REPARATION AND CHARACTERIZATION OF LIQUID GLUCOSE FROM THE SWEET POTATO STARCH OF *POMOEA BATATAS* L. (SHWE-KAN-ZUN-U)

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Abstract

The present work deals with isolation and identification of liquid glucose from sweet potato tuber *Ipomoea batatas* L. (Shwe-ka-zun-u). From the preliminary phytochemical investigation, alkaloids, carbohydrates, α-amino acids, flavonoids, glycosides, saponins, steroids, tannins, terpenoids, phenolic compounds, starch, organic acids, reducing sugars were found to be present and the cyanogenic glycosides was absent in the tuber of sweet potato. In addition, sweet potato tuber contains the content of water (42.15 %), total solid (57.85 %), nitrogen (0.192 %), ash (1.91 %), fat (0.72 %), starch (48.34 %) and crude fibre (1.97 %). According to ED XRF data analysis, K (65.705 %), Ca (21.918 %), Fe (6.025 %), Rb (3.487 %) and Mn (2.865 %) were observed. The contents of soluble matters using water, ethanol and petroleum were 6.01, 4.14 and 2.23 %, respectively. The yield percent and dextrose equivalent of liquid glucose prepared by enzymatic method and acid hydrolysis method were respectively found to be 11.95 and 11.04 %, 40.37 and 37.30., determined by Fehling's solution method. Similarly, the yield percent of liquid glucose prepared by enzymatic method and acid hydrolysis method were observed as 22.43 and 21.78 %, 75.78 and 73.58, respectively, determined by phenol-sulphuric acid assay. The prepared liquid glucose was characterized by paper chromatographic method. The characteristics of prepared liquid glucose obtained by enzyme hydrolysis and acid hydrolysis were indicated as specific gravity (1.08 and 1.02), refractive index (1.42 and 1.30), viscosity (1.5282 cP and 0.7864 cP), pH (4.4 and 4.8), water content (25.67 % and 31.95 %), total solid (74.33 % and 68.05 %) and sulphated ash (0.048 % and 0.069 %), respectively.

Keywords: *Ipomoea batatas* L., sweet potato tuber, liquid glucose, termamyl enzyme, paper chromatography

Introduction

The sweet potato (*Ipomoea batatas* L.) is a dicotyledonous plant that belongs to the bindweed or morning glory family, *Convolvulaceae*. Its large, starchy, sweet-tasting, tuberous roots are a root vegetable (Purseglove, 1968). The young leaves and shoots are sometimes eaten as greens. The plant is herbaceous perennial vine, bearing alternate heart-shaped or palmately lobed leaves and medium-sized sympetalous flowers. Sweet potato cultivars with white or pale yellow flesh are less sweet and moist than those with red, pink or orange flesh. The sweet potato (*Ipomoea batatas*) is one of the most important food crops in the world and provides not only staple food but also important as an industrial raw materials. Originating in South America, it is now grown all over the world spreading throughout the tropical and sub-tropical countries. The sweet potato tubers are rich in starch, sugars, minerals and vitamins. In Asian countries, some edible tubers are also used as traditional medicine.

The production of glucose syrup from sweet potato actually is produce the glucose from sweet potato starch, because the sweet potato with more protein and fiber. Liquid glucose is also a main ingredient of candies and sweets. In the pharmaceutical industry, it is used as a cost-effective replacement to sugar syrup preparations and is also used in tablets for coating and as a granulating agent.

Liquid glucose is widely used in the confectionery, biscuit and food canning industries, as a thickener, sweetener and to modify the mouth feel of food preparations. Glucose liquids are

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obtained by the hydrolysis of starch in which the long-chain carbohydrate molecules are broken down into series of low molecular weight carbohydrates. Liquid glucose is an aqueous solution of nutritive saccharide obtained by starch hydrolysis, by using corn and rice as raw material, which is purified and concentrated to required solids. It is usually odorless and clear yellow colored viscous liquid sweet syrup which is processed and stored under hygienic conditions.

