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Lipase-catalysed synthesis of erythorbyl laurate in acetonitrile

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ABSTRACT

Erythorbic acid, a stereoisomer of ascorbic acid, has been used as an antioxidant, but has poor lipophilicity. To overcome this problem, lipase-catalysed esterification between erythorbic acid and lauric acid was performed to synthesise the erythorbyl fatty acid ester in acetonitrile; subsequently, the time course of the enzymatic esterification was monitored, using HPLC equipped with RI- and UV-detectors. A steady state was achieved after 8 h, from initiation of the esterification, and the degree of esterification (molar conversion yield) reached 78.5%. After the purification procedure, the product synthesised by immobilised lipase (Novozym® 435) was identified as erythorbyl laurate (6-*O*-lauroyl-erythorbic acid) by LC–ESI-MS, ^{1}H, and ^{13}C NMR analysis. The results suggest that erythorbyl laurate, produced in this study, could be used as an emulsifier with antioxidant activity to retard oxidation of lipid foods.

1. Introduction

Polyphenols, which have high reducing power, are widely used as antioxidants in the food and cosmetic industries (Rice-evans, Miller, Bolwell, Bramley, & Pridham, 1995). Ascorbic acid is a natural antioxidant that has limited application in lipid-based products, due to its low lipophilicity (Karmee, 2009).

Synthetic polyphenols, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), are typical antioxidants, used to retard lipid oxidation of foods. However, they may possibly degrade into carcinogenic or toxic components under physiological conditions during storage (Criado, Allevi, Ceballos, & Garcia, 2007; Ito, Fukushima, & Tsuda, 1985; Kaitaranta, 1992).

For these reasons, research on synthesis of naturally occurring antioxidants and their lipophilic derivatives has garnered much attention (Shahidi & Wanasundara, 1992; Stamatis, Sereti, & Kolisis, 1999). Among the methods for production of ascorbyl fatty acid esters, enzymatic synthesis would be recommended on account of its high regioselectivity and mild reaction conditions (Song & Wei, 2002; Watanabe, Adachi, Nakanishi, & Matsuno, 1999).

The enzymatic synthesis of ascorbyl fatty acid esters has been extensively studied, with regard to the effect of various reaction parameters on the yield of conversion (Humeau, Girardin, Coulon, & Miclo, 1995; Kuwabara, Watanabe, Adachi, Nakanishi, & Matsuno, 2003; Watanabe, Kuwabara, Adachi, Nakanishi, & Matsuno, 2003). This research has included studies on a variety of acyl donors and organic solvents for the enzymatic synthesis (Song, Wei, Zhou, Xu, & Yang, 2004; Viklund, Alander, & Hult, 2003; Watanabe, Adachi, Nakanishi, & Matsuno, 2001) and optimisation of conditions for the enzymatic esterification by response surface methodology (Chang, Yang, Chen, Akoh, & Shieh, 2009).

No report on the enzymatic synthesis of erythorbyl fatty acid esters has been published. Erythorbic acid, a stereoisomer of ascorbic acid and a food additive, has antioxidative activity and should be available at relatively low cost because calcium 2-ketogluconate is readily converted to erythorbic acid during the fermentation of glucose (Swern, Stirton, Turer, & Wells, 1943). It has also recently been reported that erythorbic acid possesses the ability to enhance bioavailability of non-haem iron (Fidler, Davidsson, Zeder, & Hurrell, 2004).

The objective of this study was to synthesise erythorbyl laurate, which could be used as an antioxidant for lipid-based foods and as a functional emulsifier. The lipase-catalysed esterification between erythorbic acid and lauric acid, a medium-chain fatty acid, in an organic solvent system, was investigated in this work and the product was identified using LC–ESI-MS, ^{1}H, and ^{13}C NMR analysis.

2. Materials and methods

2.1. Materials

Immobilised lipase, from *Candida antarctica* (triacylglycerol hydrolase, EC 3.1.1.3; Novozym® 435), was kindly provided by

Abbreviations: HPLC, high performance liquid chromatography; LC–ESI-MS, liquid chromatography–electrospray ionisation-mass spectrometry; NMR, nuclear magnetic resonance; DMSO, dimethyl sulfoxide.

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Novozymes (Bagsvaerd, Denmark) with a reported catalytic activity of 7000 PLU/g (the activity of PLU refers to the millimoles of propyl laurate synthesised per minute at 60 °C). Erythorbic acid (⩾99.0%) and lauric acid (⩾99.0%) were purchased from Fluka Co. (Buchs, Switzerland) and Sigma–Aldrich Co. (St. Louis, MO, USA), respectively. HPLC-grade acetonitrile (J.T. Baker Co., Phillipsburg, NJ, USA) was dehydrated by molecular sieve 4 Å (Dae Jung Chemicals and Metals, Siheung, Korea) and filtered using a membrane filter (0.45 μm) prior to use as a reaction medium. All other chemicals were of extra pure grade and were used without further purification.

2.2. Procedure for lipase-catalysed esterification

Erythorbic acid (0.12 mmol) and lauric acid (0.60 mmol) were placed in a screw-capped glass vial with 20 ml of acetonitrile and pre-incubated at 50 °C for 30 min using an orbital shaking water bath (200 rpm). The reaction was initiated by adding 200 mg of immobilised lipase to the mixture. The temperature was kept constant at 50 ± 1 °C during the reaction. The reaction mixture was sampled at appropriate intervals and filtered through a membrane filter (0.45 μm); then, 20 μl of each aliquot were injected into the HPLC for further analysis.

