Food Sci. Technol. Int. Tokyo **3** (4), 370-372, 1997

Note

Studies on the Optical Rotation of Whey Syrup Prepared by Immobilized β-Galactosidase

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Received April 11, 1997; Accepted August 29, 1997

Whey syrup was prepared by immobilized β-galactosidase and its specific rotation was determined. The rate of lactose hydrolysis and the specific rotation of whey syrup increased as the reaction time increased. The specific rotation of whey was +52.41° and that of whey syrup markedly increased and finally reached +78.82° by enzymatic reaction. The increase in specific rotation in the whey syrup indicates that its sweetness increases, and therefore, the whey syrup can be used in such foods as canned fruit, soft drinks, ice cream, and frozen yogurt as a sweetener. There was a positive correlation between the changes in specific rotation and the rate of lactose hydrolysis within a definite reaction time; therefore, a novel method based on the measurement of specific rotation can be used to calculate the rate of lactose hydrolysis and sweetness in the process of whey syrup production. Changes in specific rotation coincided with the changes in galactose content during the reaction; thus, galactose production is the most important factor which determines the changes in specific rotation in the whey syrup.

Keywords: optical rotation, specific rotation, whey syrup, immobilized β-galactosidase

In recent years, immobilized β-galactosidase has been used to produce hydrolyzed lactose products in order to improve the nutritional quality of milk and solve the whey surplus problem in the dairy industry (Bakken *et al.*, 1990; Bodalo *et al.*, 1995; Chen & Zall, 1983; Pivarnik *et al.*, 1995). When the whey is processed by immobilizing β-galactosidase, whey syrup is produced and its applications are expanded in the food processing (Chambers & Ferretti, 1979; Chiu & Kosikowski, 1985; Sprossler & Plainer, 1983; Yang & Silva, 1995). Most studies suggest that, besides glucose and galactose, many kinds of oligosaccharides are formed by mono- saccharides via the galactoside linkages during the lactose hydrolysis (Asp *et al.*, 1980; Mozaffar *et al.*, 1985; Pivarnik *et al.*, 1995; Prenosil *et al.*, 1987; Toba *et al.*, 1985).

According to the stereospecificity of saccharide, all saccharides in the whey syrup are optically active compounds, which brings about a change in optical rotation in the whey syrup during the reaction. Thus, optical rotation is an important physical property of whey syrup, and it correlates with saccharide constituents, contents and flavor, such as sweetness. In order to study the optical rotation of whey syrup, the whey was processed by immobilizing β-galactosidase to prepare whey syrup. To express the optical rotation in a meaningful way so that comparisons can be made, the specific rotation of whey syrup was determined under given conditions. Moreover, the relationship between specific rotation and rate of lactose hydrolysis, and saccharide content were investigated in the present experiment.

Materials and Methods

Immobilized β-galactosidase A commercial immobilized β-galactosidase was obtained from Sumitomo Chemical Co., Ltd., Osaka. The β-galactosidase was derived from *Aspergillus oryzae*, which was immobilized using a macroporous amphoteric-ion-exchange resin with an activity of 2000 glucose units per 1 g.

Whey In the present experiment, the whey was prepared from a demineralized whey powder which was obtained from Doom Food Ingredients, Ltd., Beilen, Netherlands. The whey powder was dissolved in deionized water, and whey proteins were removed by acidifying with HCl until the pH reached 4.2, heating at 55°C for 5 min and then centrifuging at 7000×*g* for 5 min. The obtained whey was composed of, 13.50% lactose, 0.47% protein, 0.96% minerals, and 84.48% water. As a substrate, the above whey was adjusted to pH 4.5, and the lactose content was diluted to 13.3% for the further experiments.

Sample preparation Two grams of immobilized β-galactosidase was added to 100 ml of whey and then shaken at a speed of 100 rpm at 45°C. Samples were taken and filtered rapidly to stop the reaction at different times.

Analysis of saccharide constituents of whey syrup The constituents of whey syrup were analyzed by a high performance liquid chromatography (HPLC) system which consisted of an LC-6AD pump, an RID-6A RI detector (Shimadzu, Ltd., Kyoto) and an Asahipak NH2P-50 column (4.6×250 mm, Showa Denko Co., Ltd., Tokyo). The mobile phase, a 70 : 30 mixture of acetonitrile and water, was used at a flow rate of 1 ml/min. Lactose, glucose and galactose contents were determined from standard curves, and the oligosaccharide content was determined relative to the total saccharide content.

