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HYDROLYSIS OF MACROMOLECULES IN CORN FLOUR AND THEIR FILTRABILITY

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ABSTRACT

Glucose syrup was prepared by the direct hydrolysis of starch in corn flour by a single step process compared to the standard two step process of purifying starch from corn flour and then converting the starch to glucose syrup. With increase in hydrolysis time of starch in corn flour, release of proteins, reducing sugars and colour into the hydrolysate and filtration time of the hydrolysate increased with a decrease in residual matter. Addition of beta-glucanases and proteases did not improve the hydrolysis of corn flour or filtration of corn flour hydrolysate (CFH). However the filtration time of CFH decreased by a change in pH to 4.5. Addition of charcoal to CFH at pH 4.5 further decreased the filtration time.

INTRODUCTION

Preparation of glucose syrups had been reported by the hydrolysis of purified starch and by the direct use of starch containing materials [1,2,3] Preparation of dextrose, maltose or corn syrup requires purification steps after saccharification. Direct use of starch containing raw materials introduces more impurities than purified starch. However in both situations downstream processing is essential. Hence direct use of starch containing raw materials may reduce the cost required for primary purification step.

Preparation of dextrose syrup by the synergistic action of alpha-amylase and glucoamylase on raw starch (in corn flour) has already been achieved [2] . This paper describes the effects of proteases and beta-glucanases on the hydrolysis of corn flour and filtrability of the hydrolysate. Studies were made to improve the filtrability of corn flour hydrolysate (CFH).

MATERIALS AND METHODS

Materials

Corn was purchased from local market and pulverized. Alpha-amylase (*Termamyl* 60L, 67.5 KNU g^{-1}), glucoamylase (*Spiritamylase* 150L, 159.9 AGU ml^{-1}), beta-glucanases (*Cereflo* 200L, 200 BGU g^{-1}; *Finizym* 200L, 200 BGU g^{-1} and *Novozym* 280L, 8800 PGU g^{-1}) and protease (*Neutrase* 0.5L, 1500 AU g^{-1}) were from NOVO Industries, Denmark. Pepsin (Pepsin P 700, 525 AU g^{-1}) and trypsin (Trypsin T 8128, 1050 BAEE g^{-1}) were from Sigma Chemical Company, USA. Charcoal (activated) was from BDH Chemical Company Ltd., London.

ANALYTICAL METHODS

Assay for reducing sugars and proteins

Reducing sugar was determined by 3, 5 dinitrosalicylic acid method[4] and reported in terms of glucose. Soluble and total protein were respectively determined by the methods of Lowry *et al.*,[5] and Kjeldhal[6].

Assay of colour

Colour of corn flour hydrolysate was measured at 440 nm.

Release of components during the hydrolysis of corn flour

Corn flour suspension (16% (w/w) in 0.025M acetate buffer, pH 5.0) was hydrolysed by synergistic action of alpha-amylase (0.225 KNU ml^{-1}) and glucoamylase (0.4 AGU ml^{-1})[2] at 70°C and the CFH was analysed for reducing sugars, soluble protein and colour at 1, 2, 3h. At the same period the time taken for the filtration of 750ml of CFH through a buchner funnel containing Whatman No. 1 filter paper (7cm) was determined. Dry weights of the residues were noted.

Additive effects of beta-glucanases and proteases with the mixture of alpha-amylase and glucoamylase on the hydrolysis of corn flour and on the filtration time of the CFH

Corn flour (16% (w/w) suspension in 0.025 acetate buffer, pH 5.0) was hydrolysed by different combinations of enzymes (Table 2) at 70°C. Glucose formed and residues left at 1, 2 and 3h and filtration time at 3h were determined.

Filtrability of CFH

The filtration time of CFH incubated with a mixture of *Cereflo* (0.05 BGU ml^{-1}), *Finizym* (0.05 FBU ml^{-1}) and *Novozym* (4.33 PGU ml^{-1}) at pH 5.0 and 30°C for 1h was determined. As an alternative CFH was incubated for 1h at 30°C with three different proteases of the same concentration (2% w/v) and the filtration time of the treated CFH was determined. Then the pH values of CFH was adjusted from 1.0 to 14.0 and filtration time was noted. CFH treated with varying amounts of charcoal for 15min at 30°C and its effect on removing proteins and colour, and on the filtration time were observed.

