APPLIED BIOCHEMISTRY AND BIOTECHNOLOGY 7, 27–29 (1982)

A Comparison of the Formation of Intermediary Products During Lactose Hydrolysis with Free and Immobilized Thermophilic Lactase

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Accepted February 18, 1981

Index Entries: Lactose hydrolysis; immobilized lactase; immobilized enzymes, immobilized thermophilic lactase; bacillus lactase; intermediary products.

Introduction

During the last decades it became increasingly clear that the disposal of whey and whey permeate, both highly polluting byproducts from cheese manufacturing, was a serious problem.

This pollution can be avoided by using the byproducts as food or feed. A good sweetener for the food industry can be produced by hydrolysis of the lactose in whey permeate and subsequent desalting.

At NOVO Research Institute we have developed laboratory preparations of an immobilized thermophilic lactase for continuous lactose hydrolysis in whey permeate.

Process Characteristics

Our thermophilic lactase is produced by a Bacillus species, and immobilized by glutaraldehyde crosslinking of the crude cell homogenate. A laboratory column operated at 60°C, pH 7.2, with 20% w/w aqueous solution of lactose as substrate

0273–2289/82/0912–0027$02.00

showed an operational half-life equal to 600 h. The flow was adjusted to maintain 85% monosaccharides in the outlet stream. Initial activity was 1.1 g lactose/g enzyme/h. Complete hydrolysis to galactose and glucose is possible, since this lactase is less inhibited by galactose than yeast lactase.

Characterization of the Formation of Intermediary Products

This lactase and other lactases are known to be galactose transferring enzymes. The galactose moiety from a β-galactoside is transferred to an acceptor containing a hydroxyl group. The hydroxyl groups in water, carbohydrates, and some alcohols can act as acceptors.

Both monosaccharides, disaccharides, trisaccharides, and higher saccharides are possible products when lactase reacts in an aqueous lactose solution. The formation of intermediary products in the hydrolysis of lactose to monosaccharides is dependent on enzyme affinity and concentrations of possible acceptors in the microenvironment of the enzyme.

Complete hydrolysis of lactose to monosaccharides with the thermophilic lactase is a complex pattern of sequential reactions with saccharides as intermediary products. From chemical reaction theory, it is well known that the maximum concentration of intermediary products decreases when diffusion resistance is introduced in a system with sequential reactions.

Such a decrease in maximum concentration of intermediary products is seen when the thermophilic lactase is immobilized by crosslinking with glutaraldehyde. Of course the immobilization has introduced a diffusion resistance and thus generated concentration gradients of the possible acceptors in the particle. Figure 1 shows the difference in trisaccharide formation with free and immobilized enzyme.

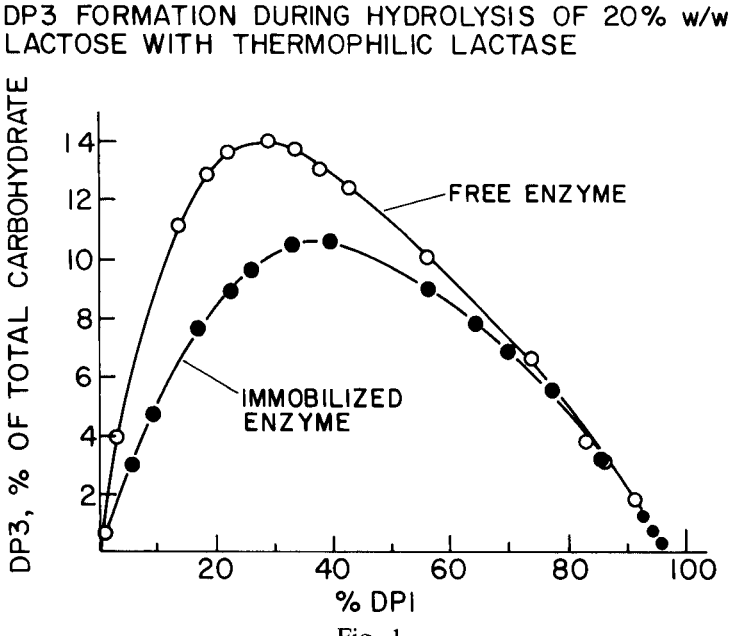

Fig. 1

TABLE I

Lactose, % w/w	Max. DP_3 immob. enzyme	Max. DP_3 free enzyme	Decrease, %	Max. DP_4^+ immob. enzyme	Max. DP_4^+ free enzyme	Decrease, %
5	5.1	6.6	22.7	1.1	1.1	0.0
10	8.6	10.2	15.7	1.4	2.1	33.3
20	10.6	13.7	22.6	2.8	4.2	33.3

The lactose concentration also affects the concentrations of intermediary products. Table 1 shows maximum DP_3 and DP_4^+ amount for 5, 10, and 20% w/w lactose with DP_3 and DP_4^+ given as % of total carbohydrate.

Conclusion

Immobilization of the thermophilic lactase causes a decrease in the formation of intermediary products. This means that partially hydrolyzed lactose syrups contain less of these intermediary products when the immobilized thermophilic lactase is used in the hydrolysis process than when a soluble lactase is used. In addition, the decrease in intermediary product formation gives a higher enzyme efficiency in the process.

Reference

Novo Enzyme Information no. 241: Novozym ™231 Immobilized Heat-stable Lactase.