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Production of glucose syrup by the hydrolysis of starch made from rotten potato

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ABSTRACT

Glucose syrup production from rotten potato was investigated. The starch was extracted from rotten potato after steeping for 12hours and purified by sedimentation method and had the yield of 82.17%. The glucose was recovered by enzymatic hydrolysis using alpha amylase produced 42.20g reducing sugar from rotten potato starch and it was also recovered by acid hydrolysis using sulfuric acid solution produced 40.54g reducing sugar. Starch from rotten potato exhibited good potential as a substrate for glucose syrup production. Acid and enzymatic hydrolysis of rotten potato starch to glucose were investigated and compared. Enzymatic hydrolysis produced higher yield of glucose as compared to acid hydrolysis.

Keywords: Rotten potato starch, Hydrolysis, Alpha amylase, Glucose

INTRODUCTION

Starch is an important polysaccharide which is found in plant sources. The microscopic appearance of starch is in the form of granules. It contains two types of molecules i.e. the linear helical amylose which contains alpha amylase linkage and the branched amylopectin which contains both alpha amylase and beta amylase linkages. Starches are generally insoluble in water at room temperature. There are two types of linkage in starchy structures: α-1,4 and α-1,6, linkages. Amylose, a kind of starch, is an unbranched, single chain polymer containing 500 to 2000 glucose subunits with only α-1,4 glycosidic links.[1] The presence of α-1,6 glycosidic linkages in some starchy materials results in a branched glucosepolymer called amylopectin.[2] The breaking down of the α-1,4 and α-1,6 linkages to small units of glucose (monosaccharide) is made possible by the actions of α-amylase and glucoamylase (enzymes) respectively.[3]Starch is used as a raw material for the production of low molecular weight products such as glucose, maltose which is widely applied in sugar, brewing as well as in textile industries.[4]The conversion of starch to glucose is achieved through either chemical (acid) or enzymatic hydrolysis. The use of enzyme has more advantages to the former due to the formation of breakdown products.[5]

The bio-fuels production is nowadays one of the main approaches in developing a sustainable economy. According todata presented by U.S. Energy Information Administration total World bio-fuels production in 2001 was equal to 54,511 m^3 day^{-1}, whereas in 2011 production of bio-fuels reached a value of 304,587 m^3 day^{-1} and 302,290 m^3 day^{-1} in 2012. This means thatproduction of bio-fuels increased during 10 years by more thanfive times. Bio ethanol can be produced from biomass by fermentation of sucrose or glucoseusing commercial yeast such as Saccharomyces Ceveresiae.[6]

Works done for glucose production from different materials like cassava,[7-10] corn,[11] whey permeate,[12] sugar cane,[13-14] potato,[15-19] maize,[20] etc. using different enzymes like *Saccharomyces Cerevisia, Z. mobilies, C. Beijerinckii,*[21] *C. Acetobutylicum,*[22] etc. have been used by the researchers and it was found that till now no research has been carried out by for glucose production by using the rotten potato starch.

The processing of starch to glucose involves gelatinization, liquefaction and saccharification processes using alpha enzymes. Carbohydrates based agricultural products like starch from potato occur abundantly and can be used as a raw material for the production of energy. Glucose, an important industrial product of starch hydrolysis finds application as sweetener in the food and pharmaceutical industries. This study is aimed at the production of glucose syrup by the enzymatic as well as acid hydrolysis of rotten potato.

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MATERIALS AND METHODS

2.1 Raw Materials

Rotten raw potatoes were obtained from the local market at Civil lines, near Indian Institute of Technology Roorkee. Alpha amylase was obtained from the culture collection unit of MTCC, Chandigarh. The chemicals used during course of this study include sulfuric acid solution, Sodium hydroxide solution, Distilled water, 3,5- Dinitrosalicyclic acid (DNSA), Potassium sodium tartrate and Iodine Solution.

2.2 Extraction of starch from rotten potato

Potatoes were peeled and cut into smaller pieces, and initial weight was recorded. Then the potatoes were grinded in a mixer grinder with sufficient water. The homogenized slurry of potatoes was collected into a beaker and enough water was added. Slurry was filtered through a muslin cloth to remove the particles. Filtrate was allowed to settle. Starch rapidly settles at the bottom. Starch free supernatant was decanted carefully. Compact mass of starch was collected and was dried in hot air oven at 80^0C for 4hrs. Final weight of isolated starch was recorded and the yield was calculated.

