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The Effect of pH, Temperature and Alkali Metal Ions on the Hydrolsis of Whey Lactose Catalysed by β-Galactosidase from *Kluyveromyces marxianus*

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Abstract: In the present study, attempts have been made in optimising the conditions for whey lactose hydrolysis to prepare whey based soups or syrups. β-galactosidase enzyme was extracted from *Kluyveromyces marxianus* and partially purified. Two different alkali Potassium hydroxide (KOH) and Sodium hydroxide (NaOH) were used to bring the whey to required pH. Then, the whey lactose hydrolysis was carried out at two different temperatures, 25 and 37°C using whey with different pH (6.6 and 7.0). Out of eight combinations of alkali metal ions (K^+, Na^+), pH (6.6 and 7.0) and temperature (25 and 37°C) used, yielded increased lactose hydrolysis than that of Na^+ ions. pH 7.0 was found to be effective in lactose hydrolysis than pH 6.6. Hydrolysis incubation temperature 37°C showed more hydrolysis percentage than 25°C. From the above results, it may be concluded that, pH 7.0, temperature 37°C and alkali metal ion K^+ combination yielded the possible highest lactose hydrolysis percentage (86.8).

Key words: Whey lactose, β-galactosidase, lactose hydrolysis, pH, temperature, alkali metal ions

Introduction

Whey is a byproduct of cheese and casein manufacture. Its disposal into the environment causes pollution because of its high BOD value. In the light of its nutritional value and realizing the importance of whey solids in human food system, it is logical to use this 2° product of cheese production in newer food systems. But cheese whey utilization is limited due to its very high lactose content (about 70% on a dry weight basis). Lactose levels also limits the consumption of whey by individuals who have a deficiency of the small intestinal enzyme lactase (Kretchner, 1972; Simmons, 1973). As a result whey disposal has become a major problem for the dairy industry, where only a third of the annual production of whey is incorporated into human and animal foods (Anderson, 1970).

One of the best whey of reducing the lactose content of whey is hydrolysis with the enzyme β-galactosidase. The main catalytic activity of β-galactosidase is the hydrolysis of terminal non-reducing β-galactose residues. The hydrolysis reaction can be applied to the production of lactose-hydrolysed Whey drinks (Garman *et al*., 1996). Studies have been made on whey lactose hydrolysis using free-galactosidase (Kar *et al*., 1996; Victor Bernal and Pavel Jelan, 1985) and whole cell

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of *Kluyveromyces* (Decleire *et al*., 1985). But reports are hardly available on the effect of different pH, temperature and alkali metal ions, on whey lactose hydrolysis. Cheese whey cannot be a good substrate for β-galactosidase, because of its low pH. Required pH of enzyme is brought in whey by adding alkali, which influences enzyme activity. So, in the present study, β-galactosidase was extracted from *Kluyveromyces marxianus*, partially purified, added to whey, hydrolysis rates were followed with respect to pH, temperature and alkali metal ions.

Materials and Methods

The study was carried out in the Department of Biochemistry, V.V.Vanniaperumal College for women, Virudhunagar, Tamilnadu, India in 2002. Whey sample was obtained from Aavin, Kodai dairy, Kodaikanal.

Kluyveromyces marxianus (MTCC., 1989) was bought from microbial type culture collection center, Chandigarh. It was maintained in slants at 4°C. The cultures were activated by subculturing 3 times in sterilized whey. Required biochemicals were purchased from Himedia laboratrories Pvt Ltd., Mumbai and also from local companies.

Yeast Preparation

Yeast was propaged in whey medium according to the method of Kar *et al*. (1998).

Enzyme Extraction

β-galactosidase was extracted according to the method Citti *et al*. (1965).

Lactase Purification

The autolysate showing maximum activity from best time temperature combination was partially purified through salting out by ammonium sulfate followed by dialysis.

