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Two-Steps Procedure for the Purification of Corn Flour Hydrolysate

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When corn flour hydrolysate filtrate (CFHF) was treated with 100% (w/v) $(NH_4)_2SO_4$ for purification, 80.5% of the protein was removed. Among the pH values studied, at pH 1.5, 90.2% of the total protein was precipitated from CFHF and supernatant was removed by decantation. The corn flour hydrolysate was purified with Amberlite and DEAE-cellulose after precipitating the protein at pH 1.5. Charcoal was better than cation- and anion- exchange resins for the removal of proteins, amino acids and pigments from the hydrolysate.

Zweistufen-Verfahren zur Reinigung von Maismehlhydrolysat. Wenn das Filtrat von Maismehlhydrolysat (CFHF) mit 100% (w/v) $(NH_4)_2SO_4$ zur Reinigung behandelt wurde, wurden 80,5% des Proteins entfernt. Unter den untersuchten pH-Werten wurden bei pH 1.5 90,2% des Gesamtproteins aus CFHF und der überstehenden Lösung niedergeschlagen und durch Dekantation entfernt. Das Maismehlhydrolysat wurde mit Amberlit und DEAE-cellulose nach Niederschlagung des Proteins bei pH 1,5 gereinigt. Aktivkohle war besser als Kation- und Anionaustauscherharze für die Entfernung von Proteinen, Aminosäuren und Farbtoffen aus dem Hydrolysat.

1 Introduction

Current procedure for dextrose production from starch can be separated into starch hydrolysis, refining, evaporation, crystallization, centrifugation, washing and drying [1–5]. Different works have been reported for the hydrolysis of purified starch preparations [6] and direct use of starch containing raw materials [7, 8]. Preparation of dextrose, maltose or corn syrup require purification steps after saccharification. Direct use of starch containing raw materials introduces more impurities than purified starch. However, in both situations down-stream processing is essential. Thus direct use of starch containing raw material may reduce the cost required for the primary step, i.e. starch purification from raw material.

Preparation of dextrose syrup by the synergistic action of α-amylase and glucoamylase from raw starch in corn flour has already been achieved [7]. This paper describes the studies made on the purification of corn flour hydrolysate to obtain dextrose monohydrate crystals of high purity level.

2 Experimental

2.1 Materials

Corn was purchased from local market and pulverized. α-Amylase (Termamyl®, activity 67 KNU · g^{-1} [9]) and glucoamylase (Spiritamylase®, activity 150 AGU g^{-1} [10]) were from NOVO Industries (Denmark). Charcoal (decolourizing powder, activated, BDH Chemical Company Ltd., London), DEAE-cellulose (anion-exchanger, Sigma Chemical Company, U.S.A) and Amberlite IR-120 (NA) (Avondale Laboratories, England) were used for purification.

2.2 Analytical methods

The reducing sugars in the hydrolysate were determined by 3.5-dinitrosalicylic acid method [11] and represented in terms of glucose. Protein was determined by the modified method of *Lowry* et al. [12]. Total amino acids present in the samples were estimated by the method of Rick [13] and the pigment as estimated as described elsewhere [14].

2.3 Preparation of corn flour hydrolysate filtrate (CFHF)

Corn flour was hydrolyzied by the synergistic action of α-amylase and glucoamylase [11]. The hydrolyzate was strained through a muslin cloth, pH was adjusted to 4.5, filtred through a Whatman No. 1 filter paper and used for the experimants [14].

2.4 Purification of CFHF

2.4.1 Ammonium sulphate precipitation

Varying amounts of ammonium sulphate were used to precipitate the protein from CFHF and the protein removed by the precipitation was estimated.

2.4.2 By isoelectric precipitation

The pH of CFHF was adjusted from 1 to 14 with 0.5 pH unit difference. Protein content of the precipitates and supernatants were estimated.

2.4.3 By anion-exchange resin

To remove the impurities, CFHF after precipitating the proteins at pH 1.5 (CFHF-1, at pH 11.5) was treated in batch process with varying amounts of activated DEAE-cellulose for 3h at 30°C.

2.4.4 By cation-exchange resin

The CFHF-1 (at pH 1.0) was mixed with different amounts of activated Amberlite IR-120 (NA) to remove the impurities.

2.4.5 By cation- and anion-exchange resins

The CFHF-1 (at pH 1.0, CFHF-11) was first treated with optimum amount of activated Amberlite IR-120 (NA) for 3h at 30°C and the pH of the decant (CFHF-111) was adjusted to 11.5 (CFHF-IV), mixed with optimum amount of activated DEAE-cellulose for 3h at 30°C. The supernatant was decanted

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(CFHF V). CFHF-11, 111, IV and V were analyzed for proteins, pigments, amino acids and reducing equivalents as described in the preceding.

2.4.6 By activated charcoal

CFHF-1 was treated with different amounts of activated charcoal at 30°C for 15min.

