Studies on Utilization of Residue from Tapioca Starch processing Industry

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A number fo industrial units on large scale as well as on a small scale manufacture of starch and sago from tapioca tubers and chips are there in our country. In the process of manufacture, about 20 percent of waste fibrous residue is obtained which is not being utilised efficiently though it contains a high percentage of starch. The paper presents details of its chemical composition and the results of studies undertaken for its utilization to obtain glucose syrup. Analysis of this byproduct indicated a starch content of 60-65 percent. Results of hydrolysis of the material by acid, acid-enzyme or enzyme process indicated a conversion of 92-95 percent. The hydrolysate was found to contain other sugars in addition to glucose. Data are presented on the optimisation of certain parameters for the above two processes. The possibilities of utilizing the hydrolysate for the production of alcohol by fermentation was also explored. The fibrous residue was found to contain 75-90 ppm of hydrocynic acid on dry weight basis. About 0.5 and 1.0 percent of the hydrocynic acid present in the material was found to remain in the hydrolysates prepared by acid-enzyme and enzyme-enzyme process respectively.

Cassava (Manihot esculenta Crantz), popularly known as tapioca, is one of the major tuber crops of the world, being cultivated extensively in tropical countries and perhaps, provides a major source of calories to about 300 million people in the world[1]. Though a native of Brazil, it is produced in about 80 countries on the total world area of a magnitude of about 9.4 mha[2]. The world production in 1973 was over 90 million tons and was worth about U.S.$70 milion a year[3]. It was introduced into cultivation in India before over 160 years and about 700,000 acres were under cultivation in Kerala State alone in 1970[4]. On dry weight basis cassava contains about 80-82 percent starch of which 55-60 is recoverable as starch.

In India, the most important products of cassava is industrial starch and a sizable portion of the cassava produce is utilised for the production of starch and sago by a number of industrial units both on a large and on small scale. In the process of manufacture of starch about 20 percent of waste fibrous residue is obtained which is not utilised efficiently at present though it contains over 50 percent starch. In the past, the largescale units were drying this material for selling as a cattle feed. However, due to the increase in fuel costs the drying has been discontinued and its disposal is posing serious problems. Some of the small scale units sun dry the fibrous waste and dispose it as cattle feed.

Work was therefore undertaken to study the possibilities of utilising the fibrous waste for obtaining glucose syrup by hydrolysing starch present in it with acid, acid-enzyme and enzyme-enzyme processes and subsequent conversion to valuable fermentation products. The present communication describes its chemical composition, its hydrolysis to glucose and data on the utilization of the hydrolysate for the production of alcohol.

Experiments and Discussion

Chemical composition : Samples of the sundried fibrous residue were obtained from a largescale unit as well as from a small scale industry and were analysed for their major chemical constituents by standard methods of analysis[5] Hydrocyanic acid content was estimated by acid-hydrolysis method[6,7,8]. For estimation of pentosans, the powdered sample was treated overnight with 0.2N sodium hydroxide solution and the filtrate was used for determination of pentoses by Dische and Borenfreunds' method [9].

Acid hydrolysis of the fibrous residue : A slurry of the residue was made with acidified tap water at pH 1.8 and was autoclaved at 15 psi for 60 minutes. The reducing sugar in the hydrolysate was estimated by Shaffer and Hartman method[10]. Studies were carried out to find out suitable concentration of the fibrous residue in the slurry for effective hydrolysis as well as the effect of pH on hydrolysis.

Enzyme hydrolysis with α-amylase : A slurry of the residue with water was made and the pH was adjusted to 6 0. The slurry was kept in a water bath maintained at 60°C and experiments were conducted with different concentrations of α-amylase and a reaction time of 60 minutes. The enzyme reaction was stopped by heating to 80°C for 10 min. and the hydrolysate was analysed for the reducing sugars. In a similar set up studies were also carried out to determine the optimum contact time of the enzyme with the starchy waste.

Acid-enzyme hydrolysis : 10 percent slurry of the residue in acid solution at a pH of 1.5 was prepared and autoclaved at 15 psi for 60 minutes. After cooling and adjusting the pH to 4.2 amyloglucosidase at 0.4% (v/v) level was added and incubated at 56°C for 48 hrs. Reducing sugar was estimated in the hydrolysate.