RESULTS AND DISCUSSION

Release of components during the hydrolysis of starch in corn flour

When corn flour (16% w/w) was hydrolysed with alpha-amylase and glucoamylase, the concentration of glucose obtained at 1h was 11.9% (w/w) and at 2 and 3h was 13.1% (w/w) (Table 1). At these time intervals theoretical starch hydrolysis was 90.2%, 99.2% and 99.2% respectively. However the residues obtained from corn flour hydrolysis at 1, 2 and 3h were 45.6, 42.9 and 40.5g respectively which is 32.4, 30.5 and 28.7% of the corn flour,

on a dry weight basis (12% moisture). With increase in hydrolysis of corn flour, more glucose, proteins and colour forming substances were released into the hydrolysate, concomitantly reducing the dry weight of the residue and increasing the filtration time (Table 1). Filtration time of CFH (through Whatman No.1 filter paper) has notably increased with time. This could be due to the block of filter paper by proteins and colloidal materials released into the CFH[3] (Table 1). Correct selection of filter cloth and filtration method are important for downstream processing. Further, release of these clogging particles from residual material support the reduction in dry weight of the residue from 1h to 3h. During saccharification, the increase in colour of the CFH could be due to the reaction of amino groups in the proteins and amino acids with the carboxyl groups of glucose[7]. Thus the difference in optical density measurements made at 440nm for colour quantitation could be due to Millard reaction[7] in addition to the release of some pigments in corn flour.

Additive effects of beta-glucanases and proteases on corn flour hydrolysis and on the filtrability of CFH

Hydrolysis of corn flour by different combination of enzymes showed no difference in glucose formation and in turn on DE (Table 2). Since glucoamylase is an exo-amylase and alpha-amylase is an endo-amylase, random attack of alpha-amylase on starch would have released more starch/dextrins and other attached materials into solution than glucoamylase. Hence the residue left after the action of alpha-amylase was less than that left by the action of glucoamylase. Addition of beta-glucanases and proteases did not improve the filtration property of CFH (Table 2). Thus different studies should be made to improve the filtrability of CFH for downstream processing.

Filtrability of CFH

Studies were made to improve the filtrability of CFH so as to support and exploit the corn flour hydrolysis instead of corn starch. Starch in corn flour was hydrolysed by a mixture of alpha-amylase and glucoamylase at 70°C and pH 5.0. In this set of experiments the coarse residue was removed from CFH with the help of muslin cloth. It is generally assumed that residual glucans and colloidal proteins could be the reason for the difficulty faced with filtration. Therefore CFH was treated with a mixture of beta-glucanases and different proteases. Time taken for filtration of CFH with and without the addition of beta-glucanases were almost same (Table 3). Thus addition of beta-glucanases to improve the filtrability of CFH had no use. However enzyme preparation which breaks down high molecular weight proteins, dextrins and non starchy polysaccharides had doubled the filtration rate of beer [8]. As an alternative, effect of proteases on the filtrability of CFH was studied to remove colloidal proteins clogging the pores of filter paper. Results obtained were discouraging, because CFH which was not exposed to the proteases required almost the same time as those incubated with the proteases (Table 3). In this set of experiments choice of the respective pH values were according to pH optima of the proteases. In case of pepsin, change in pH value from 5.0 to 1.8 might have precipitated some proteins leading to the observed decrease in filtration time rather than the proteolytic effect per se. These results indicated that the observed differences in the time taken for the filtration could be due to