Lactase Activity

Lactase activity on O-nitrophenyl β-D-galactopyranoside (ONPG) was determined by incubating 0.1 mL of suitably diluted enzyme with 4 mL of 1.25 mM ONPG containing 0.1 M Potassium phosphate buffer at pH 7.0 and 37°C. After 5 min, the reaction was stopped by adding 1 mL of 0.5 M sodium carbonate. The absorbance of the nitrophenolate ion in an alkaline solution was measured at 420 nm. One ONPG unit of enzyme activity is defined as the amount of enzyme which liberates 1mMol of O-nitrophenol per minute under the conditions described.

Lactose and glucose were determined according to the methods of Nickerson *et al*. (1975). Protein was determined by a modified Folin Ciocalteau method (Toennies and Feng, 1965).

Lactose Hydrolysis in Cheese Whey

Whey with initial pH about 3.9 was taken. Two different alkali substances, sodium hydroxide and Potassium Hydroxide (5M) were used to adjust the pH of whey. Then the whey with two different pH 6.6 and 7.0 were incubated at two different temperatures, 25 and 37°C for 3 h. After the completion of the specified time intervals of 30 min, the enzyme was inactivated by placing the samples in boiling water for 3 min. Then the sample was withdrawn and analysed for the amount of glucose produced from lactose hydrolysis. From this value, the percentage of lactose hydrolysis was calculated as

$$\%H = \frac{\text{mM glucose produced}}{\text{mM initial lactose}} \times 100$$

Results and Discussion

The efficiency of whey lactose hydrolysis by lactase is compared between KOH and NaOH neutralized Whey at various temperature (25 and 37°C) and pH (6.6 and 7.0) (Table 1-4).

The highest hydrolysis percentage was observed in KOH treated whey than NaOH treated whey. 86.8% of whey lactose was hydrolysed in KOH added whey whereas NaOH addition to whey led to 63.8% hydrolysis at the end of 150 min of incubation time at pH 7.0, at 37°C. At other conditions of 37°C at pH 6.6, 74.2 and 58.5% lactose hydrolysis was obtained in KOH and NaOH treated whey, respectively after 120 min of incubation time (Table 1 and 2).

Similar effects of KOH on whey lactose hydrolysis were observed at pH 7.0, temperature 25°C and pH 6.6. Irrespective of pH and temperature, KOH activated lactase enzyme, which results in maximum lactose hydrolysis than NaOH (Table 3 and 4). This is due to the fact that the association of monovalent cations with β-galactosidase was on the basis of ionic radius with Na^+ being more tightly bound than K^+ and that both ions affected activity by inducing conformational changes in enzyme structure (Becker and Evans, 1969). So, our results suggest that KOH is much more suitable pH adjusting agent than NaOH to bring pH of the acid whey to optimum of β-galactosidase. This fact is supported by Victor and Jelen (1985). Mahoney and Adamchuk (1980); Burguess and Shaw (1983) also reported that sodium inhibits the activity of β-galactosidase, potassium increases it.

Our finding is also supported by the results of German *et al*. (1996) who reported that in the absence of added ions no activity was detectable for the enzyme from *Lactis* sp. 7692. The addition of 2 mM mg^{2+} increased the activity of the enzyme. The further addition of 100 mM K^+ led an increase in activity.

Two different pH 6.6 and 7.0 were selected based on the previous reports, at which whey lactose hydrolysis was studied by lactase from different strains. At pH 7.0, highest whey lactose hydrolysis (86.8%) was obtained than at pH 6.6 (74.2%) (Table 5). This indicates that the β-galactosidase enzyme activity was maximum at pH 7.0 for *Kluvyveromyces marxianus* (MTCC 1989) than at pH 6.6. So, pH 7.0 is the optimum pH for β-galactosidase from *K. marxianus* in whey lactose hydrolysis. So, fall in enzyme activity at pH 6.6 may be due to decreased saturation of the enzyme with substrate, due to decrease affinity or an effect of pH on the stability of the enzyme, which may become irreversibly destroyed on either side of the optimum and also due to change in the state of ionization of the components of the system as the pH changes from optimum.