Enzyme-enzyme hydrolysis: To a 10 percent slurry hydrolysed with α-amylase (0.4% v/v) at 60°C for 60 minutes, different concentrations of amyloglucosidase were added after adjusting the pH to 4.5. The reaction was continued for 64 hrs and reducing sugars were estimated. Under a similar set up, studies were also carried out to determine the optimum contact time of amyloglucosidase.

Paper chromatography of the hydrolysate : The hydrolysate was qualitatively analysed by paper chromatography by using various solvent system.

Alcohol production from hydrolysates: The hydrolysates both from acid-enzyme and enzyme-egzyme process were used as a substrate for alcohol production by a alcoholic strain of *Saccharomyces carevisiae*. Hydrolysates with or without concent-

ration were supplemented with minerals with the following composition : $(NH_4)_2SO_4$ (0.1%). $(NH_4)_2HPO_4$ (1%) and $MgSO_4.7H_2O$ (0.03%) and pH adjusted to 4.2 were inoculated with the vegetative cells of the yeast and incubated at room temperature (28°C) for 5 days. The alcohol formed was distilled and estimated by a standard method.

Chemicals : All the chemicals used in the studies were of laboratory grade. α amylase and amyloglucosidase were produced in the laboratory by utilising the know-how developed for these enzymes in this Laboratory. [11,12,13,14]

Chemical composition : The chemical composition of the waste from large scale unit according to the present analysis along with the data reported by Subramanyan at al[15] are given in Table 1. Except for slightly higher starch content in sample from

TABLE 1. CHEMICAL COMPOSITION OF WASTE FIBROUS RESIDUE FROM TAPIOCA STARCH PROCESSING INDUSTRY

Constituent	gm/100 gm sun-dried waste — Sample from large-scale industry	Analysis reported by Subramanian et al. [15]
Moisture	12.50	11.20
Starch	61.80	56.20
Crude fibre	12.80	10.60
Crude protein	1.50	0.85
Total ash	0.58	1.45
Free reducing sugars	0.37	1.20
Hydrocyanic acid	0.0075	—
Pentosan expressed as Xylose	1.95	—
Fat	—	0.30
Hemicellulases, Other polysaccharides, lignin etc. (by difference)	8.4925	18.20

small scale unit other constituents are more or less similar in samples both from small and large scale industries.

Acid hydrolysis of the fibrous residue : The results of the acid-hydrolysis of the waste material in the slurry at different concentrations are given in Table-2. The conversion of starch to glucose decreases as the concentration of the waste in the slurry increases. The percentage of conversion of starch was in the range of 24 to 58. Studies were also carried out on the effect of initial pH of the slurry on the saccharification and the results are presented in Table-3. The results indicated that the percentage of hydrolysis decreases with the increase in pH of the slurry. With pH 1.0, the conversion rates were 80.38 and 104.6 percent for samples from large scale and small scale units respectively. At pH 2.0 and above, the conversion was less than 1 percent.

TABLE 2: ACID HYDROLYSIS OF THE RESIDUE FROM LARGE SCALE STARCH PROCESSING UNIT

% material in the slurry		Percent starch in the slurry	% conversion to sugar	
Sun dried	Dry wt. basis		Based on starch	Based on material (dry wt.)
2.5	2.1875	1.427	58.40	38.10
5.0	4.3750	2.853	44.51	29.03
7.5	6.5625	4.280	32.99	21.52
10.0	8.7500	5.707	24.57	16.03

Slurry of the material was made with acidified tap water at pH 1.8. Autoclaved for 1 hr at 15 psi. Sugar was estimated in the filtrate.