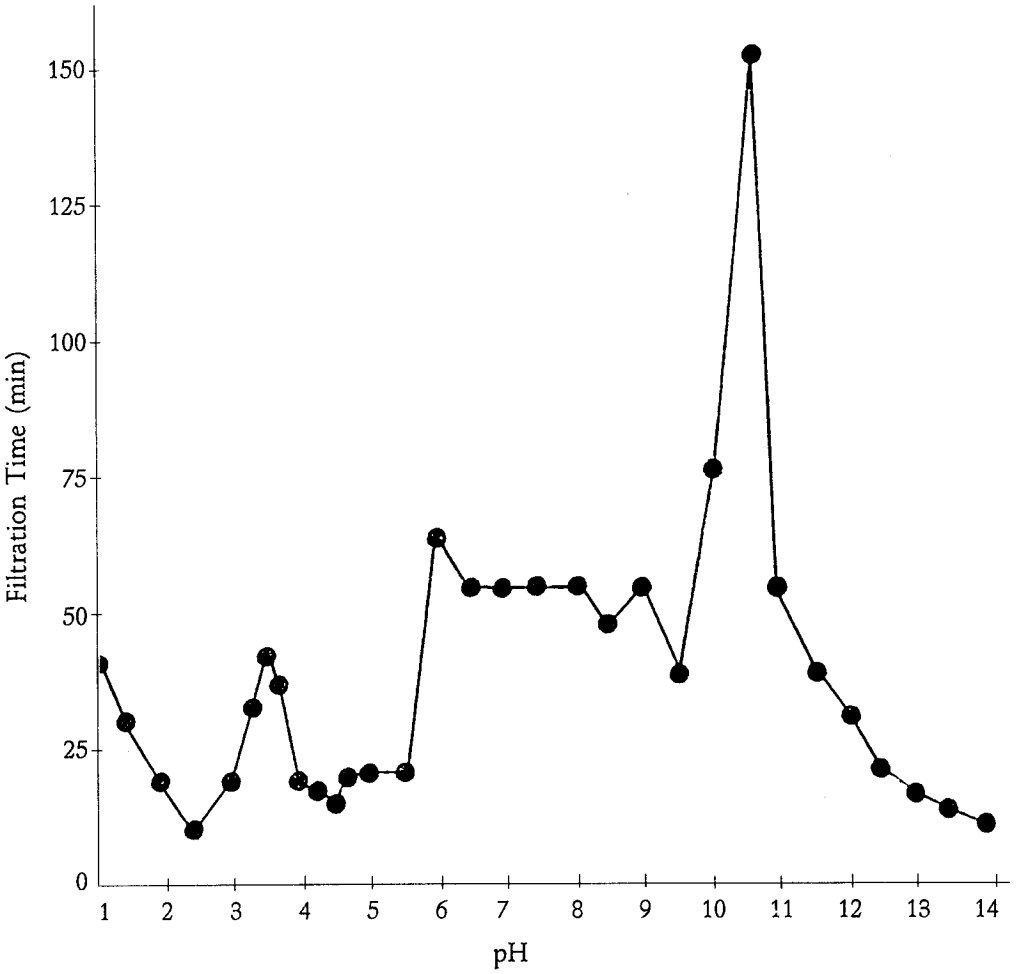

Fig. 1 Effect of pH on the filtration time[a] of CFH.

[a] Filtration time was measured as said in Table 1.

TABLE 1. Hydrolysis of starch in corn flour suspension (16% w/w in 0.025M acetate buffer, pH 5.0) by a mixture of alpha-amylase and glucoamylase and its effect on the release of proteins, colour and filtrability.

Substance	Time (h)		
	1	2	3
Starch hydrolysed (%)	90.2	99.2	99.20
Glucose (%, w/w)	11.9	13.1	13.10
Soluble proteins (mg ml-1)	0.12	0.19	0.26
Colour (Absorbance at 440nm)	0.25	0.28	0.30
Filtration time[a] (min)	11.1	14.4	19.20
Residue[b]	45.6	42.9	40.50

[a] Filtration time was determined by noting the time required to filter 750ml of CFH through a buchner funnel containing Whatman No. 1 filter paper (7cm).

[b] Residue obtained when 160g corn flour (suspended in 840g of 0.025M acetate buffer, pH 5.0) was hydrolysed by alpha-amylase and glucoamylase under the above mentioned conditions.

TABLE 2. Dextrose equivalents and residue left after the hydrolysis of 16% (w/w) corn flour suspension (0.025M acetate buffer, pH 5.0) by different combination of enzymes.

