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Digestion of residual β-cyclodextrin in treated egg using glucoamylase from a mutant strain of *Aspergillus niger* (CFTRI 1105)

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Abstract

Glucoamylase enzyme isolated from the cultures of a mutant strain of *Aspergillus niger* (CFTRI 1105) was found to digest β-cyclodextrin in standard 1% solutions as well as in β-cyclodextrin-treated egg yolk and whole egg samples. About 70–95% of β-cyclodextrin in samples was digested with both dilute (reducing sugar activity 870 units) and concentrated (34,800 units) enzyme solutions at 40°C and 70°C for incubation periods of 15–360 min.

1. Introduction

Contents of chicken egg (whole egg as well as egg yolk) can be treated with β-cyclodextrin to remove cholesterol (Cully & Vollbrecht, 1992; Smith, Award & Gill, 1995; Awad, Bennink & Smith, 1997). This process leaves residues of β-cyclodextrin in egg. Although some countries like Japan, Hungary, France, Spain and Holland have approved the use of β-cyclodextrin in food systems, the lack of approval in others has prompted research work on the need for a procedure to break down β-cyclodextrin. The LD_{50} value for β-cyclodextrin in rats (oral administration) was found to be 0.6 g/kg body weight (Saenger, 1980). It is reported that only insignificant amounts of β-cyclodextrin in intact form are absorbed when administered orally. Most of the β-cyclodextrin is metabolized by the microflora in the colon to acyclic maltodextrins, maltose and glucose and these can be further broken down into CO_2 and H_2O in the same way as starch (Szejtli, 1994). Cleavage of β-cyclodextrin by bacterial and fungal α-amylases (1,4-α-D–Glucan glucohydrase, E.C. 3.2.1.3.) has been reported (Kohlrausch, Cully & Vollbrecht, 1994; Ros & Gijsbertus, 1996). In the process developed for the removal of cholesterol in chicken egg, elimination of residual β-cyclodextrin appeared effective using glucoamylase produced by a mutant strain of *Aspergillus niger* (CFTRI 1105). This process was efficient since it resulted in almost complete degradation of β-cyclodextrin residues. The details are reported in this paper.

2. Materials and methods

β-Cyclodextrin from M/s Cyclolab (Hungary), phenolphthalein and sodium acetate from BDH (India) were used. Glucoamylase employed for these experiments was obtained from a mutant strain of *Aspergillus niger* (CFTRI 1105). The organism was grown in conditions optimal for enzyme production and the crude culture filtrates had very little α-amylase and other glucosidase activities (Suresh, Dubey & Umesh Kumar, 1997, unpublished results). The enzyme activity was determined by estimating the reducing sugar released from starch (Bernfeld, 1955) as substrate using glucose as standard since the major product was glucose as identified by paper chromatography (Trevelyan, Proctor & Harrison, 1950). The activity unit of the enzyme employed is expressed as starch hydrolyzing activity throughout this work. One unit of this activity is defined as milligrams of glucose released from a 2% solution of starch/h at 70°C/ml of the enzyme employed at pH 4.2. Commercial glucoamylase enzyme purchased from Novo Laboratories, Denmark was employed for comparison with the enzyme produced by the mutant strain of *Aspergillus niger*.

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Table 1
Digestion of standard solutions of β-cyclodextrin with enzyme from a mutant strain of *Aspergillus niger* (CFTRI 1105) and commercial sample

Activity of enzyme[a], (units)	15 min		30 min		60 min		90 min		90 min commercial sample[e]		
	Reducing sugars (in mg)[b]	βCD remaining[c] (in mg)	Reducing sugars (in mg)	βCD remaining (in mg)	Reducing sugars (in mg)	βCD remaining (in mg)	Reducing sugars (in mg)	βCD remaining (in mg)	Activity of enzyme (unit)	Reducing sugars (in mg)	βCD remaining (in mg)
0	d	9.95		9.95	d	9.95	d	10.54	0	d	9.95
870	d	d		5.60	d	d	d	d			
1740	0.93	6.28		4.44	8.26	3.53	10.4	2.97	8750	5.1	0.94
3480	1.16	4.71	d	6.00	10.4	2.97	10.4	2.65	17500	6.2	0.89
4350	d	d		3.33	d	d	d	d			
5220	1.12	4.69		d	11.24	2.23	8.33	1.42	6250	7.0	0.79
6960	1.36	4.44		2.65	12.36	2.10	5.41	1.39	3500	07.5	0.84
8700	1.34	4.71		2.36	7.97	1.87	3.43	1.39	37500	8.5	0.83

[a] 10 mg/ml concentration of standard β-cyclodextrin solution in acetate buffer, pH 4.2 digested with enzyme at 70°C. Activity unit given as mg of glucose released from hydrolysis of 2% starch solution per hour at 70°C per ml of enzyme used.
[b] Estimation of reducing sugar by DNS method.
[c] Estimation of β-cyclodextrin by phenolphthalein method.
[d] No data.
[e] Activity = 250,000 units.