TABLE 3 ACID HYDROLYSIS OF WASTE RESIDUE—EFFECT OF INITIAL pH OF THE SLURRY

pH of the slurry	Sample from large scale unit				Sample from small-scale unit			
	Conversion to Glucose (%)		Presence of starch in		Conversion to Glucose (%)		Presence of starch in	
	On starch basis	On material basis (dry wt.)	Residue after hydrolysis	Filtrate	On starch basis	On material basis (dry wt.)	Residue after hydrolysis	Filtrate
1.0	80.38	52.42	—	—	104.6	75.75	+	—
1.5	21.56	14.06	+	—	27.27	19.75	++	+
2.0	0.75	0.49	+++	+++	0.72	0.52	+++	+++
2.5	0.69	0.45	+++	+++	0.22	0.16	+++	+++

10% slurry of the sundried material was used. pH was adjusted by HCl. Autoclaved for 1 hr at 15 psi. Sugar was estimated in the filtrate. Presence of unhydrolysed starch was tested by iodine solution.

Enzyme-hydrolysis with ∝*-amylase :* Table 4 shows the effect of various concentration of ∝-amylase on the hydrolysis of the waste material. The conversion rate in the sample from small-scale industry increases with the increase in concentration of ∝-amylase. However, the conversion rate in sample from large scale unit remained nearly, constant at concentrations higher than 2 percent ∝-amylase (v/v) in the slurry. The data on determination of optimum contact time of the enzyme with substrate are given in table-5. An incubation time of 60 min. was optimum for the hydrolysis and any further increase in the contact time did not improve the percent conversion.

TABLE : 4 ENZYME HYOROLYSIS OF THE WASTE RESIDUE--EFFECT OF ∝ - AMYLASE CONCENTRATION

∝-amylase conc. ml/100 ml. slurry	Sample from large scale unit				Sample from small scale unit			
	Percent conversion to Glucose		Presence of starch in		Present conversion to Glucose		Presence of starch in	
	On starch basis	On material basis (dry wt.)	Residue after hydrolysis	Filtrate	On starch basic	One material basis (dry wt.)	Residue after hydrolysis	Filtrate
0.4	45.65	29.77	+++	+++	26.37	19.10	+++	+++
1.0	53.40	34.83	+++	+++	66.53	48.18	+++	+++
2.0	72.52	47.30	+++	+++	72.56	52.55	+++	+++
3.0	N. D.	N. D.	N. D.	N. D.	79.45	57.53	+++	—
4.0	68.64	44.77	+++	—	89.62	64.90	+++	—
6.0	64.00	42.00	+++	—	93.93	68.02	+++	—

10% slurry of the sun dried material (pH 6.0) was autoclaved for 1 hr. at 15 psi and incubated in a shaker water bath at 60°C for 60 min. after the addition of the enzyme. (The enzyme had an activity of 2300 units/ml). Sugar was estimated in the filtrate. Unhydrolysed starch was detected by iodine test.

N. D.—Not detected.

TABLE 5 HYDROLYSIS OF THE WASTE MATERIAL BY ∝—AMYLASE ... EFFECT OF TIME OF INCUBATION

Reaction time of ∝-amylase (min.)	Sample from large scale unit				Sample from small scale unit			
	Percent conversion to Glucose		Presence of starch in		Percent conversion to Glucose		Presence of starch in	
	On starch basis	On material basis (dry wt.)	Residue after hydrolysis	Filtrate	On starch basis	On material basis (dry wt.)	Residue after hydrolysis	Filtrate
30	18.34	11.96	+++	++	15.41	11.16	+++	++
60	36.68	23.92	+++	—	34.81	22.20	+++	—
90	15.40	10.04	+++	—	28.19	20.41	+++	—
120	25.57	16.68	+++	—	46.16	33.43	+++	—

10% slurry of the sun dried material was prepared by autoclaving for 1 hr. at 15 psi; cooled and ∝—amylase was added at 0.4% (v/v) level. Incubated at 60°C on shaker water bath for different periods. Reducing sugar was estimated in the filtrate. Presence of unhydrolysed starch was detected by iodine test.

Acid-enzyme hydrolysis: The hydrolysis of the waste by acid treatment showed 24-27 per cent conversion of starch to glucose and the subsequent reaction of the acid hydrolysate with amyloglucosidase resulted in 96-98 per cent conversion of starch to glucose (Table 6.)