Enzyme[a]	Time (h)	Glucose (%, w/w)	DE[b] (%)	Residue[c] (%)	Filtration time[d] (min)
Alpha-amylase	1	6.5			
	2	6.9	52.3	43.7	—
	3	6.9			
Glucoamylase	1	4.7			
	2	5.4	45.5	57.0	—
	3	6.0			
Alpha-amylase	1	11.9			
Glucoamylase	2	13.1	99.2	25.3	19.3
	3	13.1			
Alpha-amylase	1	12.0			
Glucoamylase	2	13.1	99.2	25.4	19.5
Neutrase	3	13.1			
Alpha-amylase					
Glucoamylase	1	12.2			
Cereflo	2	12.9	99.2	24.6	19.0
Finizym	3	13.0			
Novozym					
Alpha-amylase					
Glucoamylase	1	11.5			
Neutrase	2	12.7	97.1	24.3	18.5
Cereflo	3	12.8			
Finizym					
Novozym					

[a] For one gram of reaction mixture, 0.225 KNU alpha-amylase, 0.4 AGU glucoamylase, 0.615 AU *Neutrase*, 0.05 BG *Cereflo*, 0.05 PGU *Finizym* and 4.33 PGU *Novozym* were added.

[b] Dextrose equivalent (%) = $\frac{\text{Glucose formed at 3h by enzymatic hydrolysis of corn flour}}{\text{Glucose formed by acid hydrolysis of corn flour}} \times 100$

[c] Residue (%) = $\frac{\text{Dry weight of residue}}{\text{Dry weight of corn flour}} \times 100$

[d] As in Table 1

TABLE 3. Effect of beta-glucanases and proteases on the filtrability of CFH.

pH	Enzymes	Filtration time [a] (min)
5.0	Nil	19.2
	Beta-glucanases (*Cereflo, Finizym* and *Novozym*)	18.7
7.5	Nil	52.5
	Protease (*Neutrase*)	54.0
	Protease (Trypsin)	52.5
1.8	Nil	18.7
	Protease (Pepsin)	19.4

[a] Filtration time was determined as in Table 1.

TABLE 4. Effect of charcoal on the filtration time and on removal of protein and colour from CFH.

Charcoal[a] (%, w/v)	Filtration time[b] (min)	Protein removed[c] (%)	Colour removed[c] (%)
0.00	19.2	-	-
0.10	17.6	66.2	50.1
0.25	17.2	74.6	71.4
0.50	13.9	83.1	76.8
0.75	6.1	85.8	82.1
1.00	5.8	90.4	91.1
2.00	4.8	96.2	96.4
3.00	4.2	100.0	99.1
4.00	2.5	100.0	99.1
5.00	2.1	100.0	99.1

[a] Charcoal of different amounts were added to 750ml of CFH at pH 4.5 and mixed for 15min at 30°C.

[b] As in Table 1.

[c] Substance removed (%) = $\frac{\text{Substance in CFH} - \text{Substance in CFH after treatment}}{\text{Substance in CFH}} \times 100$

the differences in pH values. Figure 1 shows that the filtration time varied with pH and was least (10.7 min) at pH 2.5 and 14.0. A second considerable decrease in the filtration time was observed at pH 4.5 (15 min). Time taken for the filtration was maximum in the pH range from 6.0 to 11.5. As the filtrate obtained at pH 2.5 was turbid, pH 4.5 was preferred to pH 2.5 even though filtration time was least (10.7 min) at pH 2.5. This agrees with the former studies where the pH of the crude maltose/dextrin solution was adjusted to 4.6 - 4.8 with sodium bicarbonate[9]. Further, the original pH of CFH was 5.0, and it was decided to adjust the pH to 4.5 to carry out the filtration as it would reduce dilution and addition of extra chemicals (acid/alkali) to CFH. Further decrease in filtration time of CFH was achieved by treating it at pH 4.5 with activated charcoal (Table 4). Activated charcoal of high concentrations (1 - 5%, w/v) was also very effective in removing proteins and colour (Table 4). About 99% of colour and 100% of proteins present in CFH were removed when activated charcoal used was above 3% (w/v) concentration.

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