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Optimization of liquefaction and saccharification times for laboratory scale production of glucose syrup from Cassava starch and scaling up process of optimized conditions at pilot scale

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Abstract

Cassava tubers (Manihot esculenta Crantz) are locally available natural source for starch and commercially unexploited in the production of glucose syrup in Sri Lanka. Presently, there is an increasing demand for glucose syrup at local market due to its wide application in food industry such as bakery, confectionery, beverage and dairy. Since there is a high importation cost for glucose syrup to the country, this study was focused to optimize liquefaction and saccharification times at constant selected enzyme concentrations in laboratory scale production of glucose syrup from Cassava starch with an intention to scaling up process. Recommended commercial Cassava variety (MU-51) was analyzed for its composition and extractable starch content. Extracted starch was subject to liquefaction under known, constant alpha-amylase concentration (0.03% w/w, dry basis) and DE was measured at constant time intervals until the DE reached to the expected DE value of 8-15. Liquefied slurry obtained at expected optimized liquefaction time was subject to saccharification under known, constant glucoamylase concentration (0.07% w/w, dry basis) and DE value was obtained at different time periods in order to optimize saccarification step to obtain glucose syrups with required intermediate or high DE values. Study was shown that the estimated optimum liquefaction time was 15 min under the given conditions. Minimum saccharification times spent to obtain glucose syrups with intermediate and high DE values were 15 min and 75 min respectively. In each case the total enzymatic reaction time spent for laboratory scale production of glucose syrup was less than 2 h.

Keywords: Glucose syrup, Cassava syrup, Cassava, Liquefaction, Saccharification, Enzymatic hydrolysis.

Introduction

Cassava or manioc (*Manihot esculenta* Crantz) is a nutty flavored, starchy tuber of the spurge family (*Euphorbiaceae*) from the South-American origin. Its sweet crunchy underground tuber is a popular edible root since centuries in many parts of Africa, Asia and South America[1]. Cultivation of manioc is identified to be 24,200 hectares and whole production of the country is 307,950 MT in growing seasons of 2013 *Yala* and 2013/2014 *Maha*[2]. It was reported that only 2732 MT of this produce was exported to generate income to the country[3]. Typical mature roots have an average composition of 60 – 70% water, 30 – 35% carbohydrate, 1 – 2% fat, 1 – 2% fiber and 1 – 2% protein, with trace quantities of vitamins and minerals[4]. Cassava is very low in fat and protein than in cereals and pulses. Like in other roots and tubers, cassava is also free from gluten.

Glucose Syrup is a viscous liquid of starch hydrosylate of mono, di and higher polysaccharides. It meets the demand of sweetening mixtures and brings additional functionality (thickener and as humectants) to many sectors such as beverage, confectionery, dairy and many others. Further it contributes to the texture, colour stability and flavour of the final product while adding economical advantage also[5]. Mainly it is imported from other countries and the annual expenditure spent for the importation is about 450 million Sri Lankan rupees[3].

Although corn flour is widely used to produce glucose product in Western and European countries some of the Asian and African countries have tried it with cassava flour and rice flour and obtained successful results[6].

The process of production of glucose syrup is mainly involved in two steps i.e liquefaction and saccharification which breaks down the starch into dextrins in a first step and followed by simple sugars in a second step. In the present study, starch hydrolysis was carried out using enzymatic hydrolysis method since they are the ideal catalysts for the food industry in process development. There are many advantages occurred when using enzymes in a process optimization step such as specific action, ability to work under mild conditions, increment of reaction rate, operation without contamination by microorganisms and high purity of end product. The main enzymes which involved in this process are thermo stable α- amylase and glucoamylase respectively. The α- amylase (E.C 3.2.1.1) hydrolyzes α (1→4) linkages of starch components and related carbohydrates by

their nature of an endo type action. Thermostable α-amylases originated from *Bacillus* species of *B. amyloliquefaciens, B. licheniformis, B. streothermophilus* and *B. subtilis*[8,9]. They are very important enzymes for industrial uses and hydrolysis products are mainly maltose and maltotriose. The enzyme has the optimum pH range between 6.0 to 7.0 and capable of amylolytic action above the gelatinization temperature of starch that is above 70^{o}C[10]. At liquefaction step, gelatinization is required to destroy the starch granule and so as to make starch easily breakable by the amylolytic enzymes[11]. Gelatinization is achieved by heating starch with water at very high temperatures.

The glucoamylase is also known as amyloglucosidase or β-amylase and named as E.C 3.2.1.3 of the international enzyme nomenclature. It is in nature of exoenzyme type which has an ability to hydrolyze both α (1→4) and α (1→6) linkages in starch by removing glucose units. Therefore, it referred as a saccharifying enzyme in the food industry and in the production of sugar syrup which is converted to dextrose and low molecular weight polysaccharides. They are produced from microorganisams such as *Aspergillus, Penicillium* and *Rhizopus*. Optimum operating pH has been found that 4.5.and temperature as 55-60^{o}C[12]. End of saccharification step, the mixture of carbohydrates of oligosaccharides, maltodextrins and low molecular sugars such as glucose, maltose were resulted.