Our finding is supported by the reports of Dickson *et al*. (1979) who reported that the optimum pH of 6.9 -7.3 for the lactase from *K.lactis*. It is also coincides with the results of Kuby and Lardy (1953) and German *et al*. (1960) that pH of lactase from *S. thermophilus* is 7.0 and Lactic acid bacteria is 7.0, respectively.

Our results contradicts the result of Kar *et al*. (1998) and Victor and Jelen (1985), who reported the optimum pH for lactase from *K. marxianus* and *K. fragailis* was 6.6 and 6.8, respectively in whey lactose hyrolysis.

When the effectiveness of lactase on whey lactose hydrolysis at two different temperature (25 and 37°C) was compared (Table 6), it was found out that highest lactose hydrolysis (86.8%) was observed at 37°C than at 25°C (74.2%). Decrease in percentage of hydrolysis rate below 37°C may be due to effect of temperature on the stability of the enzyme, actual velocity of breakdown of the complex determined by the heat of activation and the reaction and enzyme-substrate affinity.

Table 1: Effect of alkali metal ions on whey lactose hydrolysis for different incubation period at pH 7.0, Temperature 37°C

	Lactose hydrolysis in (%)	
Incubation time (min)	KOH	NaOH
30	40.7	22.4
60	67.6	38.2
90	82.1	61.3
120	84.0	64.2
150	86.8	63.8
180	85.3	63.17

Table 2: Effect of alkali metal ions on whey lactose hydrolysis for different incubation period at pH 6.6, Temperature 37°C

	Lactose hydrolysis in (%)	
Incubation time (min)	KOH	NaOH
30	23.4	16.4
60	60.2	26.2
90	69.4	39.2
120	74.2	58.5
150	73.4	57.6
180	73.9	56.5

Table 3: Effect of alkali metal ions on whey lactose hydrolysis for different incubation period at pH 7.0, Temperature 25°C

	Lactose hydrolysis in (%)	
Incubation time (min)	KOH	NaOH
30	13.6	9.4
60	24.9	28.6
90	52.5	34.8
120	53.2	41.9
150	57.2	40.7
180	56.5	41.2

Table 4: Effect of alkali metal ions on whey lactose hydrolysis for different incubation period at pH 6.6, Temperature 25°C

	Lactose hydrolysis in (%)	
Incubation time (min)	KOH	NaOH
30	15.4	12.4
60	23.4	22.4
90	50.5	25.9
120	50.5	38.4
150	51.6	38.1
180	50.9	37.6

Table 5: Effect of pH on whey lactose hydrolysis for different incubation period at 37°C whey is neutralized with KOH

	Lactose hydrolysis in (%)	
Incubation time (min)	pH 6.6	pH 7.0
30	23.4	40.7
60	60.2	67.6
90	69.4	82.1
120	74.2	84.0
150	73.4	86.8
180	73.9	85.3

Our findings is supported by the result of Kar *et al*.(1998) and Adamchuk (1980) who observed maximum lactase activity in lactose hydrolysis at 37°C . But the enzyme from other strains like lactic acid bacteria has optimum temperature of 38°C (German *et al*., 1996) and lactase from *K.fragilis* has 38°C (Victor and Jelen, 1985).

Table 6: Effect of temperature on whey lactose hydrolysis for different incubation period whey pH is adjusted to pH 7.0 with KOH

	Lactose hydrolysis in (%)	
Incubation time (min)	25°C	37°C
30	13.4	40.7
60	24.9	67.6
90	52.5	82.1
120	53.2	84
150	57.2	86.8
180	56.5	85.3

From the above results it is concluded that pH 7.0, temperature 37°C is the optimum condition to attain maximum percentage of lactose hydrolysis in whey using β-galactosidase from *K.marxianus* (MTCC 1989). Addition of K^+ ions in neturalising acid whey to bring it to the optimum pH of enzyme is more preferable than Sodium ions. Though this works seems to be an added data to the already existing literature review, β-galactosidase from *K. marxianus* (MTCC 1989) characterization for getting highest whey lactose hydrolysis forms a strong base to carryout further work in whey lactose hydrolysis with an aim to prepare whey lactose hydrolyzed drinks.

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