The present study was focused on the preliminary phytochemical tests, chemical analysis, preparation and characterization of liquid glucose from sweet potato tuber by using Termamyl enzyme and HCl hydrolysis methods.

Materials and Methods

Collection and Preparation of Plant material

The tuber of *I. batatas* (Shwe-ka-zun-u) was collected from Taikkyi Township, Yangon Region. The tuber sample was transformed into powder and stored in air-tight container.

Preliminary Phytochemical Test

A few grams of dried tuber powder sample of *I. batatas* was subject to the tests of alkaloids, carbohydrates, α-amino acids, flavonoids, glycosides, saponins, steroids, tannins, terpenoids, phenolic compounds, starch, cyanogenic glycosides, organic acids and reducing sugars according to the standard procedures (Finar, 1968; M-Tin Wa, 1972; Marini-Bettolo *et al*., 1981; Robison, 1983; Shriner *et al*., 1980; Trease and Evans, 1996).

Chemical Analysis of Sweet Potato Tuber

The water content of dried powder tuber sample was determined by the Dean and Stark method (AOAC, 1995), total solid content by the oven drying method (Pearson, 1976), nitrogen content by the Micro-Kjeldahl distillation method (AOAC, 1995), the ash content by the gravimetric method, fat content by the soxhlet extraction method (Joslyn, 1956), water-soluble matter, ethanol-soluble matter and petroleum ether-soluble matter contents by the British Pharmacopoeia method. The starch content was calculated by multiplying the sugar content with factor 0.93. The crude fibre content was determined by the acid and alkali digestion method, shown in Table 2. The relative abundance of elements was determined by Energy Dispersive X-ray Fluorescence, shown in Table 3.

Preparation of Liquid Glucose from Sweet Potato Tuber

Determination of optimum saccharification time

Starch 100 g was mixed with 500 mL of distilled water and cooked with pressure cooker for about 30 min until the starch solution become sticky and waxy in nature. Liquefaction of the starch slurries was carried out using a thermostable α-amylase (125 mL of termamyl enzyme). The pH of the slurry was adjusting to 6.0 and reaction was carried out in a stirred reactor with enzyme at 65 ºC for 2 h. The resulting solution (prepared liquid glucose) was cooled to 30 ºC. Saccharification was carried out for 5 days at 30 ºC.

Dextrose equivalent (D.E) of the saccharified solution was determined daily by phenol-sulphuric acid assay method. The results are recorded in and the standard calibration curve was plotted by dextrose equivalent against the saccharification time. From this curve, the optimum saccharification time was obtained.

Preparation of liquid glucose from sweet potato tuber by using enzyme and acid

For enzyme, the dried powder tuber of sweet potato 100 g was mixed with 500 mL of distilled water and cooked with pressure cooker for about 30 min until the starch solution become sticky and waxy in nature. Liquefaction of the starch slurries was carried out using a thermostable α-amylase (125 mL of termamyl enzyme). The pH of the slurry was adjusting to 6.0 and reaction was carried out in a stirred reactor with enzyme at 65 ºC for 2 h. The resulting solution (liquid glucose) was cooled to 30 ºC. Saccharification was carried out for 72 h which was the optimum saccharification time obtained from determination of optimum saccharification time at 30 ºC and was stopped by heating to 90 ºC for 10 min. The hydrolysate was double filtered using a nylon cloth and filter paper so as to get the clear hydrolysate. The hydrolysate was clarified with 10 g of animal charcoal and filtered, and then measured the value of reducing sugar (glucose).