The production of glucose syrup by enzymatic method is recommended, as it is an advanced technique in process development with higher yields, wide range of products, higher product quality and saving energy[13].

Thus, the objective of the present study is to optimize the enzymatic liquefaction time and saccharification time to obtain potentially high yield of glucose syrups with suitable Dextrose Equivalent values in relatively short enzymatic reaction time using commercially grown Sri Lankan cassava variety (MU-51).

Materials and methods

Materials: Commercially grown cassava variety of MU-51 was obtained from Root and Tuber Crop Division, Horticulture Crop Research and Development Institute, Gannoruwa, Sri Lanka.

Starch hydrolysis enzymes of Liquozyme® SC (thermostable alpha amylase E.C. No. 232-565-6) and Saczyme® (glucoamylase E.C. No. 232-877-2) were purchased from Novozymes A/S, Denmark.

Activated Carbon granules (Grade CTC 70) and mesh size 4x8 were used for removing color pigments in the sugar solution during laboratory scale trials.

Methods: Composition of Cassava tubers: Proximate composition analysis of edible portion of Cassava tuber was performed according to the methods specified in Association of Official Analytical Chemists[14].

Laboratory scale trial for the production of glucose syrup: Extraction of starch: Schematic diagram for laboratory scale extraction of starch from Cassava tubers was given in Figure-1.

Cassava tubers were washed, cut in to pieces, peeled and sliced using hand slicer. Cassava slices were ground using wet grinder and passed through 750 µm and 250 µm sieves respectively. To the resultant slurry 1% sodium metabisulphite was added and allowed to stand for 3 h to settle starch by gravity separation. Starch was separated by decanting the supernatant and stored at 4^{o}C until used.

Optimization of enzymatic liquefaction time: Schematic diagram for the laboratory scale enzymatic hydrolysis of starch was given in Figure-2.

A slurry concentration of 10% (w/v, dry basis) was prepared and heated to gelatinize. As gelatinization started, 0.03% of industrial grade alpha-amylase (w/w, dry basis, Novozymes) was added and heating was continued. Once the temperature reached to 80 – 82^{o}C which is the optimal temperature for liquefaction, aliquots were sequentially drawn from the slurry at 15 min intervals (0, 15, 30, 45, 60, 75 and 90 min) and ^{o}Brix values were measured. Reducing sugar contents were also measured using 3,5-di-nitro-salicylic acid (DNS) colorimetric method[15,16] and DE values were calculated according to following equation.

$$DE = \frac{\text{Reducing sugars expressed as glucose (g)}}{\text{Total soluble solids (g)}} \; x\; 100 \qquad (1)$$

Optimization of enzymatic saccharification time: Liquefied slurry obtained from previously optimized liquefaction time was cooled to room temperature, pH was adjusted to 4.5 and incubated with 0.07% industrial grade glucoamylase (w/w, dry basis, Novozymes) for 15, 30, 45, 60, 75 and 90 min. After each incubation time saccharified solution was heated to 80^{o}C for 5 min to inactivate the enzyme. Solution was cooled to room temperature, centrifuged at 10,000 rpm for 20 min and decanted. Resultant supernatant was clarified by passing through an activated charcoal column (Grade CTC 70) and filtered through filter paper (Whatmann No. 4). After clarification, solution was evaporated to over 70 ^{o}Brix in a rotary evaporator and DE of final syrup was calculated (Equation-1).

Analysis of simple sugars in glucose syrup samples (Liquid Chromatographic Method): Analysis was carried out with modifications to the AOAC official method 977.20[14].

2.0000 g of syrup sample was weighed into a 100 mL beaker and transferred to 50 mL volumetric flask with 25 mL of H_2O. Then it was immediately diluted to volume with CH_3CN and filtered through syringe filter (clarification kit – Syringe filter 0.25mm, 0.22 µm PVDF) into a 1.5 mL vials. Sample was injected (10 µL) to the liquid chromatograph (Equipped with

quaternary gradient solvent delivery system, autosampler, Refractive Index detector, thermo stated column compartment and recording/ computing integrator) and separation of sugars was done by specific carbohydrate column (250mm × 4.6mm, Agilent ODC carbohydrate column with particle size 5µ, Agilent, USA) using mobile phase – LC grade acetonitrile diluted with H_20 (78+22).

The concentrations of simple sugars were obtained by comparing peak area of samples with reference standards (Fructose, Glucose and Maltose).