2.3. HPLC analysis

Quantitative analysis of synthesised erythorbyl laurate was carried out using an HPLC (LC-2002, Jasco, Tokyo, Japan) equipped with a silica-based column (5 μm, 4.6 × 150 mm: Luna C_{18}, Phenomenex, Torrance, CA, USA), a refractive index detector (RI-2031, Jasco), and an ultraviolet detector (UV-2075, Jasco). The mobile phase was acetonitrile/water/acetic acid (90:5:5, v/v/v) at 1.0 ml/min flow rate for 15 min. Peaks in the HPLC chromatograms were identified using retention times of erythorbic acid standards and its lauric acid ester, purified in this work. The degree of esterification (molar conversion yield, %) was defined by the following equation and estimated using the peak areas at 256 nm integrated by Borwin software (Ver. 1.21, JASCO Corp.).

$$\text{Degree of esterification } (\%) = \frac{\text{erythorbyl laurate}}{\text{erythorbic acid} + \text{ erythorbyl laurate}} \times 100$$

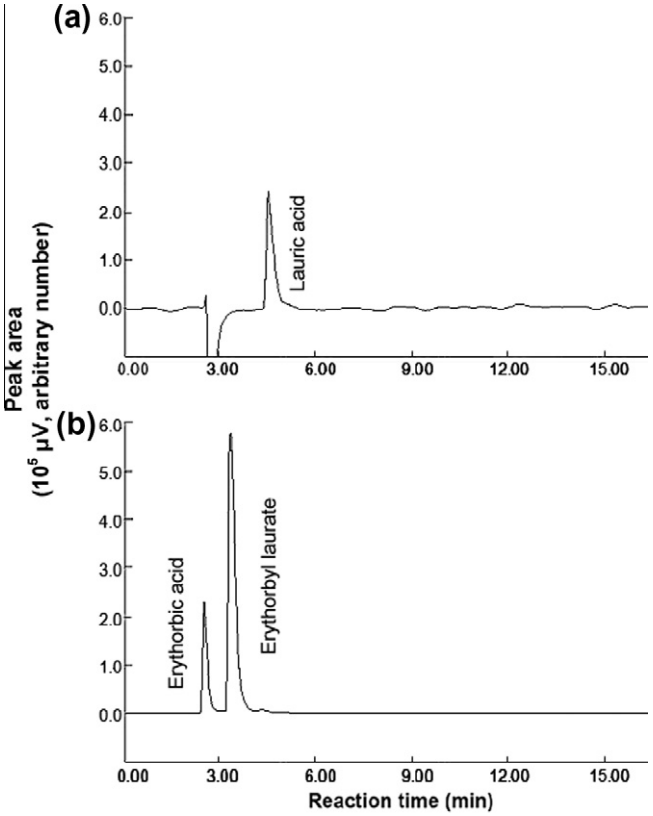

Fig. 2. HPLC chromatograms for the components obtained from the immobilised lipase-catalysed esterification between erythorbic acid and lauric acid in acetonitrile (a, RI-; b, UV-detector).

All of the data were the averages of triplicate samples and were reproducible within ±10%.

2.4. Purification and structural analysis of erythorbyl laurate by LC–ESI-MS, ^1H, and ^{13}C NMR

After enzymatic esterification, erythorbyl laurate was isolated from the reaction mixture according to the reported method in

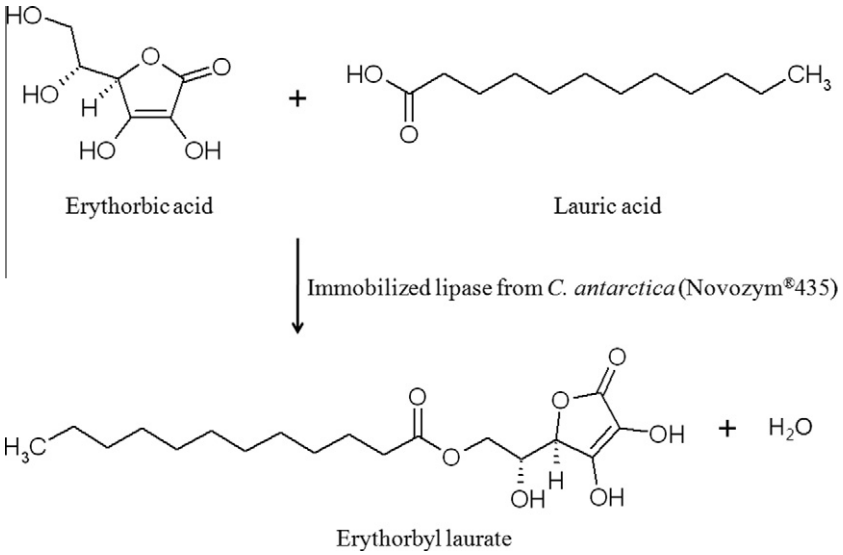

Fig. 1. Scheme of lipase-catalysed synthesis of erythorbyl laurate in acetonitrile.