In acid hydrolysis method, the dried powder tuber of sweet potato 100 g was mixed with 500 mL of distilled water and cooked with pressure cooker for about 30 min until the starch solution become sticky and waxy in nature. The starch solution was removed from the pot and cooled to 30 ºC. Aqueous solution at pH 5.5 was treated with 5 % fuming hydrochloric acid at 40 ºC with stirring for 20 min. The mixture was cooled to 30 ºC and after 72 h, 0.1 M Na_2CO_3 was added into this slurry to neutralize. The hydrolysate was double filtered using a nylon cloth and filter paper so as to get the clear hydrolysate. The hydrolysate was clarified with 10 g of animal charcoal and filtered, and then measured the value of reducing sugar (glucose).

Quantitative Determination of Glucose in the Prepared Liquid Glucose from Sweet Potato Tuber

Fehling's solution method (Volumetric method)

First, Fehling's solution was standardized with glucose solution. The standardization of Fehling's solution was carried out with the standard glucose solution. The results showed that the volume of the sugar solution required to reduce 10 mL of Fehling's solution must be in the range of 17 mL to 34 mL (equivalent to 0.15 % to 0.3 % reducing sugar solution) so that the error for titration would be minimum. Standard glucose (ca. 2 g) was weighed, dissolved in distilled water and made up to mark in a volumetric flask (1000 mL). Standard glucose solution was freshly prepared to standardize Fehling's solution. Fehling's solution 10 mL was pipetted into a conical flask and glucose solution 15 mL was added from a burette. The mixture was heated on a hot plate till it boiled. When the solution in the flask had boiled for about 15 s, the blue colour of the solution turned red and the major portion of copper was precipitated as cuprous oxide. Methylene blue indicator 1 mL was then added and the liquid was boiled for another 2 min. Small quantity of standard liquid glucose solution 1 mL was added in portion, keeping the liquid boiling, till the colour of the indicator disappeared. The titration was carried out so that it was completed within 5 min. And then standard liquid glucose solution 1 mL was added and boiled for 15 s. Methylene blue indicator 3 drops was added and these mixture solution was boiled for another 2 min and titrated with glucose solution till the colour of the indicator disappeared. The volume of glucose solution required for the reduction of 10 mL of Fehling's solution was noted. From the titre the amounts of glucose required to reduce10 mL Fehling's solution was calculated (AOAC, 1995).

Phenol-sulphuric acid assay method (UV spectrophotometric method)

For preparation of standard glucose solution, 0.2 g of glucose was exactly weighed and dissolved in 100 mL of distilled water. 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 mL of these solutions were down out and placed in each 100 mL of volumetric flask and diluted to the mark with distilled water. These solutions contained 20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 µg of glucose per mL, respectively.

One mL each of the prepared glucose liquid solution and the above ten standard glucose solutions (20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 µg of glucose per mL) were introduced into each test tube. One mL of 5 % phenol solution was added to each test tube and mixed. A blank was prepared with 1 mL of distilled water instead of sugar solution, 5 mL of concentrated sulphuric acid was added again to each test tube. Each test tube was agitated during the addition of acid. After about 10 min, the tubes were shaken again and placed in water-bath at 30 ºC for 20 min. The yellow orange colour was stable for several hours. Absorbance was measured at 490 nm using CIBA Corning Spectrophotometer 259 (Pearson, 1976).

A standard curve was plotted by the absorbance of the standard glucose solutions against the concentration in µg per mL. Using this standard curve, the concentration of glucose in the sample and dextrose equivalent was then calculated (Conn and Stumpf, 1972).

Characterization of the Prepared Liquid Glucose by Paper Chromatography

The prepared liquid glucose by enzymatic method and acid hydrolysis method was screened by means of ascending paper chromatography using appropriate standards and solvent system. The solvent in this technique moved upward against the gravitational pull (Whistler and Wolfrom, 1964). The paper was cut into small paper (7.5 × 15 cm in size). About 5 µL of samples (two isolated liquid glucoses, standard glucose, standard galactose) were spotted on the paper using a capillary tube. The paper was placed in a chromatographic chamber with *n*-butanol: pyridine: water (10: 3: 3) solvent system. When the solvent reached the height of about 15 cm from the place of origin, it was taken out. The paper chromatogram was dried in an oven (at a temperature 60 ºC). The spots on paper chromatogram were detected by viewing directly under UV 254 nm and 365 nm light and by spraying the dried paper with aniline phthalate reagent. Then, the paper was heated at 80-110 ºC in an oven for exactly 3 min. The separated sugars were revealed as brown spots for hexoses and the paper chromatogram. The R_f values of liquid glucose were measured (Whistler and Wolfrom, 1964).

