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Lipase-mediated synthesis of water-soluble plant stanol derivatives in *tert*-butanol

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ABSTRACT

The effects of solvents with different log *P* values, and of lipases on the synthesis of water-soluble plant stanol derivatives were investigated. Results showed that conversion in solvents with log $P < 0.37$ was mainly controlled by the hydrophobicity of the solvent and subsequent complete or partial deactivation of the enzyme. The solubility of substrate was the leading factor for the conversion in solvents with log $P > 0.37$. Lipozyme RM IM and *tert*-butanol was the most suitable biocatalyst and solvent, respectively. The highest yield (>51%) of plant stanyl sorbitol succinate was obtained under the selected conditions: 50 μmol/mL plant stanyl hemisuccinate, 1:3 molar ratio of plant stanyl hemisuccinate to D-sorbitol, 80 mg/mL 3 Å molecular sieves and 100 mg/mL Lipozyme RM IM in *tert*-butanol, 150 r/min and 55 °C. Fourier transform infrared spectroscopy, mass spectroscopy and nuclear magnetic resonance spectroscopy were adopted to determine the structure of product, suggesting that water-soluble plant stanol derivatives were successfully synthesized.

1. Introduction

Plant sterols (phytosterols) such as beta-sitosterol, stigmasterol, campesterol and brassicasterol can be found in small quantities in plant foods, such as vegetable oils, nuts, beans, seeds, cereals (Chien et al., 2010) and are generally extracted from the deodorizer distillates produced during vegetable oil refining and from tall oil, a by-product of the paper pulping industry (Fernandes and Cabral, 2007). Plant stanols (phytostanols), consisting of sitostanol and campestanol, are the less abundant hydrogenated counterparts of plant sterols. Usually, plant stanols can be obtained from plant sterols by chemical hydrogenation (He et al., 2010). Plant sterols and stanols have many beneficial properties, including cholesterol-lowering properties (Takeshita et al., 2008; Weingärtner et al., 2011; Hallikainen et al., 2011), anti-inflammatory, anti-atherogenic, antipyretic and antioxidant activities, as well as anti-cancer effects (Rudkowska, 2010; Brufau et al., 2008).

Many studies confirmed that plant stanols were more effective and safer than plant sterols for lowering cholesterol (Calpe-Berdiel et al., 2009; Thompson and Grundy, 2005), but, plant stanols are only minimally absorbed in vivo. In general, phytosterols are poorly absorbed in the intestine (0.4–3.5%), while phytostanols absorption (0.02–0.3%) is even lower. Plant stanols are more resistant to oxidation than plant sterols, but practical application of free plant stanols in foods is limited due to their poor solubility and low bioavailability. Free plant stanols are water insoluble and hardly soluble in fat and oil. Esterification or transesterification of free plant stanols with fat-soluble compounds, such as fatty acids, can significantly improve their lipid-solubility (Weber et al., 2001; Kim and Akoh, 2007; Morinaga et al., 2011; Hellner et al., 2010; Sengupta and Ghosh, 2011; Robles-Manuel et al., 2011; Teixeira et al., 2011). Recently, phytostanyl esters of long-chain fatty acids (C_{12}–C_{18}) were prepared by esterification using Novozym 435 lipase B from *Candida antarctica* in non-aqueous media and the cholesterol-lowering effects of plant steryl and stanyl laurate were investigated (He et al., 2010, 2011).

Some researchers have attempted to employ emulsification technique to increase the water solubility of plant stanol derivatives by incorporating phytosterols into oil-in-water microemulsions (Leong et al., 2011). However, this method is limited by poor stability of emulsion, which affected the product quality when used in foods (Engel and Schubert, 2005). In the present study, the effects of several organic solvents with different log *P* value and different lipases on enzymatic synthesis of the water-soluble plant stanol derivative stanyl sorbitol succinate were investigated. Sitostanyl sorbitol succinate was synthesized by a two-step sequence of chemical acylation with succinic anhydride followed by lipase-catalyzed esterification of D-sorbitol with sitostanyl hemisuccinate. In addition, substrate solubility, enzyme load, reaction temperature, substrate molar ratio and substrate concentration were also investigated.

2. Methods

2.1. Materials

D-Sorbitol, succinic anhydride, dimethyl sulfoxide (DMSO), acetonitrile, acetone, *tert*-pentanol, *tert*-butanol, *n*-hexane, methanol,

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