β-Cyclodextrin was estimated by the phenolphthalein method (Basappa, Rao, Rao, & Divakar 1998). β-Cyclodextrin digestion by the enzyme was carried out using 1% β-cyclodextrin solution in 0.01 M acetate buffer (pH 4.2), as substrate after which β-cyclodextrin content was estimated by the method mentioned above. In case of standard β-cyclodextrin solution, 1ml of the solution was made up to 25 ml with a 50 μM phenolphthalein solution and the pH was adjusted to 11.0 before measuring the absorbance at 554 nm. The amount of β-cyclodextrin in the sample was estimated using the equation $\log X = (\text{Intercept/Slope}) - (\log Y/\text{Slope})$, where X in the amount of β-cyclodextrin in mg/ml, Y the Absorbance of the solution at 554 nm, Intercept/Slope = −1.56 and Slope = −0.528 (for the above concentration range of β-cyclodextrin).

Contents of egg (egg yolk or whole egg) after treatment with β-cyclodextrin were estimated to contain 1100–1800 ppm of β-cyclodextrin, depending on the treatment procedure. Enzyme digestion of 20 ml of β-cyclodextrin-treated yolk was carried out for 90 min at 70°C with appropriately diluted enzyme (Tables 1 and 2). Aliquots of enzyme-treated yolk (3 ml) were taken at 0, 15, 30, 60 and 90 min for β-cyclodextrin estimation. In the case of whole egg, 900 ml (815 g) of the sample was digested with the appropriate amounts of the enzyme at 40°C for a period of 2 h to prevent denaturation of the egg white, which occurs at 70°C (Table 2).

In the case of egg yolk or whole egg samples, aliquots (3 ml) were treated with 5 ml of 10% methanolic KOH solution and left overnight. The alkali was neutralized with dilute HCl and the aqueous layer after two successive extractions with chloroform was concentrated, filtered and made up to 100 ml with a 100 μM phenolphthalein solution. The pH of this solution was adjusted to 11.0 before measuring the absorbance at 554 nm using a Shimadzu 160A UV-visible spectrometer. The absorbance at 554 nm for enzyme blanks (without β-cyclodextrin and phenolphthalein) was found negligible.

3. Results and discussion

The digestion experiments were performed with standard 1% β-cyclodextrin solutions as well as with egg samples. Breakdown of β-cyclodextrin by digestion with the enzyme was observed both with standard 1% β-cyclodextrin solutions and egg samples containing yolk or whole egg.

Fig. 1 shows the digestion of 1% β-cyclodextrin solution with various amounts of enzyme for different periods of incubation. Extent of digestion increased with incubation period, ranging from 35% at 15 min to 70% at 90 min. Further increase in enzyme amounts, at 15, 30, 60 and 90 min, resulted in further digestion of only 10–25% of β-cyclodextrin. While the enzyme digestion data at 30 min showed progressive digestion with increase in enzyme activity units, those of 15, 60 and 90 min were found to be independent of the amount of enzyme after a certain threshold amount of activity units of enzyme employed. At higher enzyme concentrations, the catalytic effect appears to be lost. The reason for this is not known.

Reducing sugar estimations, for the experiments conducted with standard 1% β-cyclodextrin solution at 15, 30, 60 and 90 min mentioned above, showed maximum values of reducing sugar with enzyme solution of activity 6960 units (Table 1). While β-cyclodextrin values showed a steady decrease with increase in enzyme volume as well as digestion period, anomalies were observed in reducing sugar values. The total amount of sugars, that is, reducing sugars and the remaining undigested β-cyclodextrin put together, was always found to be higher than the amount of 10 mg/ml of β-cyclo-

Table 2
Digestion of egg yolk and whole egg samples containing β-cyclodextrin with enzyme from a mutant strain of *Aspergillus niger* (CFTRI 1105) and from commercial source

Enzyme activity	Digestion time (in min)	β-CD remaining (in mg)
Treated egg samples		
Yolk[a]	0	93.7
0	15	22.3
17400	30	6.0
17400	45	3.5
34800	60	2.5
34800	90	1.8
Whole egg[b]		
0	0	1057
5000	120	676
8750	240	277
12500	360	208

[a] 20 ml sample, digestion temperature 70°C, enzyme glucoamylase from a mutant strain of *Aspergillus niger*, pH 4.2.
[b] 900 ml (815 g) of sample, digestion temperature 40°C, enzyme glucoamylase from a commercial source, pH 4.2.