Operating Conditions of liquid chromatograph: Flow rate – 1.2 ml/ min (62 bars), column temperature – 30.00°C, Refractive Index temperature – 30.00°C.

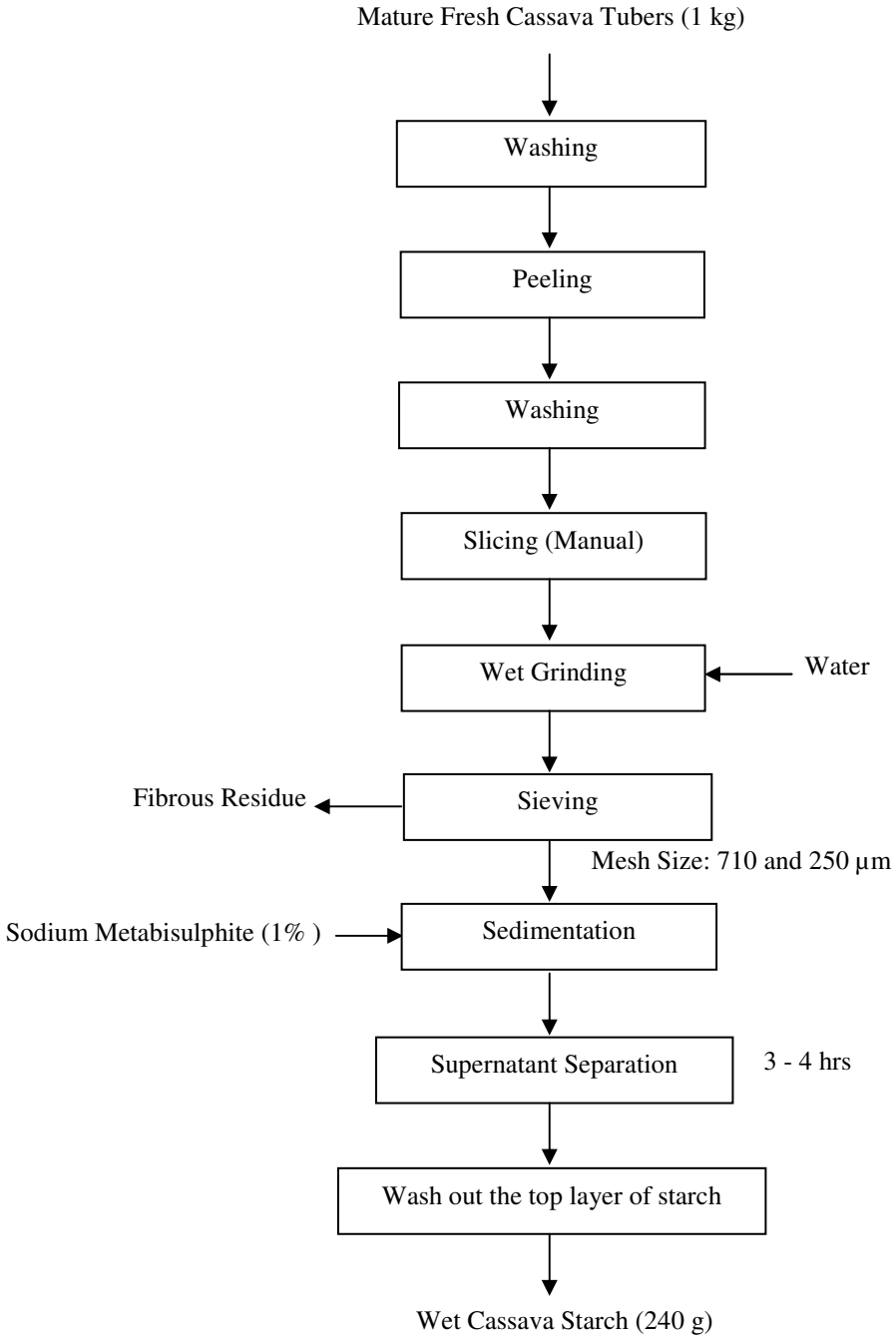

Figure-1: Laboratory scale extraction of starch from Cassava tubers.

