrolases according to the classification of Henrissat (1991). An important feature of the enzymes from the -amylase family is that they all employ the -retaining mechanism but that they vary widely in their substrate and product specificities. The degree of hydrolysis determines the sweetness of starch syrup. Complete hydrolysis of starch results in the formation of only glucose or dextrose. The main drawback of the -amylases used is that they are not active at a pH below 5.9 at the high temperatures used. During the last three decades, these amylases have been exploited by the starch-processing industry as a replacement of acid hydrolysis in the production of glucose from starch. This enzyme is also used for the removal of starch in beer, fruit juices, or from clothes and porcelain [25].

Shadila binti Alias studied using Enzymatic Hydrolysis the effects of pH and temperature on glucose production from tapioca starch. Its Objective was to determine the effects of various factors like pH and temperature on the glucose produced from hydrolysis of cassava starch and to evaluate the optimum temperature and pH for steps liquefaction and saccharification which maximize the glucose concentration. Using enzymatic hydrolysis glucose has been produced which consists of two main processes i.e. liquefaction and saccharification. The whole experiment was performed to determine the glucose yield produced from locally available cassava starch. The study included various liquefaction temperature, saccharification temperature, liquefaction pH and saccharification pH. Agitation speed, reaction time and enzyme loading were kept constant during the experiment and by using DNS method the glucose produced was analyzed. There are two methods to obtain glucose syrup, they are enzymatic or acid hydrolysis. Enzymatic hydrolysis is more preferable as it gives better yield, less byproduct and easy to maintain, due to its non-corrosiveness, though the glucose percentage is very less and needs further enzyme treatment. In the liquefaction step, gelatinization increased the rate of hydrolysis while saccharification is a step to further hydrolyze the liquefied starch. The latter process resulted to about 96 % glucose yield, and about 4% byproduct. Similarly for temperature, where there is slight increment in amount of glucose there is an increase in the temperature. Yield of hydrolysis also depends on substrate concentration, type of starch, enzyme dose, time taken, and speed of agitation, granule size and viscosity of the raw starch. Lesser substrate concentrations are preferable so as to avoid substrate inhibition. Greater hydrolysis time and high enzyme dose showed the highest increase in glucose yield percentage as temperature rises [26].

Bai and Ghazali investigated the Cassava Syrup production with Glucoamylase Immobilised to Acid-washed Charcoal. By binding to water-insoluble supports like charcoal, porous glass beads, cellulose, chitin, ion-exchange resins and other biopolymers, the enzyme can be immobilised. This prevents the continuous conversion of the substrate to the product. It permits re-use of the enzyme and as compared to conventional prossesing plant it requires smaller plant space. The saccharification of Liquefied cassava starch was affected by Glucoamylase immobilised to acid-washed animal charcoal. In liquefied starch solution the addition of glucoamylase, increased the DE significantly due to the fact that in starch hydrolates, glucoamylase is a hydrolase that acts upon 1, 4 and 1, 6 -linkages. When the substrate was fed at a low rate of 1.0 mL /min into a packed-bed column reactor of immobilized glucoamylase, a 69 DE cassava syrup was produced from 7 DE liquefied cassava starch solution. An asymptotic relationship between the flow-rate and the extent of saccharification was observed. The brown colour that developed in the liquefaction process was removed at lower flow rates, resulting in a fairly colourless syrup. The conversion rate was greatest in the first 30 minutes of the reaction and then eventually slowed down for all the other substrate concentrations. The extent of saccharification of liquefied starch depends on the particle size of the activated charcoal to which the glucoamylase was immobilized [27].

III. CONCLUSION

Thus, accepted and improved quality of glucose syrup can be produced by the enzymatic hydrolysis of cassava and other starches using various grains like rice, sorghum, maize etc., as the enzyme source. This glucose syrup finds wide applications in brewing, baking, dairy and confectionaries. The coupled enzymatic starch hydrolysis –permanganate decolourisation reaction is an effective procedure for monitoring amylase activity in malt crude extracts prepared from germinating cereals. This can be used to ensure maximum degradation of starch to glucose and give better yield.

One of the greatest challenges of food processing in the developing countries is the transformation of traditional food processing method into modern industrial operations. These processing techniques for making traditional food product differ within and between countries because of differences in food culture, available raw materials, and processing equipment utilized. The analysis of the chemical composition of cassava proves that it is a principal source of carbohydrates for the consumers. It is therefore very important to convert cassava into more utilizable products. Effort in this regard is gradually developing for home as well as industrial purposes.

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