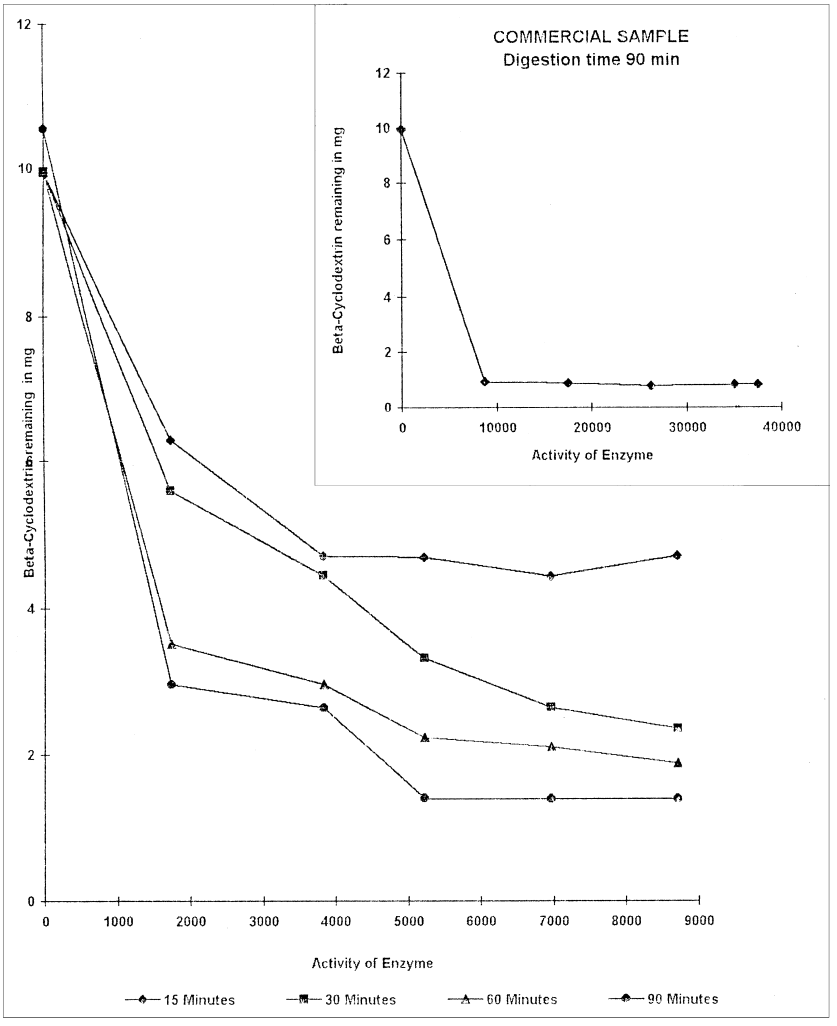

Fig. 1. Digestion of standard solution of β-cyclodextrin with glucoamylase from a mutant strain of *Aspergillus niger* (CFTRI 1105). 10 mg/ml of β-cyclodextrin was digested at 70°C. Inset: Digestion of standard solution of β-cyclodextrin with glucoamylase from Novo Laboratories. 10 mg/ml of β-cyclodextrin was digested at 40°C. β-Cyclodextrin was estimated in both cases by the phenolphthalein method (Refer text).

dextrin employed. Digestion of β-cyclodextrin by the enzyme does not convert β-cyclodextrin completely into glucose units. Instead, oligomers are formed which on assay by the DNS method do not yield the exact amount of degraded compounds. The phenolphthalein method gives an estimate of intact β-cyclodextrin in the digested samples but not of degraded β-cyclodextrin.

High concentrations of the enzyme solution were employed to achieve maximum digestion of β-cyclodextrin. Accordingly, 87% of β-cyclodextrin in the standard, 98% of β-cyclodextrin in the yolk and 80% of β-cyclodextrin in the whole egg samples were digested. Experiments performed with whole eggs treated with β-cyclodextrin reduced the level of the latter from 1100–1800 mg to about 250 mg on digestion with the enzyme. However, even a dilute sample of the enzyme solution of reducing sugar activity of 200 units was sufficient to digest 80% of β-cyclodextrin in the whole egg sample. Such a dilute solution was found to be very active even at a temperature of 40°C for 2 h which enabled the digestion experiment to proceed smoothly without denaturation of the egg white in the samples which did occur in the digestion experiments carried out at 70°C.

The performance efficiency of the enzyme produced by a mutant strain of *Aspergillus niger* (CFTRI 1105) was compared (Table 1) to that of glucoamylase from Novo, Denmark (Reducing sugar activity-250,000 units). Maximum reduction of β-cyclodextrin of up to 92% was obtained for an enzyme solution of activity 37,500 units for a β-cyclodextrin solution of concentration of 10 mg/ml. Though glucoamylases are known to have *exo* activities on starch, the need for higher concentrations of the enzyme for β-cyclodextrin degradation suggests some affinity of the enzyme for this substrate as well. Higher amounts of enzyme may result in slight *endo* activity of this enzyme on β-cyclodextrin. However, this was not studied in detail.

These experiments clearly showed that removal of undesired β-cyclodextrin in food samples could be easily accomplished by digestion with glucoamylases, especially from a mutant strain of *Aspergillus niger*. Not only can the digestions be carried out under mild conditions, but effective removal of β-cyclodextrin can also be achieved, making it an attractive and desirable procedure.

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