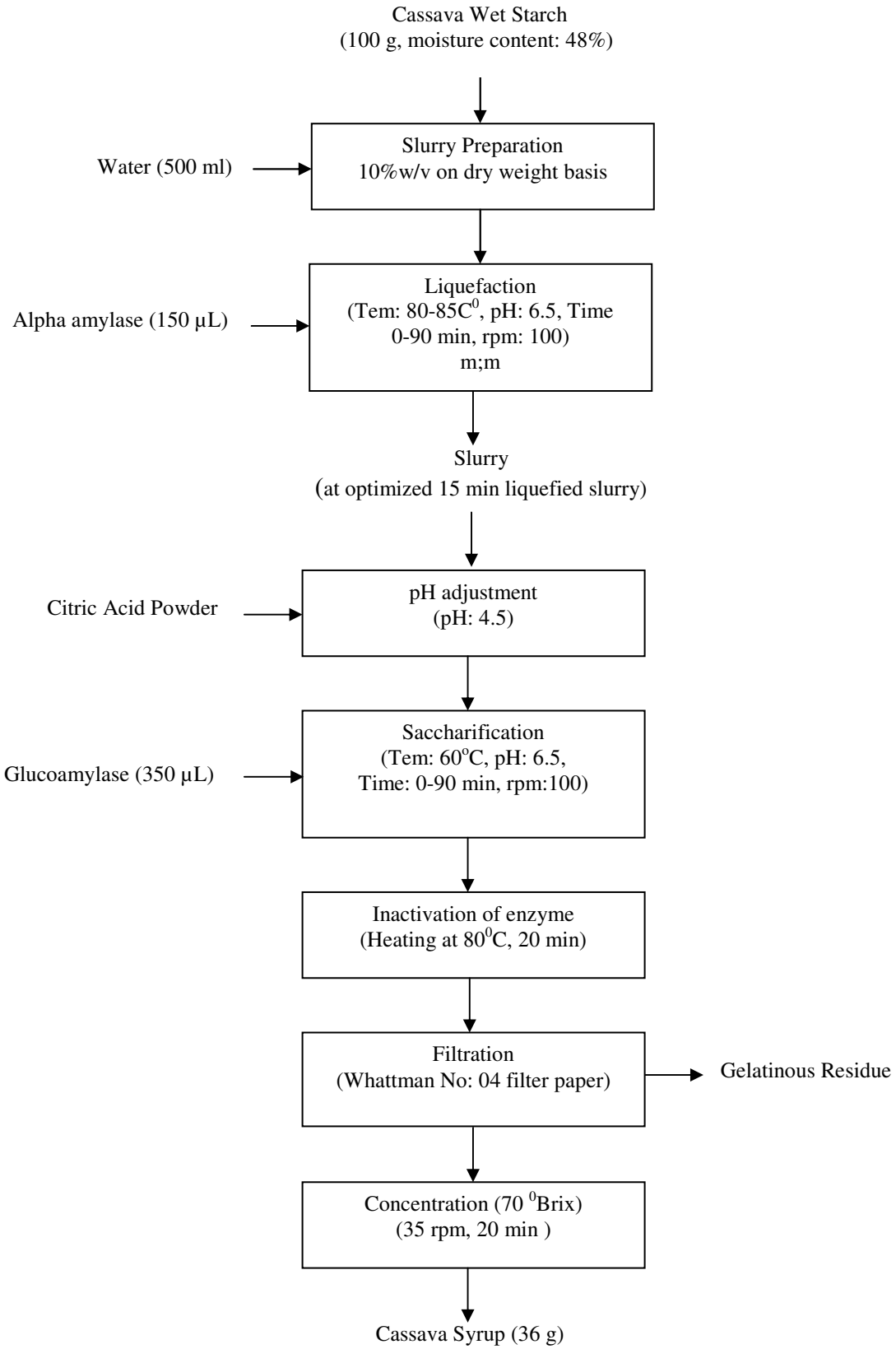

Figure-2: Laboratory scale production of syrup from cassava starch.

Pilot scale trial for the production of glucose syrup: Extraction of starch: Schematic diagram for pilot scale extraction of starch from Cassava tubers was given in Figure-3.

100 kg of fully matured raw Cassava (MU 51) was collected from Department of Agriculture, Gannoruwa, Sri Lanka. Tubers were washed thoroughly and peeled off manually. Peeled off tubers were washed using chlorinated water and they were sliced using a slicing machine to cut into slices with thickness of 2 mm to 3 mm. The pieces were ground using a wet grinder (SAWA BOY, JAPAN). The starch slurry was sieved using a rotating sieve (mesh size 0.5mm) to remove fibrous and extraneous materials. Then the slurry was passed through a 250 µ sieve to separate out the finest portion of the wet starch. The separated starch was decanted and was stored at 4^{o}C until further processing.

Syrup production: Schematic diagram for the laboratory scale enzymatic hydrolysis of starch was given in Figure-4.