TABLE 6: HYDROLYSIS OF THE WASTE BY ACID-ENZYME PROCESS

Details	Sample from large-scale unit	Sample from small scale unit
1. Weight of sundried waste material	500 gm	500 gm
2. Percent moisture in the waste	12.5	13.0
3. Dry weight of the waste	437.5 gm	435.0 gm
4. Volume of acidified water	5000 ml	5000 ml
5. Weight of starch in the slurry (dry wt.)	285.34 gm	315.00 gm
6. pH of the slurry	1.4	1.4
7. Starch conversion to glucose after acid hydrolysis		
i) based on sundried waste	13.75%	17.19%
ii) based on waste material (dry wt.) basis	15.72%	19.75%
iii) based on starch wt.	24.10%	27.28%
8. Starch conversion to glucose after further hydrolysis by amyloglucosidase.		
i) based on sundried waste material	55.06%	62.29%
ii) based on waste material (dry wt. basis)	62.93%	71.59%
iii) based on starch wt.	96.49%	98.87%

The slurry in acidified water was taken in a bio reactor vessel and was autoclaved for 1 hr. at 15 psi. Cooled and pH was adjusted to 4.2 and amyloglucosidase (activity 9100 units/ml) was added at 0.4% (v/v) level and was incubated at 56°C for 2 days stirring at 230 rpm. Reducing sugar was estimated in the filtrate.

Enzyme-enzyme hydrolysis: The samples hydrolysed with α-amylase were subsequently treated with different concentrations of amyloglucosidase and the data obtained are presented in Table 7. With samples from large and small scale industries the rate of conversion increases with the increase in enzyme level. The studies on the contact time of amyloglucosidase indicated an optimum contact time of 24 hrs (Table 8). Further increase in incubation time did not improve the conversion rate.

TABLE 7 ENZYME-ENZYME HYDROLYSIS OF THE WASTE MATERIAL-EFFECT OF AMYLOGLUCOSIDASE CONCENTRATION

Amyloglucosidase concentration ml/100 mg slurry	Sample from large scale unit		Sample from small scale unit	
	Percent conversion to Glucose			
	On starch basis	On material basis (dry wt.)	On starch basis	On material basis (dry wt.)
0.4	N. D.	N. D.	66.29	47.98
1.0	80.80	52.70	69.35	50.22
2.0	98.53	64.26	81.47	58.99
4.0	107.71	70.25	83.89	60.75
6.0	N. D.	N. D.	100.67	72.90

10% slurry of sundried material (pH 6.0) was autoclaved for 1 hr. at 15 psi and treated with α-amylase (0.4% v/v) for 60 min. at 60°C. The pH was adjusted to 4.5 and amyloglucosidase was added and incubated for 24 hrs. at 50°C. Sugar was estimated in the filtrate.

TABLE 8. ENZYME—ENZYME HYDROLYSIS OF WASTE MATERIAL EFFECT OF TIME OF REACTION WITH AMYLOGLUCOSIDASE

Reaction time with amyloglucosidase (hr.)	Sample from large scale unit		Sample from small scale unit	
	Percent conversion to Glucose			
	On starch basis	On material basis (dry wt.)	On starch basis	On material basis (dry wt.)
24	90.39	58.95	83.35	60.36
48	86.00	56 09	88.62	64.17
65	88.73	57.87	85.01	61.56
72	85.84	55.99	89.24	64.62

10% slurry of the sundried material (pH 6.0) was autoclaved for 1 hr. at 15 psi, cooled and treated with α—amylase (0.4% v/v) for 60' min. at 60°C. Then pH adjusted to 4.5 and 0.4% (v/v) amyloglucosidase was added and incubated at 50°C for different duration.

Hydrocyanic acid levels: The hydrocyanic acid content in the fibrous waste as well as in the hydrolysates from acid-enzyme and enzyme-enzyme process are given in Table 9. Though the levels are comparitively high in the fibrous residue the levels were reduced to less than 1 ppm in the hydrolysates.

