Bioresource Technology 128 (2013) 156–163

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Enhancement of the selective enzymatic biotransformation of rutin to isoquercitrin using an ionic liquid as a co-solvent

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HIGHLIGHTS

- Ionic liquid-containing buffer system was first applied in conversion of rutin.
- Both [Bmim][BF_4] and [Emim][BF_4] improve selective synthesis of isoquercitrin.
- [Bmim][BF_4]-glycine-sodium hydroxide buffer (pH 9.0) (10:90, v/v) is the best.
- The highest rutin conversion and isoquercitrin yield were 93.40% and 91.41%.

ARTICLE INFO

Article history:
Received 16 July 2012
Received in revised form 19 October 2012
Accepted 23 October 2012
Available online 1 November 2012

Keywords:
Biocatalysis
Co-solvent
Hesperidinase
Ionic liquid
Isoquercitrin

ABSTRACT

An ionic liquid (IL)-containing buffer system was first applied in the conversion of rutin to isoquercitrin. High substrate solubility was achieved to enhance the selectivity and efficiency of hesperidinase-catalyzed reaction. Ten ILs were selected as co-solvents to assist catalytic reactions in this biotransformation process. The transformed products of rutin were identified by LC–MS. The [Bmim][BF_4]-glycine-sodium hydroxide buffer (pH 9) (10:90, v/v) was found to be the best medium for the biotransformation of isoquercitrin from rutin with higher selectivity and efficiency. The reaction time was reduced by 0.33-fold while the conversion of rutin and the yield of isoquercitrin were increased by 1.67-fold and 2.33-fold. The results suggest that IL co-solvents have great potential to enhance the selectively enzymatic hydrolysis of rutin for isoquercitrin production.

1. Introduction

Isoquercitrin, a rare flavonol glycoside distributed around the world, possesses a wide range of biological activity (i.e., anti-oxidant, anti-hypertensive, anti-cancer, anti-influenza, anti-proliferative, and diuretic activities) (Gasparotto et al., 2012; Masuoka et al., 2012; Wang et al., 2012a). Isoquercitrin is a key synthetic intermediate for the production of enzymatically modified isoquercitrin (EMIQ), which was recently approved as a multiple food additive in Japan and in the USA (Emura and Tanaka, 2010; Schmandke, 2010; Shimada et al., 2010). The application of isoquercitrin in the medicinal and food industries is also believed to hold much promise for the future (Makino et al., 2009; Nielsen et al., 2006). However, the isolation of this highly valuable isoquercitrin from extracts obtained from a plant is inefficient, time-consuming, and uneconomical (Weignerová et al., 2012). To produce isoquercitrin at a reasonable price, the transformation of rutin to isoquercitrin has been proved to be a feasible procedure because rutin is much more widely distributed in numerous plants than isoquercitrin (Gerstorferová et al., 2012; Wang et al., 2012b; You et al., 2010).

Several methods for the transformation of rutin to isoquercitrin have been investigated, including acid hydrolysis (Wang et al., 2011), heating (Weignerová et al., 2012), microbial transformation (Rajal et al., 2009; Tamayo-Ramos et al., 2012), and enzymatic transformation techniques (Gerstorferová et al., 2012; Wang et al., 2011). Among these methods, enzymatic hydrolysis was regarded as the most promising method for the preparation of isoquercitrin via the selective hydrolysis of rutin at the rhamnose moiety due to high specificity, yield, and productivity. We recently found that a commercial hesperidinase was an excellent biocatalyst for the production of isoquercitrin from rutin with high selectivity (Wang et al., 2012a). Hesperidinase (hesperidin-α-1,

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http://dx.doi.org/10.1016/j.biortech.2012.10.098

6-rhamnosidase, EC 3.2.1.40) is an enzyme preparation containing two active enzymes, α-L-rhamnosidase, which splits rutin into rhamnose and isoquercitrin, and a β-D-glucosidase (EC 3.2.1.21), which subsequently splits the isoquercitrin into glucose and quercetin (Wang et al., 2011). Compared with those crude and recombinant α-L-rhamnosidases, the commercially available hesperidinase may become more technically feasible catalyst for the biotransformation of rutin to isoquercitrin via controlling pH instead of temperature (Wang et al., 2012a). However, a further industrial application of the hesperidinase-catalyzed transformation is limited by low substrate solubility, slow catalytic efficiency, and a long reaction time.