Determination of Physiochemical Properties of the Prepared Liquid Glucose

The prepared liquid glucose was determined the specific gravity by Baume's hydrometer, the refractive index by an Abbe 60, refractometer (Jacobs, 1958), the viscosity by the U-tube viscometer (ASTM, 1966), the colour by the Lovibond Tintometer, water content by oven drying method (Pearson, 1976), sulphated ash by the gravimetric method and pH is measured by pH meter.

Results and Discussion

Preliminary phytochemical analysis was performed in order to know the different types of compounds present in sweet potato tuber. The results on phytochemical testes are summarized in Table 1.

The Dean and Stark method is more accurate than oven drying method because the water content from oven drying method contains bound water, adsorbed water and bulk or free water. Nitrogen content was determined by using the Micro-Kjeldahl distillation method. Ash is the inorganic residue remained after the organic matter has been burnt away, shown in Table 2. Fats were determined by the soxhlet extraction method using petroleum ether (b.pt 40-60 ºC). The petroleum ether cannot extract non-fat constituents such as starches and proteins. Determination of water soluble matters, alcohol soluble matters and petroleum ether-soluble matters were carried out to know the amount of total solids soluble in water, in alcohol and in petroleum ether (b.pt 40-60 ºC). Starch content in the sweet potato tuber was also determined by using distilled water and dilute sulphuric acid. Starch is a water-soluble complex, carbohydrate found naturally in many

vegetable products. The crude fibre is the insoluble and combustible organic residue with remains after the sample has been treated under prescribed conditions. The results are shown in Table 2.

X-ray spectrometer permits simultaneous analysis of light element to heavy element. Shimadzu EDX-700 spectrometer can analyze the element from Si to U under vacuum condition. In the present work, relative abundance of element present in sweet potato tuber sample was determined by ED-XRF spectrometer. It can be seen that essential minerals for human such as potassium and calcium in tuber was predominant. Potassium (K) is important in regulating the body fluid volume and also widely distributed in foods. Potassium is essential in maintaining water or intracellular fluid balance. The daily intake may range from 1900-5600 mg. Calcium (Ca) with which decreases the toxicity of other ions, is a major mineral constituents of the body (Monier, 1950). Ca is important for the health of bone and teeth, but it also affects muscles, hormones and nerve function. Iron (Fe) is essential nutrients for men (Bowman, 1980). Rubidium (Rb) in its chemical properties, it is closely resembles potassium. Among them, potassium (65.705 %) was higher than other elements in the sample, shown in Table 3.

Liquid glucose from sweet potato was prepared by using termamyl enzyme for 5 days at 30 ºC. The dextrose equivalent (D.E) of the saccharide solution was measured daily by phenol-sulphuric acid assay method and these values are shown in Table 4 and Figure 1. According to the Figure 1, the optimum saccharification time was 48 h and the dextrose equivalent was 75.78. Dextrose equivalent is very important for sugar chemistry. The dextrose equivalent value (D.E) of commercial liquid glucose is the reducing sugar content, as dextrose, calculated on the basic of the solid matter.

The amounts of glucose in the prepared liquid glucose from sweet potato by enzymatic and acid hydrolysis methods were determined by the Lane and Eynon's Method (Lane and Eynon, 1923) and phenol-sulphuric acid assay method (Duboiset *et. al.,* 1956). Standard glucose curve was used for quantitative determination and standard calibration curve was shown in Figure 2 and Table 5. The amount of glucose in the prepared liquid glucose and dextrose equivalent are shown in Table 6.