Cassava slurry of 70 kg with a solid concentration of 10% (w/v) was used for the trial. Slurry was heated in a jacketed pan (Ca; 80 L) while agitating at a speed of 75 rpm using 1.1 bar saturated steam pressure. Pre-determined optimized amount of alpha amylase was added (1.5 ml) at 50^{o}C and continued stirring and heating for 15 minutes maintaining the slurry temperature at 85^{o}C. The slurry was cooled to 35^{o}C by supplying water to the jacketed pan. pH of the slurry was adjusted to 4.5 using Citric acid. Similarly pre-determined optimized amount of glucoamylase (3.6 ml) was added and the slurry was heated up to 60^{o}C. The temperature achieved was maintained for 1 hour and 30 minutes. The slurry was cooled and allowed for several hours to settle down the part of the suspended materials to the bottom. Then the slurry was filtered using a filter press (plate and frame) until get reached to the desired clarity. Then the clear solution was concentrated in a rotary vacuum evaporator (Ca; 30 L) at 60^{o}C and 25 rpm until the concentrated solution reaches to 78 ^{o}Brix.

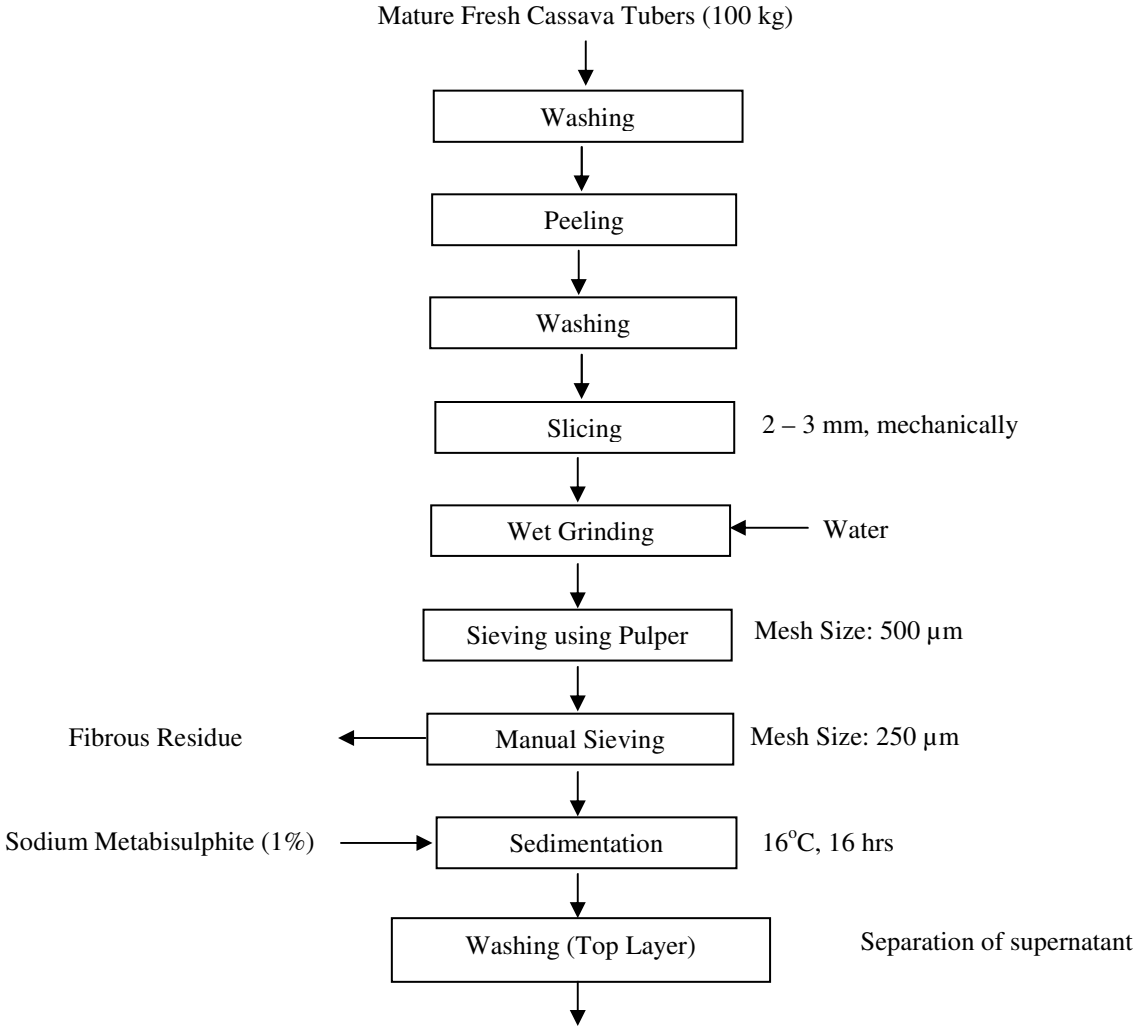

Figure-3: Pilot scale starch extraction from Cassava.