TABLE 9. HYDROCYANIC ACID IN THE WASTE MATERIALS AND THEIR PRODUCTS

Material and its products	Hydrocyanic acid (ppm) Sample from large scale unit	Sample from small scale unit
Waste material (sun dried)	75.0	87.5
Waste material (dry wt. basis)	85.7	100.6
Hydrolysate from acid–enzyme process	0.625	0.349
Hydrolysate from enzyme—enzyme process	0.894	0.675

Alcohol production : In Table 10 are presented the data on the production of alcohol from the hydrolysates of the waste fibrous residue. The original hydrolysates from acid-enzyme and enzyme-enzyme process having a concentration of 6.9 and 6.7° Brix respectively showed an alcohol production of 32.54 and 39.48 (w/w) whereas concentrated hydrolysate helped in an higher yield of alcohol i.e., 37.93 and 41.26 (w/w) respectively as compared to a theoritical yield of 51. 1. (w/w).

TABLE 10. PRODUCTION OF ALCOHOL FROM HYDROLYSATES OF FIBROUS RESIDUE FROM STARCH PROCESSING INDUSTRY

Hydrolysate from	Sugar conc. used Brix	Percent alcohol produced W/W	V/W
Acid-enzyme process	6.9	32.54	35.07
	20.00	37.93	46.83
Enzyme-enzyme process	6.7	39.48	43.28
	20.0	41.26	51.18

Original as well as concentrated (to 20° Brix) hydrolysates were fortified with $(NH_4)_2So_4$ (0.1%), $(NH_4)_2HPO_4$ (0.1%) and $MgSO_4.7H_2O$ (0.03%) and pH adjusted to 4.2 were fermented with an alcohol strain of *Saccharomyces cerevisiae* for 5 days. Alcohol was distilled and estimated.

In the process for manufacture of starch and sago from cassava, three types of wastes are obtained. These include outer skin which essentially is a dry waste product and is resistant to biodegradation due to its low organic matter content. This waste is economically insignificant and is used for land fill[16]. Before the peeled roots are disintegrated with a help of the rasper in a stream of water, the inner rinds

are removed which constitute about 10-15% value of the tubers and contain about 10-12 percent starch. Balagopal and Maini [17] investigated the production of protein from the rinds by using amylotic fungi and reported that the protein production was directly proportional to the starch concentration.

The major waste that is formed in manufacture of starch and sago from cassave is the fibrous residue retained on different mesh sieves which also referred as tapioca spent pulp [18], tapioca refuse [19] in the literature. It contains high concentration of starch which is presumably present in intact cells not ruptured during the rasping process[15] and thus causes heavy pollution of the environment [20-22]. Treatment of the waste before discharge in the nature was studied[15], though ideal solution would be either effective economic utilization or by-product recovery to off set costs involved in waste treatment. Reports on the utilization of fibrous waste as poultry feed[18], fertilizer[19], substrate for microbial protein production [16], and in the manufacture of alcohol [23,24] or recovery of starch[15] are known but are characterized by high transportation costs[19] or low value product [16]. The possibility of hydrolysing the starch of the waste to glucose syrup by saccharification have not received due attention and may offer definite economic advantages to the starch industry and better return to the agriculturists.

The presence of toxic factor in cassava was known as early as 1605 [25] and was attributed to the presence of hydrocyanic acid (HCN) in 1836 [26]. It occurs in the form of cyanogenic glycosides. Linamarin [27-30] and methyllinamarin [31-33] which liberates free HCN under enzymatic hydrolysis with linamarase [34-36] or acid hydrolysis. In active healthy tissue of the growing plant, the enzyme and substrate are kept apart but their contact occurs upon mechanical damage of the tissue, loss of physiological integrity, post-harvest deterioration or wilting of the leaves. The normal range of cyanide content is 15-400 ppm calculated as mg HCN/Kg fresh weight but occasional samples as low as 10 mg/Kg or over 2000 mg/Kg have been reported [37].

Cyanogenic glucosides of cassava are known to be responsible for both acute and chronic toxicity in humans and animals. High cassava intakes are associated with the incidence of tropical ataxic neuropathy and disturbances in iodine metabolism. In the presence of marginal iodine and low protein intake, it may lead to the development of goitre and cretinism[1]. Indian Standards Institution has set a limit as high as 300 mg HCN/Kg in dried cassava products meant for animal feeding [38,39]. The low HCN content of the hydrolysates obtained either by acid, acid-enzyme and enzyme-enzyme processes, does not pose any problem for its utilisation.

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