Determination of specific rotation The specific rotations of whey syrup were determined with a model DIP-1000 polarimeter (Japan Spectroscopic Co., Ltd., Tokyo) after 1 h of reaction. By convention (Morrison & Boyd, 1987), the

Table 1. Saccharide constituents and contents of whey syrup at different reaction times.[a)]

Saccharide constituents and content (g/100 ml)	Reaction time (min)								
	0	10	20	30	40	50	60	90	120
Lactose	13.30	11.66	8.45	5.53	4.30	3.43	2.66	0.50	0.23
Glucose	0	0.80	2.33	3.82	4.43	4.80	5.21	6.31	6.45
Galactose	0	0.41	1.36	2.13	2.56	3.73	4.36	5.93	6.33
Oligosaccharide	0	0.43	1.16	1.82	2.01	1.34	1.07	0.56	0.29

[a)]Average of five replications.

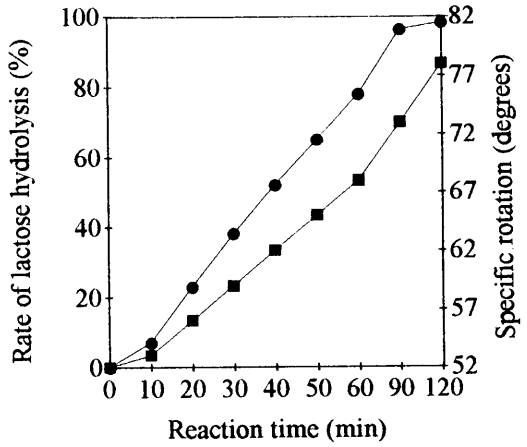

Fig. 1. Changes in rate of lactose hydrolysis and specific rotation in the whey syrup with reaction time. ● rate of lactose hydrolysis, ■ specific rotation.

conditions of determination were given as follows: a sample containing 1.00 g/dl of total saccharide was loaded into a 1-dm tube and determined by the D line (wavelength=589 nm) of a sodium lamp at 20°C. The determinations were repeated five times.

Results and Discussion

The saccharide constituents of whey syrup were analyzed by HPLC at different reaction times, and the results are shown in Table 1. The results indicated that the saccharide constituents of whey syrup were changed as the reaction time increased. Seven kinds of saccharides were detected in the whey syrup. Besides lactose, glucose and galactose, some oligosaccharides, such as tri-, tetra- and pentasaccharide were also present in the whey syrup.

Figure 1 shows the changes in lactose hydrolysis rate and specific rotation in the whey syrup with reaction time. The rate of lactose hydrolysis was not high during the first 10 min of reaction; this may be due to unstable reaction conditions. After 10 min of reaction, the rate of lactose hydrolysis increased rapidly and was close to 80% at 60 min of reaction. After 120 min of reaction, the rate of lactose hydrolysis was nearly 100%, and almost all the lactose was hydrolyzed in the whey syrup.

As shown in Fig. 1, the specific rotation of whey syrup also increased as the reaction time increased. Before reaction, the specific rotation of whey was +52.41°, which was very close to the standard value of lactose (+52.00°). After reaction, the specific rotation markedly increased and finally reached +78.82°. The major saccharides in the whey syrup, such as lactose, glucose and galactose, exist as two different isomeric forms: α- and β-isomers. Either the α- or β-isomer has its characteristic specific rotation which is an angle. However, when the α- or β-isomer is dissolved in water, the specific rotation gradually changes with time and approaches a final equilibrium value. This change is due to the formation of equilibrium consisting of the α- and β-isomers (Lehninger, 1970; Walstra & Jenness, 1984). During the reaction, the lactose content decreased, while the glucose and galactose content increased; thus a large change was caused in the equilibrium consisting of α-and β-isomers for each saccharide. As a result, the specific rotation of whey syrup changed with the reaction time.

The increase in specific rotation with lactose hydrolysis means an increase in the sweetness of the whey syrup. Zadow (1984) has reported that hydrolyzed lactose solution has a taste twice as sweet as lactose solution. The total calories of whey and whey syrup are the same, but the sweetness of whey syrup is higher than that of untreated whey. Thus, the whey syrup can be used in such foods as canned fruit, soft drinks, ice cream, and frozen yogurt as a sweetener, and its applications will be expanded relative to whey in food processing.

Moreover, we found that a positive correlation exists between the changes in specific rotation and rate of lactose hydrolysis within 60 min of reaction, and the coefficient of correlation was 0.9563. Consequently, a novel method based on the measurement of the specific rotation can be used to calculate the rate of lactose hydrolysis and sweetness in the process of whey syrup production by immobilized β-galactosidase.

On the other hand, the specific rotation of galactose is +80.01°; this value is much higher than that of lactose (+52.00°) and glucose (+52.60°). In the whey syrup, the changes in specific rotation coincided with the changes in galactose content during the reaction. Thus, the produced galactose is probably the most important factor which determines the changes in specific rotation.

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