2.3 Starch yield

The dry mass obtained from the sedimentation procedure may contain trace amount of other residues also. The starch yield was determined as:

%Yield = (Dry weight of Starch Recovered from Extraction/ Dry weight of whole potatoes) × 100

2.4 Hydrolysis of Gelatinized Starch by sulfuric acid

50g of starch was hydrolyzed by dissolving in 150 ml of H_2SO_4 (0.2-0.1M) at 60^0C to 100^0C for 4 hours. After the specified time, 50 ml of 0.1 M sodium hydroxide was used to neutralize the solution. Fehling test was used to determine the concentration of glucose in solution.

Fehling Test –
Fehling A: 7 g of $CuSO_4$ was dissolved in 100 ml of distilled water containing 2 drops of dilute sulfuric acid.
Fehling B: 34.6 g of sodium potassium tartartate and 12 g of sodium hydroxide were dissolved in 100 ml of distilled water.

1. Known standard solution of glucose was prepared by dissolving 1.25 g of glucose in 250 ml of standard flask in distilled water.
2. 20 ml of each of Fehling's A and B were pipette out in dry conical flask and was shaken thoroughly.
3. 20 ml of this freshly prepared Fehling's solution was pipette out into a clean conical flask and was diluted with 20 ml of distilled water.
4. Solution was heated up to 60^0C over wire gauze.
5. Prepared standard solution of glucose was taken in a burette and this solution was run slowly into the boiling Fehling's solution until the blue color completely disappeared.
6. A positive test was indicated by green suspension and then a red precipitate.

Fehling A: 7 g of $CuSO_4$ in 100 ml distilled water which would equal to 0.25 N.

Fehling B: 34.6 g of sodium potassium tatarate and 12 g of sodium hydroxide in 100 ml of distilled water which would equal to 4.25 N.

Overall normality of Fehling solution = 2.25 N.
Normality $N_2 = (N_1 \times V_1) / V_2$

The amount of substance in the whole of the given solution = (Atomic weight × Normality × Volume of the solution up to made (ml)) / 1000

2.5 Hydrolysis of Gelatinized Starch by using Alpha amylase

50 g of starch was dispersed in 150ml of distilled water. The slurry obtained was heated at 80^0C for 4 hours on a magnetic stirrer. 1g of alpha amylase was added to the slurry and allowed to liquefy for 1 hour. The liquefied starch was cooled at 50^0C. Glucose Concentration of the glucose syrup was determined by Fehling test.

RESULTS AND DISCUSSION

3.1 Yield of Potato

Table 1 Weight of starch extracted from potato

S. No	Weight of potatoes (g)	Weight of starch (g)
1.	208	169
2.	227	186
3.	255	212

The given sample contains 82.17g of starch per 100g of potatoes.

3.2 Acid hydrolysis of starch

Fehling A: 7 g of CuSO4 in 100 ml distilled water which would equal to 0.25 N.

Fehling B: 34.6 g of sodium potassium tatarate and 12 g of sodium chloride in 100 ml of distilled water which would equal to 4.25 N.

Overall normality of Fehling solution = 2.25 N.

Table 2 Volumetric titration for glucose by acid hydrolysis

S.no	Volume of Titrate (ml)	Volume of Titrant used (ml)	Normality N_2	Glucose (g)
1.	20	47.5	0.947	42.665
2.	15	38	0.888	39.994
3.	10	26	0.865	38.976

Average amount of glucose in the whole of the given solution is 40.545g.

So, from 50g of starch 40.545g of glucose is obtained.

Yield = (Amount of glucose obtained)/ Amount of starch = 0.8109

Yield percentage = 81.09 %.

3.3 Enzymatic hydrolysis of starch

Fehling A: 7 g of $CuSO_4$ in 100 ml distilled water which would equal to 0.25 N.

Fehling B: 34.6 g of sodium potassium tatarate and 12 g of sodium chloride in 100 ml of distilled water which would equal to 4.25 N.

Overall normality of Fehling solution = 2.25 N.

Table 3 Volumetric titration for glucose by enzymatic hydrolysis

S. no	Volume of Titrate (ml)	Volume of Titrant used (ml)	Normality N_2	Glucose (g)
1.	20	43	0.937	42.224
2.	15	33	0.978	44.059
3.	10	24	0.935	42.201

Average amount of glucose in the whole of the given solution is 45.1137g.

So, from 50g of starch 42.201g of glucose is obtained.

Yield = (Amount of glucose obtained)/ Amount of starch

= 0.855 = 85.5 %.