Ionic liquids (ILs) represent a class of promising and 'green' non-molecular solvents that have increasingly attracted attention as the green, high-tech reaction media of the future (Yang et al., 2012; Zhang et al., 2008). Like conventional water-miscible organic solvents, these ionic solvents work as co-solvents to control the activity of water, thus inhibiting the second hydrolysis of the glycosylated products and improving the yield (Yang et al., 2012). As alternative reaction media, ionic liquids have enormous potential due largely to the ability to tailor their properties through appropriate cation, anion, and substituent selection (van Rantwijk and Sheldon, 2007). In the case of the enzymatic synthesis of isoquercitrin from rutin, substrate solubility is considered to be a major limiting factor governing the overall rate and extent of the reaction. Although rutin suspended in the buffered enzyme solution could keep the concentration saturated in the biocatalytic system at all times (the concept of an 'immobilized substrate' (Weignerová et al., 2012), the substrate concentration was not improved. It is possible to use IL as a co-solvent to increase the dissolution of the substrates that will have a significant effect on the conversion, yield and selectivity of the enzymatic reaction system. However, to the best of our knowledge, no report has been published concerning the use of this straightforward process to significantly enhance both reactivity and selectivity in the enzymatic transformation of rutin to isoquercitrin.

The aim of this study was to establish a new catalytic system containing an IL as a co-solvent for the production of isoquercitrin from rutin catalyzed by commercially available hesperidinase. Ten types of ILs were selected as co-solvents in the enzymatic reaction media, and the effects of pH value, IL concentration, substrate concentration, enzyme concentration, and reaction time on the rutin conversion, isoquercitrin yield, and quercetin yield were assessed, respectively. The transformed products of rutin in the new IL-containing co-solvent system were identified by liquid chromatography–mass spectrometry (LC–MS).

2. Methods

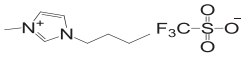
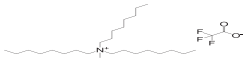
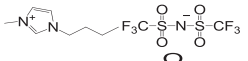
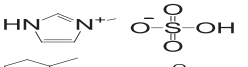
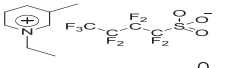
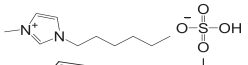
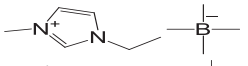
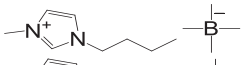
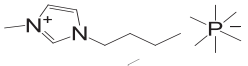
2.1. Materials

Hesperidinase (contains both α-L-rhamnosidase and β-D-glucosidase activities, ⩾1 units/g solid, produced by *Aspergillus niger*) and isoquercitrin standard were from Sigma Chemical Co. (St. Louis, MO, USA). Standards of rutin and quercetin were from the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP) (Beijing, China). Ten ILs, as listed in Table 1, were from Shanghai Cheng-Jie Chemical Co., Ltd. (Shanghai, China). The residual chloride content in these ILs was less than 50 ppm. Methanol and acetonitrile were of HPLC-grade (Tedia Co., Fairfield, OH, USA), and the other reagents and solvents were of analytical (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China). Water was purified using an Elga Purelab Option-Q purification system (Elga Labwater, High Wycombe, Bucks, UK) and its resistivity was not less than 18.0 MΩ cm. This water was used for cleaning procedures and in the preparation of all buffer solutions.

2.2. Enzymatic biotransformation

All enzymatic reactions were carried out in a temperature-controlled incubator shaker. In this study, disodium hydrogen phosphate-citrate buffer (pH 3–8) and glycine-sodium hydroxide buffer (pH 9–10) were used. In a typical experiment, rutin buffer solution was added with the IL to a 10 mL screw-capped vial. The reaction was started by adding the buffered solution of hesperidinase, and the mixtures were incubated for different amounts of time at various pH values, IL concentrations, substrate concentrations, enzyme concentrations, and reaction times while the other

Table 1
Structures of the selected ILs used as co-solvents in this study.

No.	Name	Abbreviation	Structure
1	1-Butyl-3-methylimidazolium trifluoroacetate	[Bmim][TfO]	
2	Trioctylmethylammonium trifluoroacetate	[TOMA][TfO]	
3	1-Butyl-3-methylimidazolium bis[(trifluoromethyl)sulfonyl] imide	[Bmim][Tf_2N]	
4	N-Methylimidazolium hydrosulfate	[Nmim][HSO_4]	
5	1-Ethyl-3-methylpyridinium perfluorobutanesulfonate	[EMPY][$C_4F_9SO_3$]	
6	1-Hexyl-3-methylimidazolium hydrosulfate	[Hmim][HSO_4]	
7	1-Ethyl-3-methylimidazolium tetrafluoroborate	[Emim][BF_4]	
8	1-Butyl-3-methylimidazolium tetrafluoroborate	[Bmim][BF_4]	
9	1-Butyl-3-methylimidazolium hexafluorophosphate	[Bmim][PF_6]	
10	Trioctylmethylammonium bis[(trifluoromethyl) sulfonyl]imide	[TOMA][Tf_2N]