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SHORT COMMUNICATION

EFFECT OF ENZYMES ON HYDROLYSIS AND FILTERABILITY OF CORN FLOUR

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Abstract: The optimal alpha amylase concentration for liquefaction was found to be 0.2% (V/V) when 40% (W/W) corn flour was treated with thermostable alpha amylase at pH 7.0 and at 90-95^{0}C for 1h. The dextrose equivalent (DE) was improved when liquefaction was followed by saccharification with glucoamylase at pH 4.5 and 40^{0}C for 24h. When saccharification by glucoamylase is supplemented with commercially available enzymes like cellulases, proteases and pectinases the filtration rate was not improved. The rate of filtration was improved by treatment of the hydrolysate with activated charcoal. The application of these β-glucanases and proteases reduced the dry weight of the undigested solid matter.

Starch from cassava, corn, potato, sweet potato, rice and wheat is widely used as a raw material in the production of sugars and syrups. From the practical point of view a concentration of starch of atleast 30-40% (dry weight) is desirable for high glucose production.

In our present work, corn was selected as the source of starch on account of its availability in Sri Lanka. Its annual production is 50,000 metric tons.[2] The starch content of corn is 72-75%.[3] Liquid glucose is widely used in bakeries and confectionaries. Sri Lanka imports liquid glucose worth SLR 20 million annually. The conversion of corn to liquid glucose would add value to the primary agricultural products of Sri Lanka.

The aim of this study was to determine the optimum conditions for liquefaction of starch in corn flour using alpha amylase. The effect of commercially available cellulases, proteases, pectinase and activated charcoal on the rate of filtration of the saccharified corn flour was also investigated.

Enzymatic Hydrolysis of Corn Flour

Locally purchased corn grains were powdered in a mill and sieved. Termamyl (NOVO), amyloglucosidase (Sigma), Cereflo (NOVO), celluclast (NOVO), Polygalacturonase, Neutrase (NOVO), Pepsin (Sigma) and trypsin (Sigma) were used in this investigation. Activated charcoal decolourizing powder (BDH 33032) was also used.

Hydrolysis of Corn Flour

Corn flour (40% W/W) suspension in water was liquefied by termamyl at 90-95^{0}C and pH 7.0. During this process, the residual starch was tested with I_2.[5] In order to increase the hydrolysis and rate of filtration the liquefied slurry was treated with the combination of enzymes shown in Table 1 and incubated for 24h at 40^{0}C.

Fillerability

The saccharified slurry was diluted (1:1) and filtered through a Whatman No.7 filter paper under vaccum while it was hot. The residue in the filter paper was dried at 80-90^{0}C in an oven to a constant weight. The rate of filtration, turbidity of filtrate and the dry weight of the residue were noted and given in Table 1. The amino acid content of the filtrate was also assayed.[4]

Reducing sugar obtained at the end of liquefaction and saccharification was measured by DNS.[1] The corn flour was fully hydrolysed by 1N HCL and the total reducing sugar was also measured. Dextrose equivalent (DE) was expressed as percentage of reducing sugar obtained / total reducing sugar obtained after acid hydrolysis. This process involved the incorporation of an alpha amylase to corn flour for liquefaction followed by a mixture of enzymes (Table 1) into the mixer under the experimental conditions for hydrolysis.

Liquefaction of 40% (W/W) corn flour was carried out with different concentration of alpha amylase at 90-95^{0}C for 3h (Figure 1). The starch content during the liquefaction process was tested with iodine. When alpha amylase concentration was 0.1%, 0.2% and 0.3% (V/V) the reducing sugar obtained in 1h of liquefaction was 32%, 40% and 40% respectively (Figure 1). These results indicate that the dextrose yield was not increased when the alpha amylase concentration was increased from 0.2 to 0.3%. When the alpha amylase concentration was 0.1%, the starch was present even after 3h of liquefaction. From these results, the optimal alpha amylase (Termamyl) concentration for liquefaction was 0.2% (V/V) when 40% (W/W) corn flour suspension (pH 7) was used at 90-95^{0}C for 1h.

The liquefied corn flour was highly viscous and filtration was rather slow. To avoid the filtration problem the undigested solid matter was removed by centrifugation (at 3000rpm, for 15 min). The residue was washed with distilled water and dried at 80^{0}C.

The content of dry matter obtained by this method was 28.6% W/W (Table 1). The supernatant was highly turbid and this could be due to proteins and glycans other than starch.

Hence the liquefied corn flour was treated with different combinations of enzymes (Table 1) like cellulases, proteases and pectinase with and without glucoamylase for 24h at 40^{0}C and at two different pH values 4.5 and 7 respectively. The filterability, turbidity of the filtrate, aminoacid content of the filtrate and dry

Table 1: Effect of glucanases and proteases on the separation of solid materials of the liquiefied corn flour.