Most of the physical characteristics of liquid glucose can be judged visually. The liquid glucose is light brown, viscous liquid having a bunt sugar smell. The colour was determined by using the Lovibond Tintometer. It was observed that yellow colour value of liquid glucose obtained from acid hydrolysis was very high compared to the corresponding red and blue values. The red colour value of liquid glucose obtained by enzymatic method was very high compared to the corresponding yellow and blue values. Specific gravity is the ratio of the mass of a unit volume of the sample to the mass of a unit volume of water at 30 ºC. The specific gravity of liquid glucose from enzyme and acid hydrolysis were determined by hydrometer. The amount of total solid present would be related to the specific gravity. Hydrometers constructed for the determination of specific gravity of sugar solution are called saccharometers under which name, they are known in breweries. The refractive index, as normally measured, is the ratio of the velocity of the light in air to the velocity of the light in the substance being determined. Refractive index of liquid glucose from enzyme and acid hydrolysis were determined by the Abbe-refractometer, at 30 ºC. Refractive indices of liquid glucoses have been found to provide a reliable indication of the dry weight of solid in the solution. The viscosity of a substance is the shearing resistance of a liquid film which separates two horizontal plates, one of which is being moved across the other. The viscosity of liquid glucose from enzyme and acid hydrolysis were determined by U-tube viscometer. Water contents of liquid glucose from enzyme and acid hydrolysis were determined by oven drying method. The pH of liquid glucose was obtained by using enzymatic method and acid hydrolysis method. The two prepared liquid glucose (by using enzymatic method and acid hydrolysis method)

were heated with concentrated sulphuric acid and the ash was expresses as "sulphated ash". The results are shown in Table 8.

Table 1 Result of Phytochemical Investigation on Tuber of *I. batatas*

Sr. No.	Tests	Extract	Test reagents	Observation	Remark
1.	Alkaloids	10 % acetic acid in EtOH	Mayer's reagent Dragendroff's reagent sodium picrate solution	white ppt blue-black ppt yellow ppt	+ + +
2.	Carbohydrates	H_2O	10 % α-naphthol, H_2SO_4(conc.)	pink colour	+
3.	α-amino acids	H_2O	Ninhydrin reagent	violet spot on paper	+
4.	Flavonoids	EtOH	Mg ribbon, HCl (conc.)	pink colour	+
5.	Glycosides	H_2O	10 % lead acetate	white ppt	+
6.	Saponins	H_2O	distilled water	frothing	+
7.	Steriods	Benzene	acetic anhydride & H_2SO_4(conc.)	green colour	+
8.	Tannins	EtOH	1 % $FeCl_3$ and gelatin solution	greenish-yellow colour	+
9.	Terpenoids	$CHCl_3$	acetic anhydride & H_2SO_4(conc.)	blue colour	+
10.	Phenolic compounds	H_2O	1 % potassium ferricyanide& 1 % ferric chloride	deep green colour	+
11.	Starch	H_2O	I_2 solution	bluish-black colour	+
12.	Cyanogenic glycosides	H_2O	conc: H_2SO_4	no brick red	-
13.	Organic acids	H_2O	bromocresol blue	blue colour	+
14.	Reducing sugars	H_2SO_4	Benedict's solution	brick-red ppt	+

Presence = (+), Absence = (-), Precipitate = (ppt)

Table 2 Chemical Analysis of Tuber of *I. batatas*

No.	Principal Components	Observation Value (%)	Literature Value (%)*
1.	Water Content (Wet-matter)	42.15	68.5-72.3
2.	Total solid Content	57.85	31.5-27.7
3.	Nitrogen Content (Crude Protein)	0.192	0.182-0.553
4.	Ash Content	1.91	0.7-1.0
5.	Fat Content	0.72	0.2-0.4
6.	Water-soluble Matter Content	6.01	-
7.	Ethanol-soluble Matter Content	4.14	-
8.	Petroleum Ether-soluble Matter Content	2.23	-
9.	Starch (Wet-matter)	48.34	25.6-31.0
10.	Crude Fiber Content	1.97	0.7-1.0