3 Results and Discussion

Important step in the downstream processing of CFHF is the removal of impurities [2]. To purity the CFHF, different alternatives were studied. From previous results [14] (effect of pH on the filtration rate), it was found that the change in pH has affected the filtration rate and was assumed that the proteins could be the main reason for the defective filtration rate. Hence proteins were precipitated with ammonium sulphate. However, addition of high concentration of ammonium sulphate was not efficient in precipitating proteins from the CFHF (Figure 1), because the protein precipitated by 50% (w/v) and 100% (w/v) ammonium sulphate were 66% and 80.5%, respectively. This method is not preferred as it introduced 100% (w/v) ammonium sulphate salt while removing 80.5% of protein.

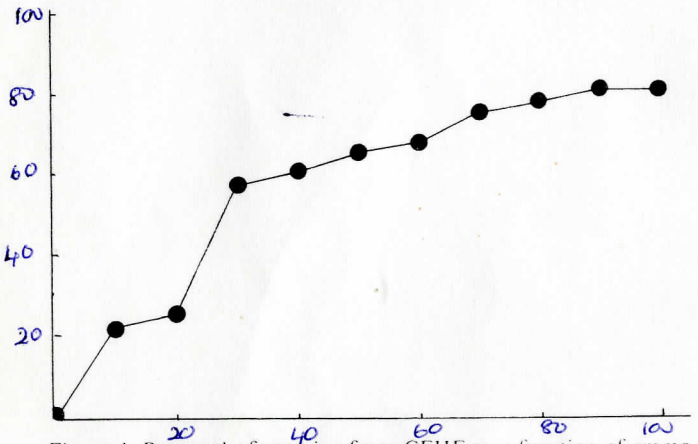

Figure 1. Removal of proteins from CFHF as a function of ammonium sulphate concentration.

When the pH of the CFHF was adjusted from 1 to 14 and left at 4°C, most of the acidic and basic proteins were precipitated in the pH range from 1.5 to 3.5 and from 8.0 to 11.0, respectively (Figure 2). Thus, isoelectric pH of the acidic and basic proteins were in the range from 1.5 to 3.5 and 8.0 to 11.0, respectively. For the precipitation of proteins, pH 1.5 was selected. When the proteins were settled to the bottom, the supernatant was taken for further studies. Since the isoelectric pH range was wide, it was decided to remove the proteins by ion-exchange resins and the pH values 1.0 and 11.5 were respectively chosen to remove the acidic and basic proteins from the CFHF-1.

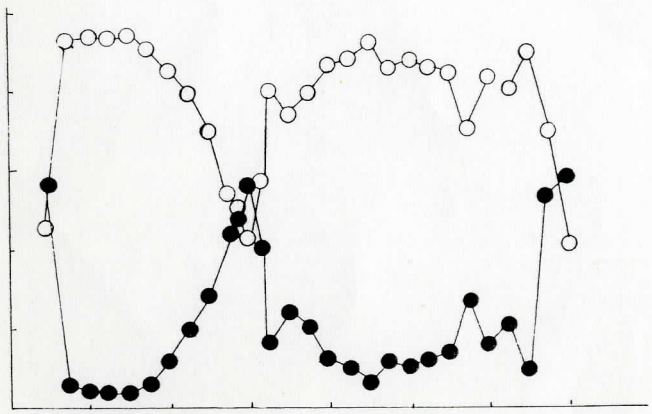

Figure 2. Effect of pH on the precipitation of protein from the CFHF. Protein in the supernatant (closed circle) and protein in the precipitate (open circle).

DEAE-Cellulose (20–50%, w/v) removed 55–63% of the total proteins, 48–55% of the total amino acids and 20–82% of the total pigments present in the CFHF-1 (Figure 3). Whereas Amberlite (20–50%, w/v) removed 48–55% of the total protein, 39–45% of the total amino acids and 60–70% of the total pigments present in the CFHF-1 (Figure 4). From these experimental results 0.25g of DEAE-cellulose and 0.2g of Amberlite were used per ml of CFHF-1. Treatment with ion-exchange resins did not affect the sugar content. Use of cation-exchange resin Duolite C-26 was previously reported for the purification of corn syrup [5].

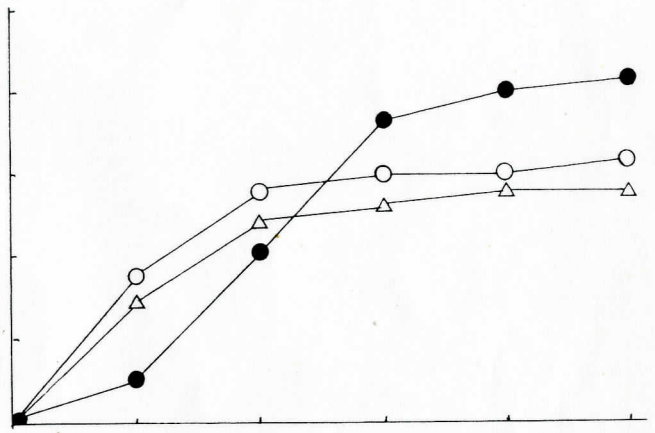

Figure 3. Effect of varying amounts of DEAE-cellulose on the removal of ● pigments, ○ proteins and △ amino acids from the CFHF-1.

