Annex 9-6.

Large Scale Preparation of Crystalline Glucose from Raw Starch in Corn Flour

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Starch in considerable amount is lost during its purification from raw materials. Further, purification costs energy and time. To avoid these, starch in corn flour was hydrolyzed by the synergistic action of α-amylase and glucoamylase while avoiding high temperature gelatinization and liquefaction processes. When 1600 g (16 %, W/W suspension) and 4000 g (40 %, W/W suspension) corn flour was hydrolyzed and purified, 76.0 % and 50.2 % glucose yields were obtained. The residues obtained were rich in protein and minerals.

1 Introduction

Hydrolysis of raw starch in corn flour by synergistic action of α-amylase and glucoamylase has already been achieved in small scale [1]. For raw starch in corn flour (16 %, w/w) hydrolysis suitable glucoamylase to α-amylase ratio has also been established [1]. Further, suitable conditions such as pH, temperature, etc. for the activities of the enzymes in mixture were elucidated [2]. After fixing suitable concentrations of the enzymes and hydrolytic conditions, an economical down stream processing of the hydrolysate was studied [3]. Having studied the above facts, here we report the hydrolysis of starch in corn flour of different concentrations in large scale.

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2 Experimental

2.1 Materials

Corn was purchased from local market and pulverized. α-Amylase (Termamyl® 60L, activity 67.5 KNU g^{-1}) and glucoamylase (Spiritamylase®, activity 150 AGU g^{-1}) were from NOVO Industries, Denmark. Charcoal (decolurizing powder, activated) was from BDH Chemical Company Ltd., London.

2.2 Analytical methods

Reducing sugars in the hydrolysates were determined by 3,5-dinitrosalicylic acid method [4] and represented in terms of glucose. Soluble and total proteins were respectively determined by *Lowry's* [5] and *Kjeldhal* [6] methods. Amino acids present in the samples were estimated by the method of *Rick* [7] and pigments were estimated as described elsewhere [8].

2.3 Large scale preparation of crystalline glucose

2.3.1 Hydrolysis of 16 % (w/w) corn flour suspension

2.3.1.1 Hydrolysis of 1600 g corn flour suspended in 8400 g water

Corn flour (16 %, w/w) suspension at pH 5.5 was hydrolyzed by synergistic action of α-amylase and glucoamylase [1] in a home made stainless steel vessel (12.0 l capacity) kept in a temperature controlled water bath at 70 °C, where continuous mixing was aided by an impeller (Gallenkamp). After 3.0 h, hydrolysate was cooled and strained (Hydrolysate – IA) through a muslin cloth to remove residual substances (Residue – IA). Residue – IA was washed with 2.0 l distilled water by mixing for 1.0 h and strained (Hydrolysate – IIA) through a muslin cloth. The residue (Residue – IIA) was analyzed for starch [11], protein [6], fiber [6], moisture [6] and ash [6] contents.

Hydrolysates – IA and – IIA were pooled together (Hydrolysate – IIIA) and analyzed for protein [5], amino acids [7] and pigments [8]. The pH of hydrolysate – IIIA was adjusted to 3.5, allowed the precipitate to settle and the supernatant was filtered through a buchner funnel containing filter paper (Whatman No. 1) (Filtered Hydrolysate A – FH – A). Charcoal (1.0 %, w/v) was mixed with FH – A for 15 min at 30 °C. The charcoal was removed by refiltration. Purified hydrolysate (PH – A) was analyzed for protein [5], amino acids [7], sugars [4] and pigments [8].

PH – A was concentrated (Concentrate – A) using a rotor evaporator and the concentrate – A was analyzed for reducing sugars [4]. Concentrate – A was left for crystallization at 4 °C. Glucose crystals were dried at room temperature and weighed (Dried Glucose Crystals – A, DGC – A).

Corn flour was also analyzed for starch [11], protein [6], fiber [6], fat [6] and ash [6] contents.

2.3.1.2 Hydrolysis of 8000 g corn flour suspended in 42 000 g water

Starch in corn flour suspension (16 %, w/w) was hydrolyzed by α-amylase and glucoamylase by taking 8000 g corn flour suspended in 42 000 g water as described before.

2.3.2 Hydrolysis of 40 % (w/w) corn flour suspension (4000 g in 6000 g water)

Starch in corn flour suspension (40 %, w/w) was hydrolyzed by α-amylase and glucoamylase as described before. In this section the hydrolysates residues etc. were named as previously and were described as B instead of A.

3 Results and Discussions

3.1 Hydrolysis of 16 % (w/w) corn flour suspension

In this work, synergistic action α-amylase and glucoamylase was exploited. Since thermolabile glucoamylase was used along with thermostable α-amylase, the temperature used for the process was 70 °C [2]. Starch in corn flour 16 % (w/w) was hydrolyzed in large scale (1600 g in 8400 g water) at 70 °C and pH 5.5 for 3.0 h using 0.4 AGU glucoamylase and 0.225 KNU α-amylase for g reaction mixture. This works out to the glucoamylase to α-amylase ratio of 1.8 AGU/KNU [1].

When the hydrolysate was strained and the residue was washed with distilled water, most of the fibers, proteins fats and minerals (ash) were retained with the residue (Residue – IIA) (Table 1). Starch content of the wet residue was 10.8 % and that of the dried residue was 48.6 %. It was found that 13.7 % of the total starch present in 1600 g of corn flour was not hydrolyzed. When 160 g of corn flour in suspension (16 %, w/w) was hydrolyzed by the synergistic action of α-amylase and glucoamylase, at 3.0 h the starch in corn flour was completely hydrolyzed [10].