*(AOAC, 1995)

Table 3 Relative Abundance of some Elements in Tuber of *I. batatas* by ED XRF Method

No.	Element	Relative Abundance (%)
1.	Potassium (K)	65.705
2.	Calcium (Ca)	21.918
3.	Iron (Fe)	6.025
4.	Rubidium (Rb)	3.487
5.	Manganese (Mn)	2.865

Table 4 Change in Sugar Content of Saccharified Solution with Termamyl Enzyme in Different Saccharification Times

Sr. No.	Saccharification time (h)	Sugar content (%)
1.	0	0
2.	12	18.01
3.	24	30.11
4.	48	41.52
5.	72	38.43
6.	96	36.78
7.	120	34.54

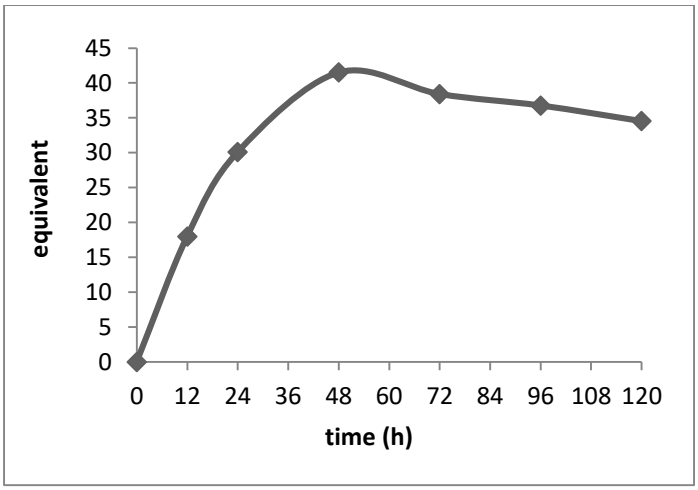

Figure 1 Plot of variation of glucose content percent for glucose syrupt with incubation period

Table 5 Absorbance of the Orange Yellow Colour of Standard Glucose Solution of Various Concentrations in the Phenol-sulphuric Acid Assay

Amount of Glucose (µg/mL)	Absorbance at 490 nm
100	0.031
200	0.062
300	0.102
400	0.129
500	0.161
600	0.193
700	0.238
800	0.262
900	0.289
1000	0.319

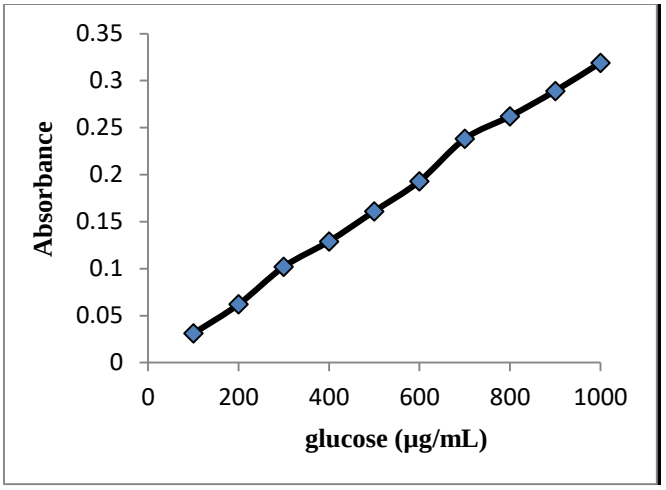

Figure 2 Plot of absorbance as a function of weight of glucose

Table 6 Glucose Content of the Prepared Liquid Glucose from *I. batatas*

Name of method	Glucose content from enzyme hydrolysis		Glucose content from acid hydrolysis	
	Glucose content (%)	D.E	Glucose content (%)	D.E
Volumetric method	11.95	40.37	11.04	37.30
Spectrophotometric method (Phenol-sulphuric acid assay method	22.43	75.78	21.78	73.58