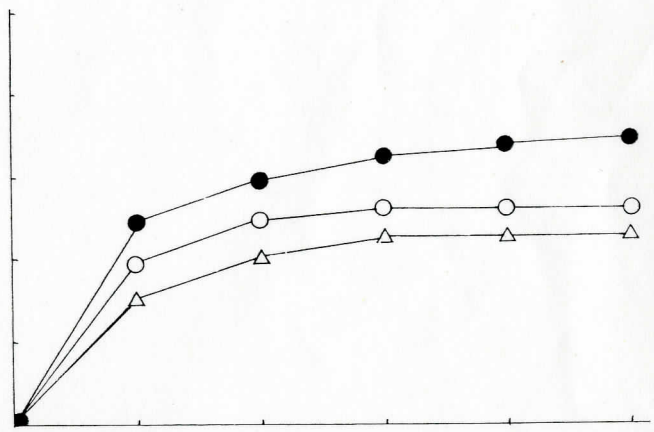

Figure 4. Effect of varying amounts of Amberlite IR-120 on the removal of ● pigments, ○ proteins and △ amino acids from the CFHF-1.

Treatment of CFHF-1 with Amberlite (at pH 1.0, 20%, w/v) and then with DEAE-cellulose (at pH 11.5, 25%, w/v) respectively removed 50% and 50% of the protein, 4.5% and 15.1% of the amino acids and 62% and 17.3% of the pigments (Table 1). CFHF-1 treated with ion-exchange resins contained 2.2% of protein, 43% of amino acids and 22% of the pigments of which were present originally. Sugar content in the CFHF was unaffected by the ion exchange resins.

Table 1. Glucose, Proteins, Amino Acids and Pigments Contents in CFHF-1 Before and After the Treatment with Cation- and Anion-Exchange Resins.

CFHF	pH	Protein (mg · ml^{-1})	Amino acid (mg · ml^{-1})	Pigment (440nm)	Glucose (%, w/w)
II	1.0	0.02	2.78	0.250*	11.3
III	1.0	0.01	1.62	0.095*	11.3
IV	11.5	0.01	1.62	0.290**	11.3
V	11.5	0.005	1.02	1.165**	11.3

* At pH 1.0 the untreated CFHF-1 had the optical density of 0.25 at 440nm against distilled water.

** At pH 11.0 the untreated CFHF-1 had the optical density of 0.75 at 440nm against distilled water.

Note: Increase in optical density of CFHF IV and V than CFHF II and III after the treatment with cation exchange resin was due to the change in pH.

As an alternative to ion-exchangers, effect of activated charcoal was studied. Optimum charcoal concentration was 1% (w/v), which had removed 96.7% of the protein, 98.3% of the amino acid and 98% of the pigments present in the CFHF (Figure 5). An activated carbon preparation (1.0%, w/v) had removed 99.5% protein and 84% colour from sugar syrup [15]. Purification of the CFHF-1 by activated charcoal took 15min. On contrast purification by ion-exchange resins took two days. (The time includes activation of the resins and purification steps). Hence, the purification by charcoal was faster and left few impurities than by ion-exchange resins. From these results it was decided to use 0.0lg activated charcoal per ml of CFHF to remove the impurities. Earlier reports state that syrup could be treated with activated powder carbon 0.5 or 1% followed by ion-exchangers (with a strong cation and week anion resins) [16]. As the purification was not sufficient two consecutive carbon treatments followed by ion-exchanger exposure were made [16, 17].

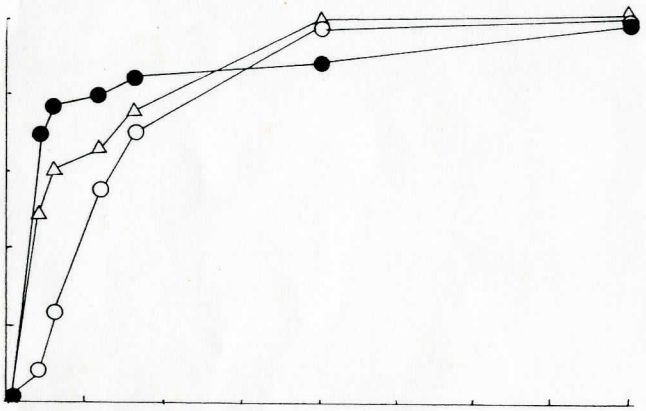

Figure 5. Effect of charcoal concentration on the removal of ● pigments, ○ proteins and △ amino acids from the CFHF-1.

4 Conclusion

The direct use of starch in corn flour to produce glucose by adopting the synergistic hydrolysis using α-amylase and glucoamylase is made feasible by following this cheap and easy method of purification. From this work, it was found that first the pH of the hydrolysate filtrate should be reduced to 1.5 and then after removing the precipitate, the supernatant should be treated with charcoal to achieve good purification. The feasibility of this process should be studied in large laboratory scale.

Acknowledgements

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