Protein content of the wet residue was 9.0 % or 40.0 % of dried residue. In addition the mineral content of the wet and dried residues were 0.4 % and 1.8 %, respectively (Table 1). In general the residue and corn flour were compared, the residue was concentrated with useful nutrients except carbohydrates. This residue is a good feed for animal and birds.

Hydrolysate – IIA contained 10.8 % (w/w) glucose, and proteins, amino acids and pigments as contaminants (Table 2). (Pigment content was taken by measuring the optical density at 440 nm and optical density of the hydrolysate – IIIA was considered as standard.) After different treatments the relative pigment content was determined as follows: Pigment (%) = Absorbance of treated hydrolysate/Absorbance of Hydrolysate – IIIA 100.

When the Hydrolysate – IIIA was treated with activated carbon (1.0 %, w/v), 98.5 % (of 0.03 %, w/v) of protein and 99.0 % (of 0.05 %, w/v) of amino acids and 100 % of pigments were removed. However the charcoal treatment has not affected the sugar content of the hydrolysate (PH – A) (Table 2). The hydrolysate was concentrated by about 8 times to get 82.8 % (w/w) glucose since above 80 % (w/w) glucose can be easily crystallized [11]. Concentrated glucose

Tab. 1. Contents of corn flour and the residues obtained after hydrolysis of 16 % (w/w) (Residue – IIA) and 40 % (w/w) (Residue – IIB) corn flour suspension at pH 5.5 and 70 °C by the synergistic action of α-amylase and glucoamylase.

Substance (%)	Corn flour	Residue			
		IIA		IIB	
		wet	dry	wet	dry
Moisture	12.0	77.7	–	64.9	–
Starch	74.5	10.8	48.6	25.8	73.5
Fat	1.1	1.0	4.4	1.0	3.0
Fiber	1.1	1.9	8.7	1.3	3.6
Ash	0.6	0.4	1.8	0.5	1.5
Protein	9.0	9.0	40.1	6.5	18.4

Tab. 2. Glucose, protein amino acids and pigments in corn flour hydrolysate, purified hydrolysate (PH) and in the concentrates obtained by the hydrolysis of 16 % (w/w) and 40 % (w/w) cornflour suspensions.

Substance (%)	Hydrolysate		PH		Concentrate	
	IIIA	IIIB	A	B	A	B
Glucose (w/w)	10.8	25.0	10.7	25.0	82.8	83.2
Protein (w/v)	0.02	0.4	0.0003	0.0004	0.0023	0.0014
Amino acids (w/v)	0.05	0.2	0.0005	0.0008	0.0038	0.0018
Pigment	100	100	–	–	–	–

syrup contained no pigments but with negligible amounts of proteins (0.0023 %, w/v) and amino acids (0.0038 %, w/v).

When 1600 g of corn flour was hydrolyzed, glucose yield was 76.0 %. When the process was carried out in higher volume by taking 8000 g of corn flour in 42.01 water, the glucose yield was 73.0 %. Thus scaling up of the process from 160 g to 1600 g has reduced the glucose yield from 100 % to 76.0 %, but scaling up from 1600 g to 8000 g has reduced the glucose yield by 3.0 %. These results indicate that the process could be further scaled up without significant loss in glucose yield.

3.2 Hydrolysis of 40 % (w/w) corn flour suspension (4000 g in 6000 g water)

In this experiment 40 % (w/w) corn flour suspension was hydrolyzed by α-amylase and glucoamylase (0.5625 KNU and 1.0 AGU/g corn flour suspension respectively, where the ratio was equivalent to 1.8 AGU/KNU) at 70 °C and pH 5.5 for 3.0 h. First a trial was made with 0.225 KNU α-amylase and 0.4 ACU glucoamylase/g corn flour suspension and the cornflour suspension used was 40 % (w/w). It was practically difficult to stir the reaction mixture and to continue the process. Hence the concentration of the enzymes were increased in proportion to corn flour concentration, to 0.5625 KNU α-amylase and 1.0 AGU glucoamylase/g cornflour suspension.

Protein and mineral contents were 18.4 % and 1.5 % respectively in dried residue. Residue – IIB contained 25.8 % and 73.5 % starch in wet and dried conditions, respectively. The starch loss accounts to 29.4 % of that present in cornflour taken for hydrolysis. Thus more starch is lost when higher concentration of corn flour was used in suspension for the hydrolysis, even though proportionately high amount of enzymes were used.

The Hydrolysate – IIIB contained 25.0 % (w/w) glucose. When the Hydrolysate – IIB was treated with charcoal (1.0 %, w/v), 99.9 % protein and 99.7 % amino acids and 100 % pigments were removed (in this case the absorbance of Hydrolysate – IIIB at 440 nm was taken as standard and compared with other hydrolysate as said for Hydrolysate – IIIA). The action of charcoal was similar to Hydrolysate – IIIA. Charcoal did not affect the sugar content in the hydrolysate as before.

The hydrolysate was approximately concentrated by 3.5 times to get glucose concentration of 83.2 % and crystallized. The crystals were dried as before, In this experiment the glucose yield was 50.2 %.

From the results it can be seen that the residues contained more moisture than that was reported earlier [12], where the filter cake contained only 5.0 % moisture. In our experiment the hydrolysate was strained through a muslin cloth manually. If a correct filter cloth and a filter could be selected moisture in the residue could be reduced while increasing the filtration efficiency. Alternatives such as use of diatomaceous earth or crushed perlite were also described for filtration [13]. Local fabrication of a filter press is in progress.

4 Conclusions

Hydrolysis of starch in corn flour without purification seems to be promising by undertaking synergistic hydrolysis and two steps purification. If high purity glucose is needed ion-exchanger treatment might be valuable to remove the residual amino acids, proteins and inorganic ions. Hence further investigation is required to produce high purity glucose.

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