D.E = Dextrose Equivalent

Table 7 Paper Chromatography of the Prepared Liquid Glucose

No.	Sugars	R_f values	Observation
1.	Liquid glucose (enzymatic method)	0.71	Brown colour
2.	Liquid glucose (acid hydrolysis method)	0.72	Brown colour
3.	Standard galactose	0.68	Brown colour
4.	Standard glucose	0.71	Brown colour

Solvent System= *n*-butanol : pyridine : water (10: 3: 3)

Spray reagent = analine phthalate reagent

S_1 = Liquid glucose (acid hydrolysis method)

S_2 = Liquid glucose (enzymatic method)

S_3 = Standard galactose

S_4 = Standard glucose

Figure 3 Paper chromatogram of the prepared liquid glucose from *I. batatas*

Table 8 Physicochemical Properties of the Prepared Liquid Glucose *I. batatas*

No.	Physicochemical properties	Liquid glucose from enzyme	Liquid glucose from acid
1.	Specific gravity	1.08	1.02
2.	Refractive Index at 30 ºC	1.42	1.30
3.	Viscosity (cP) at 28 ºC	1.5282	0.7864
4.	Water content (%)	25.67	31.95
5.	Total solid (%)	74.33	68.05
6.	Sulphated ash (%)	0.048	0.069
7.	Colour		
	(Red)	46.1	0.1
	(Yellow)	0.4	62.2
	(Blue)	0.3	0.3
8.	pH	4.4	4.8

Conclusion

From the overall assessment for the present work concerning with the phytochemical constituents and liquid glucose from enzyme and acid hydrolysis of *I. Batatas* (Shwe-ka-zun-u) tuber, the following inferences could be deduced. In the present work on the Sweet Potato tuber sample, preliminary phytochemical tests revealed the presence of alkaloids, carbohydrates, α-amino acids, flavonoids, glycosides, saponins, steroids, tannins, terpenoids, phenolic compounds, starch, organic acids and reducing sugars and the absence of cyanogenic glycosides in it.

Chemical analysis of the tuber sample revealed water, total solid, nitrogen, ash, fat, starch and crude fiber contents. Qualitative elemental analysis of the tuber sample by Ed XRF method showed that K, Ca, Fe, Rb and Mn. The results indicated relatively high contents of potassium and calcium. According to the elemental result, this sample was found to be effective for good; potassium and calcium are especially important for mineral elements which are necessary for the body in trace amount. The soluble matter for water, ethanol and petroleum ether were respectively determined.

The optimum saccharification time for liquid glucose (by using enzyme) was 48 h. The optimum saccharification time for liquid glucose (by using enzyme) was 48 h. The yield percent of liquid glucose prepared by enzymatic method and acid hydrolysis method were observed to be 11.95 % and 11.04 % determined by Fehling's solution volumetric method, and 22.43 % and 21.78 % determined by phenol-sulphuric acid assay, respectively. The dextrose equivalent of these two prepared liquid glucose were determined by volumetric and spectrophotometric methods, and found to be (40.37, 75.78) in enzymatic method and (37.30, 73.58) in acid hydrolysis method.

The prepared liquid glucoses from enzyme and acid hydrolysis were characterized by Paper Chromatographic method.

Physicochemical properties of liquid glucose prepared by hydrolysis with enzyme and acid such as specific gravity, refractive index, viscosity, water content, total solid, sulphated ash, colour and pH were determined respectively.

The prepared liquid glucoses were observed as colourless liquid and their identification test values are in close agreement with literature values.

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