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Effect of shaking velocity on mono-glycosyl-stevioside productivity via alternansucrase acceptor reaction

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ABSTRACT

In this study, effect of shaking velocity on mono-glycosyl-stevioside production by *Leuconostoc citreum* SK24.002 alternansucrase acceptor reaction was investigated, with four different level of shaking velocity (75, 100, 125 and 150 rpm) up to 24 h at 25 °C, using sucrose as donor and stevioside as acceptor. The results revealed that mono-glycosyl-stevioside yield significantly increased with increase in the reaction shaking velocity and reached maximum yield of 3.78 ± .02 mg/mL at 150 rpm shaking velocity after 6 h of reaction. And an increase in the reaction shaking velocity up to 150 rpm reduced the final reaction time and increased the productivity of mono-glycosyl-stevioside product such that the high quantity of mono-glycosyl-stevioside product was produced in only 6 h rather than 24 h. The mono-glycosyl-stevioside product was completely separated using macroporous resin AB-8 flowed by semi-preparative HPLC. Macroporous resin AB-8 showed high selectivity and capacity toward mono-glycosyl-stevioside. The structure of mono-glycosyl-stevioside was characterized, as 13-{[α-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl-(1→2)-β-D-glucopyranosyl]oxy}kaur-16-en-19-oic acid β-D-glucopyranosyl eater, a corroding to extensive 1D and 2D NMR (1H and 13C, COSY, HSQC, HMBC) and mass spectral data.

1. Introduction

Stevia rebaudiana Bertoni, is a perennial shrub of the *Asteraceae* (Compositae) family native to specific regions of South America (Paraguay and Brazil). It is grown commercially in a number of countries, especially in Japan, China, Korea, Thailand and Indonesia [1].

Generally, steviol glycosides (SGs) have been used widely in food and pharmaceutical industries [2]. Modern pharmacological researches observes that the stevioside and rebaudioside A, have beneficial effects on human health, such as antihyperglycemic, insulinotropic and glucagonostatic effects in the diabetic patients [3,4], hypotensive effects [5], immunologic enhancement [6] and as a potential chemopreventive agents for chemical carcinogenesis [7].

Stevioside presented at the highest amount among the steivol glucosyl constituents in the leaf of stevia. Its content ranges between 4 and 20% of the dried leaves (wt/wt) and was reported to be approximately 300 times sweeter than sucrose [8].

The major constituents in the leaves of *S. rebaudiana*, are the potently sweet diterpenoid glycosides, were stevioside, rebaudiosides A and D, and dulcoside A. Those compounds are all glycosides of the diterpene steviol, ent-13-hydroxykaur-16-en-19-oic acid [1], and their chemical structures are shown in Fig. 1 [9]. Rebaudioside A is known as the best sweetener on the base of sweetness intensity and taste quality. While the major steivol glucosyl was stevioside, which was isolated from the cultivated leaves in a yield of more than 10%, and has some of bitterness and an aftertaste [10].

Therefore, attempts have been made to glycosylate stevioside and other steviol glycosides present in the commercial product. An early work described the reaction of stevioside with soluble starch and cyclodextrin glucosyltransferase. Resulting in mono-, di- and tri-α-glucosylated stevioside products [11,12], the intensity and quality of the sweetness of some of these products, were

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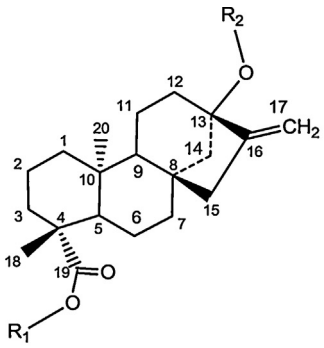

Compound	R_1	R_2
Steviol	H	H
Steviolbioside	H	β-Glc- β-Glc(2-1)
Stevioside	β-Glc	β-Glc- β-Glc(2-1)
Rebaudioside A	β-Glc	β-Glc- β-Glc(2-1) | β-Glc(3-1)
Rebaudioside B	H	β-Glc- β-Glc(2-1) | β-Glc(3-1)
Rebaudioside C (dulcoside B)	β-Glc	β-Glc- α-Rha(2-1) | β-Glc(3-1)
Rebaudioside D	β-Glc- β-Glc(2-1)	β-Glc- β-Glc(2-1) | β-Glc(3-1)
Rebaudioside E	β-Glc- β-Glc(2-1)	β-Glc- β-Glc(2-1)
Rebaudioside F	β-Glc	β-Glc- β-Xyl(2-1) | β-Glc(3-1)
Dulcoside D	β-Glc	β-Glc- α-Rha(2-1)

Fig. 1. Structure of steviol and its derivatives.

improved substantially [10]. More recently, the transglycosylation of stevioside has been achieved by using a commercial cyclodextrin glucanotransferase and cornstarch hydrolyzate. The products were mainly composed of mono- and di-glucosylated stevioside were obtained [13].

In the previous study, we conducted the biotransformation of stevioside by *Leuconostoc citreum* SK24.002 Alternansucrase acceptor reaction [15]. In this study, we investigated the effect of shaking velocity on stevioside conversion rate and mono-glycosyl-stevioside production by *L. citreum* SK24.002 alternansucrase acceptor reaction, in order to minimize the reaction time and maximize the mono-glycosyl-stevioside yield. However, the effect of shaking velocity on alternansucrase acceptor reaction has not been reported so far. Also the mono-glycosyl-stevioside was completely separated using macroporous resin AB-8 flowed by semi-preparative HPLC, and their structure was characterized using 1D and 2D NMR (1H and 13C, COSY, HSQC, HMBC) and mass spectral data.

2. Materials and methods

2.1. Chemicals and enzymes

Stevioside (purity 99%) was purchased from Wako Pure Chemical Industries, Ltd., Japan. Stevioside (purity >80%) was purchased from Aladdin Reagent Database Inc., Shanghai, China. All other chemicals were of analytical grade and were obtained from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China. Alternansucrase was prepared from *L. citreum* SK24.002 as previously described [14,15]. In brief, the cell-free culture fluid of sucrose-grown bacteria was concentrated, and the precipitate enriched in insoluble alternansucrase was harvested by centrifugation, washed and dissolved in 20 mM pH 5.4 sodium acetate buffer, and was used without further purification.

2.2. Adsorbents

Macroporous resin AB-8 was obtained from a Chemical Plant of Nankai University (Tianjin, China), the resin was pre-treated according to the manufacturer's recommendation to remove the monomers and porogenic agents trapped inside the pores during the synthesis process. In brief, before the adsorption experiments, the resin was soaked in ethanol overnight, subsequently washed with deionized water until the ethanol was thoroughly replaced by deionized water.

2.3. Effect of shaking velocity on the stevioside conversion and mono-glycosyl-stevioside production

To investigate the effect of shaking velocity on the stevioside conversion and mono-glycosyl-stevioside production, the enzymatic reactions were carried out as follow; in 10 mL tubes (15 mm diameter), 5 mL of reaction mixture contained, a fixed stevioside, sucrose and enzyme concentrations of 10 mg/mL, 10 mg/mL and