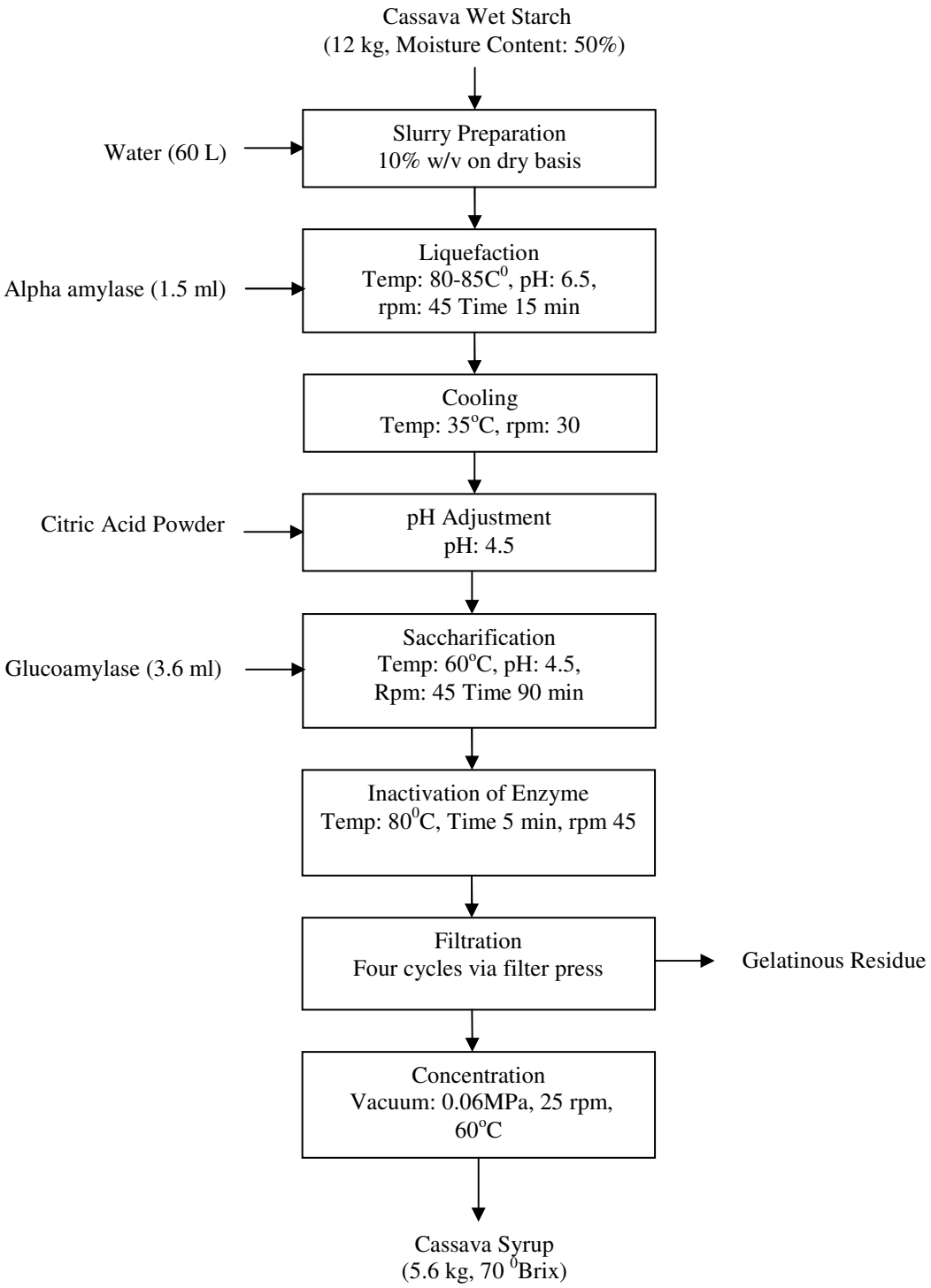

Figure-4: Pilot scale production of syrup using cassava starch.

Results and discussion

According to the results from the present study (Table-1), the percentage edible portion of Cassava tubers obtained in the laboratory scale trial was 75.2% (w/w) on wet basis while on dry basis was 29.9% (w/w). Similarly the percentage edible portion of Cassava tubers obtained in the pilot scale trial was 71.9% (w/w) on wet basis while on dry basis was 27.2% (w/w) showing slightly higher yields in laboratory scale. Non-edible outer most layers of the tubers were removed by peeing off.

Table-1: Comparison of yield in edible portion and extracted starch from Cassava MU-51.

Material	Edible Portion			Extracted Starch		
Parameters	% Moisture	% Yield* (wet basis)	% Yield* (dry basis)	% Moisture	% Yield** (wet basis)	% Yield** (dry basis)
Laboratory scale trial	61.5±0.31	75.2±0.9	29.9±1.1	47.9±0.4	23.9±1.5	16.2±1.2
Pilot scale trial	62.1±0.81	71.9±2.4	27.2±2.5	49.8±0.6	39.5±4.1	20.1±3.0

*Yield is expressed to the raw Cassava tubers (% w/w), ** Yield is expressed to the edible portion (% w/w), Values were given in Mean ±Standard Deviation of two trials.