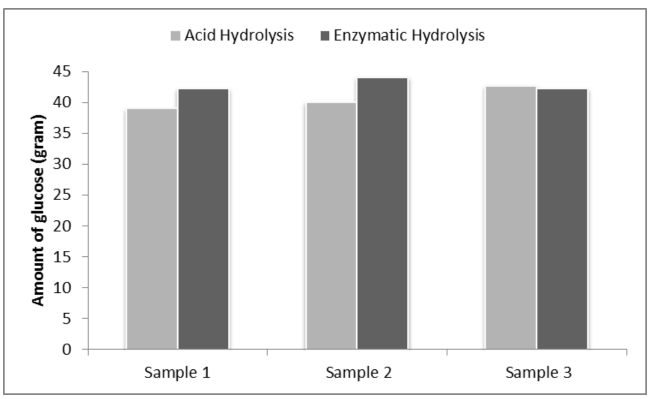


Figure 1 Comparison of glucose produced from acid and enzymatic hydrolysis

3.4 FESEM analysis of starch

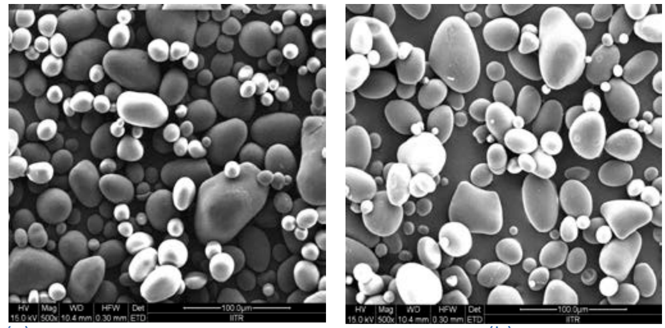
In FESEM analysis of starch, molecular structure of pure chemical starch was compared with rotten potato starch at 100µm magnification. Weight %, atomic % of carbon and oxygen element of pure starch and rotten potato starch were also compared.

Table 4 Smart Quant Results for pure starch:

S. No	Element	Weight %	Atomic %	Net Int.	Error %
1.	**C K**	62.21	68.68	173.2	5.42
2.	**O K**	37.79	31.79	62.84	11.7

Table 5 Smart Quant Results for Potato Starch:

S. No	Element	Weight %	Atomic %	Net Int.	Error %
1.	**C K**	50.58	57.69	83.01	6.78
2.	**O K**	49.42	42.31	64.16	11.2



(a) (b)

Figure 2 FESEM of pure starch (a) and potato starch (b)

3.5 FESEM analysis of Glucose

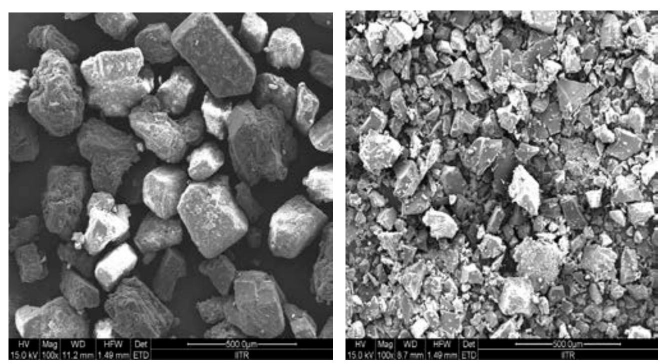
In FESEM analysis of Glucose, molecular structure of pure glucose chemical was compared with glucose from enzymatic hydrolysis at 100µm magnification. Weight %, atomic % of carbon and oxygen element of pure glucose chemical and glucose enzymatic hydrolysis were also compared.

Table 6 Smart Quant Results for pure glucose:

S. No	Element	Weight %	Atomic %	Net Int.	Error %
1.	**C K**	58.18	64.95	244.83	5.41
2.	**O K**	41.82	35.05	115.8	10.64

Table 7 Smart Quant Results for glucose from hydrolysis:

S. No	Element	Weight %	Atomic %	Net Int.	Error %
1.	**C K**	47.55	54.71	45.02	7.71
2.	**O K**	52.45	45.29	42.52	11.63



(a) (b)

Figure 3 FESEM of pure glucose (a) and glucose from potato (b)

Conclusion

Rotten Potato can be used as a raw material for the production of glucose either by acid hydrolysis or by enzymatic hydrolysis. Enzymatic hydrolysis produced the 85.5% yield of glucose at temperature of 80^{0}C during 4 hours of operation and it was found that enzymatic hydrolysis produced higher concentration of glucose of 42.201g as compared to glucose of 40.545g obtained from acid hydrolysis. Molecular structure of pure chemical starch was compared with the starch obtained from rotten potato by

FESEM analysis at a magnification of 100.0 µm and it was found that pure starch and the starch obtained from the rotten potato were exactly same round shaped. Similarly Molecular structure of pure glucose chemical was also compared with the glucose obtained from enzymatic hydrolysis of starch obtained from rotten potato and it was found that pure glucose sample was regular blocks and the glucose from the hydrolysis was irregular.

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