Expt. No.	Enzymes used ml (% V/V)	pH	Residual dry matter (%)	Concentration of aminoacid in filtrate (mg/dl)	Dextrose equivalent (DE)	Filterability (ml/min)	Turbidity of the filtrate (700nm)
1	Termamyl (0.2)	7.0	28.6	not measured	54.5	cannot be filtered	not measured
2	Termamyl (0.2) glucoamylase (80 mg)	7.0 4.5	20.0	83	87.0	18	0.90
3	Termamyl (0.2) Glucoamylase (80 mg) Cereflo (0.2) Celluclast (0.2) Pectinase (0.2) Neutrase (0.2) Pepsin (80 mg) Trypsin (80 mg)	7.0 4.5	16.0	144	92.5	20	0.80
4	Termayl (0.2) Glucoamylase (80 mg) Cereflo (0.5) Celluclast (0.5) Pectinase (0.5) Neutrase (0.5) Pepsin (80 mg) Trypsin (80 mg)	7.0 4.5	15.8	140	93.0	21	0.56

Table 1 contd.

Expt. No.	Enzymes used ml (% V/V)	pH	Residual dry matter (%)	Concentration of aminoacid in filtrate (mg/dl)	Dextrose equivalent (DE)	Filterability (ml/min)	Turbidity of the filtrate (700nm)
5	Expt. 4 was repeated without gluco-amylase	4.5	18	120	64	9.0	0.68
6	Expt. 3 was repeated without glucoamylase	7.0 7.0	22	96	53	7.0	1.5
7	Expt. 2 was repeated using charcoal*	7.0 4.5	--	10	87.5	63	0.13

*Charcoal (5%) was used for filtration.

Note: Liquefied corn flour (40 g) by 0.2% Termamyl (pH 7) was treated (110 rev/min) with the enzyme mixture at 40^{0}C and pH 4.5 for 24h (In Expt. 6; pH was 7.0). DE is Dextrose from enzymic hydrolysis/Total sugar releases by acid hydrolysis x 100.

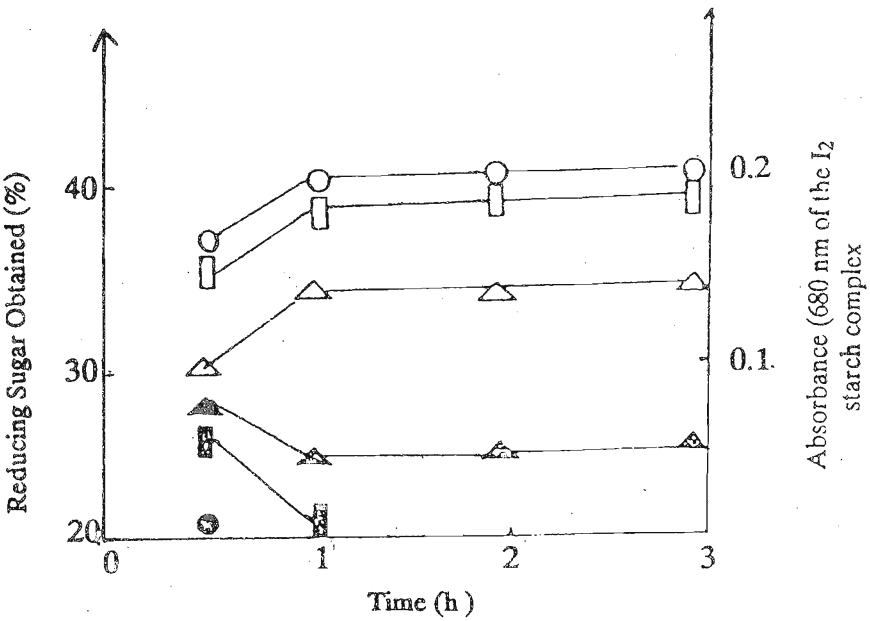

Figure 1: **Change of reducing sugar and residual starch content during the liquefaction of corn (40% W/W) at different concentration of Termamyl. The residual starch I_2 complex absorbance was measured at 680 nm.**

Reducing sugar obtained with different Termamyl concentration (% V/V).

○ , 0.3; □ , 0.2; △ , 0.1.

Residual starch with different Termamyl (%V/V).

● , 0.3; ▮ , 0.2; ▲ , 0.1.

weight of the undigested solid matter were noted. The application of these commercially available cellulases, pectinases and proteases together with glucoamylase reduced the dry weight of the undigested solid matter from 28% to 15% compared to the treatment of corn flour with alpha amylase alone (Table 1). The treatment with termamyl and glucoamylase reduced the dry weight of the residue to 20%. Saccharification with all these enzyme mixtures increased the dextrose equivalent to 93. The action of proteases on proteins increased the aminoacid content of the filtrate (Table 1). Hence the β-glucanases and proteases had acted on the undigested solid matter and further reduced the dry weight from 20% to 15%.

Our results show that the commercially available enzymes like cellulases, proteases and pectinase did not give satisfactory results on the rate of filtration. In order to increase the efficiency of filtration, the mixture was diluted 1:1 and then treated with charcoal (50g/1) at 80^0C and filtered under vacuum while it was hot. The rate of filtration was increased to 63 ml/min (Table 1). Moreover, the filtrate obtained was almost clear and colourless. The aminoacid content of this filtrate was very low (Table 1). The activated charcoal removed the colouring materials, turbidity and proteins. Hence it could be used for purification of liquid glucose.

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