The carbohydrate content of the edible portion estimated was 34.18% (w/w) on wet basis (Table-2). The extractable starch yield obtained in laboratory scale trial was 16.2% (w/w) on dry basis while in pilot scale was 20.1% (w/w) on dry basis. It is seen that the higher yield was obtained for extracted starch in pilot scale trial than the laboratory scale. It is mainly due to the industrial methods are capable of extracting starch more effectively with modern type of efficient equipments than the simple physical laboratory methods. Further amount of starch extracted depends basically on the method used, the harvesting time of the tuber and time taken to reach the processing from the harvesting.

Table-2: Proximate composition of edible portion and extracted starch from Cassava variety MU-51.

Nutrient content	Edible portion	Extracted starch
Moisture % (w/w)	61.53±0.31	50.4±0.6
Protein % (w/w)	1.16±0.08	0.4±0.0
Fat % (w/w)	0.81±0.18	0.2±0.0
Crude fibre % (w/w)	1.27±0.03	0.3±0.1
Ash % (w/w)	1.04±0.04	0.3±0.0
Carbohydrates % (w/w)	34.18	48.4

Results were expressed in Mean ± Standard Deviation of two independent readings on wet basis, Carbohydrate content was determined by difference method (calculation).

The extracted starch contained fewer amounts of fibre, fat and protein as shown in Table-2. Quality of starch is a very important factor for the final quality of the product. Starch with low protein contents ideally less than 0.3 % is recommended for syrup production[6]. In the present study, the protein content of starch was 0.4 % (w/w).

Enzymatic liquefaction: In the liquefaction step, starch granules are dispersed and gelatinized in the aqueous solution and then partially hydrolyzed by thermostable alpha- amylase. Break down of long chain molecules of starch into the simple sugars was begun at that step and it will continuously effect to the DE and ^{o}Brix value of the medium. It was seen that the amount of reducing sugars formed in the liquefaction step is increasing with the time (Table-3). It was well established that the liquefied starch at 8-12 DE is suitable for saccharification to produce syrups with DE values from 45 to 98 or more[6,17].

Table-3: Changes in total reducing sugar contents, ^{o}Brix and DE values during enzymatic liquefaction of cassava starch under constant alpha-amylase concentration (0.03 % w/w, dry basis)

Liquefaction Time/min	Reducing sugars (g/100ml)	^{o}Brix value	DE value
0	0.05±0.00	6.5	0.8±0.0
15	1.34±0.02	9.2	14.5±0.2
30	1.43±0.06	9.4	15.2±0.6
45	1.83±0.02	9.6	19.1±0.3
60	2.14±0.14	9.8	21.9±1.4
75	2.26±0.16	10.0	22.6±0.6
90	2.79±0.10	10.8	25.8±0.9

Reducing sugars and DE values were expressed as Mean ± Standard Deviation of triplicates.

The time period and α-amylase enzyme concentration will be the most important two factors which determine DE at the end of liquefaction step which required maintaining DE at 8-15. According to Table-3, ^{o}Brix and DE values had increased with the increasing liquefaction time under given conditions. Expected DE value of 8 -15 has been attained after 15 min of liquefaction time.

Most of the reported literature found that the liquefaction time varied from 30-90 min followed by long saccharification time ranging from 24 h to 96 h in production of Cassava syrup using several slurry concentrations (ranging from 10-30% w/v). Pontoh and Low reported that the liquefaction time for production of glucose syrup from Cassava starch at slurry concentration of 30% (w/v), the final DE value of 12-15 was attained after 30 min followed by saccharification for DE value of 97 was attained 24 h[18]. Silva *et al.* reported that the liquefaction and saccharification times for the production of

glucose syrup from Cassava at slurry concentration 35% (w/v) were 2 h and 48 h respectively with the given conditions[19]. Johnson and Padmaja reported that the liquefaction time for production of glucose syrup from Cassava starch at slurry concentration of 20% (w/v), the DE value of 15.5 was obtained after 1h and the saccharification time obtained for the DE value of 96 was after 48 h[13].

In the present study both liquefaction and saccharification times obtained for the production of glucose syrups with required DE values were in comparatively shorter than the reported previous studies.

Enzymatic Saccharification: Saccharification produced the low molecular weight sugars such as glucose, maltose or malto-dextrins from hydrolysis of liquefied fractions (oligosaccharides or dextrins).

In generally, saccharification time takes long time period (up to 72 h) depending on the slurry concentration, enzyme activity and its concentration. It could be accelerated by use of industrial type enzymes and applying high concentrations. The inventive step of this study is the bringing down of the saccharification time for intermediate DE and high DE glucose syrup to 15 min and 75 min respectively under described conditions. Since the total time spent for the production of glucose syrup in the present study is less than 2 h, it is benefitted to the cost reduction, time saving and the economical profit to the producers.

According to Table-4, reducing sugar contents and DE values had increased with increasing saccharification time under described conditions. According to consumer's requirement, glucose syrups with intermediate DE (38-58) or high DE (58-73) values can be obtained using the above mentioned saccharification times and conditions.

Table-4: Changes in total reducing sugar contents, ^{o}Brix values and DE values of glucose syrups produced using Cassava starch at optimized 15 min liquefied slurry followed by saccharification with glucoamylase (0.07% w/w, db) for different time periods.

Saccharification time/ min	Reducing sugars (g/100ml)	^{o}Brix value	DE value
15	26.26±0.38	72	38.6±0.6
30	27.17±0.32	71	40.6±0.5
45	33.07±0.64	70	49.4±0.9
60	37.52±0.40	70	53.6±0.6
75	43.79±0.21	73	60.0±0.3
90	46.24±0.88	70	66.1±1.1

Reducing sugars and DE values expressed as Mean ± Standard Deviation of triplicates.

It is advisable that the saccharified syrup to be filtered to remove fat and denatured protein released from the starch granules. Therefore saccharified syrup passed through the activated charcoal and ion-exchange resins for their better clarity[20].

Physico-chemical parameters of % yield, pH, DE, water activity and clarity of glucose syrup from Cassava in laboratory scale trial, pilot scale trial and the market sample were evaluated and results were presented in Table-5.

Table-5: Physico-chemical parameters of glucose syrup from Cassava MU 51 and market sample.

Parameter	Cassava syrup (90 min saccharified)	Cassava syrup pilot scale	Market glucose syrup
% Yield (w/w, to wet starch)	36%	47%	NA
Water activity	0.774 ± 0.004	0.755 ± 0.006	0.690 ± 0.004
pH	4.5 ± 0.0	6.0 ±0.0	4.5 ± 0.1
^{o}Brix	75	78	76
DE value	66.1 ± 1.1	88.1±1.6	42.9 ± 0.2
Clarity	Transparent white	Transparent light yellowish	Transparent light yellowish

Values were represented in Mean ± Standard Deviation in duplicates, NA- Not available.

According to the Table-5, it is seen that the % yield (w/w, to wet starch) of glucose syrup obtained from pilot scale trial (47 %) was significantly higher than that of laboratory scale trial (36%). Present observation can be depicted from the machinery efficiency by extracting higher yield of starch in the pilot scale trial. Similar values for the water activity were shown in both laboratory and pilot scale trials (i. e. ~ 0.7). ^{o}Brix value and DE were shown to be higher values in Cassava syrup prepared from pilot scale trial than laboratory trial due to the high rate of degradation of starch polymer when using mechanical agitation in jacketed pan. Colour and clarity of the Cassava syrup can be ranged white to light yellowish and transparent respectively.

Cassava syrup obtained after saccharification was characterized by HPLC chromatography in order to obtain the sugar profile (Table-6).

The relatively high values for reducing sugars of glucose and maltose were obtained from the pilot scale trial than laboratory scale trial. Fructose and sucrose were not found in both trials. Results could be depicted by the high rate of continuous mechanical agitation in pilot scale trial which may caused to

degrade the starch polymer chains resulting to release of more simple sugars. Those break down of resultant reducing sugars caused to increase DE and ^{o}Brix value in the medium. Market glucose sample was shown lower concentrations of reducing sugars and low DE value than the pilot scale.

Table-6: Composition of simple sugars in glucose syrup from Cassava and market sample.

Glucose syrup	Glucose (%)	Maltose (%)	Fructose (%)	*Higher sugars (%)
Cassava syrup (laboratory scale)	35.8±1.2	11.5±0.9	ND	52.7
Cassava syrup (pilot scale)	39.5±0.5	11.6±0.2	ND	48.9
Market glucose syrup	15.3±0.8	11.3±0.8	ND	73.4

Values were represented in Mean ± SD in triplicates, ND –Not Detected, *Higher sugar percentages were calculated by difference method.

Conclusion

Present study showed that the % yield (w/w, to wet starch) obtained for the pilot scale trial (47 %) was higher than the laboratory scale trial (36%). The optimum liquefaction time was 15 min under the given conditions. Minimum saccharification times spent to obtain glucose syrups with intermediate and high DE values were 15 min and 75 min respectively under the given conditions. In the present study the total enzymatic reaction time spent for laboratory scale and the pilot scale production of glucose syrup was reduced to less than 2 h. Therefore in the present study, the combination of time and enzyme concentrations will introduce an effective process method for the industry to produce glucose syrup in Sri Lanka.

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