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Preparatory production of quercetin-3-β-D-glucopyranoside using alkali-tolerant thermostable α-L-rhamnosidase from *Aspergillus terreus*

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ABSTRACT

Extensive screening for a robust producer of α-L-rhamnosidase activity from well-defined strains of filamentous fungi, including multifactorial optimization (inducers, cultivation conditions) was accomplished. Enzyme production of the optimal producer *Aspergillus terreus* (non-toxigenic) was scaled up to 50 L. α-L-Rhamnosidase, which was fully characterized, proved to be thermo- and alkali-tolerant, thus enabling effective operation at 70 °C and pH 8.0. These conditions allow for a very high substrate (rutin) load up to 100–300 g/L, thus enabling very high volumetric productivity of the reaction product quercetin-3-β-D-glucopyranoside (isoquercitrin). Here, a novel concept of "immobilised substrate" is used. Isoquercitrin is a highly effective and biocompatible antioxidant with strong anti-inflammatory activities. Rutin biotransformation was optimized and scaled up to ca 10 kg production and thus the robustness of the large-scale production was demonstrated. Isoquercitrin can be produced to a very high purity (98%) in multikilogram amounts, without any quercetin and directly applicable in nutraceuticals.

1. Introduction

Flavonoids are a large group of polyphenolic compounds that occur ubiquitously in vascular plants and therefore are a regular component of the human diet. Many flavonoids have antioxidant, antiinflammatory, antiallergenic, antibacterial, and generally chemoprotectant capabilities (Formica and Regelson, 1995; Rice-Evans et al., 1996; Hollman and Katan, 1997; Middleton et al., 2000; Terao et al., 2008). Moreover, a number of epidemiologic studies indicated the protective effects of flavonoids against cardiovascular diseases and cancer (Hertog et al., 1995; Hollman et al., 1996; Knekt et al., 1997; Xing et al., 2001). Quercetin (**3**, 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-1-benzopyran-4-one) (for all structures and the reaction scheme see Supplementary data, Fig. 1S) is one of the most abundant flavonoids present in plants; it occurs in high concentrations in onions, apples, tea, and many other natural sources. However, the low oral bioavailability of quercetin, due to its insolubility in water, limits its use as a food additive or dietary supplement. Recent studies indicate that glycosyl conjugation substantially enhanced the bioavailability of quercetin (Lesser et al., 2004). Isoquercitrin (**2**, quercetin-3-β-D-glucopyranoside; 2-(3,4-dihydroxyphenyl)-3-β-D-glucopyranosyloxy-5,7-dihydroxy-4*H*-1-benzopyran-4-one) is the most common naturally occuring quercetin derivative (denoted also as Bioquercetin) with a higher water solubility than quercetin and better intestinal absorption than rutin (**1**, 3-[[6-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranosyl]oxy]-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4*H*-1-benzopyran-4-one) (Cermak et al., 2003; Shimoi et al., 2003; Makino et al., 2009; Jung et al., 2010).

Moreover, quercetin – despite the fact that it is one of the best flavonoid antioxidant – has a rather poor reputation, giving a positive Ames test. This may be caused by its intercalation into DNA, causing topoisomerase II inhibition. Quercetin glycosides, which maintain the antioxidant activity of quercetin, do not interact with DNA (Webb and Ebeler, 2004). Therefore, the availability of, e.g. quercetin-3-β-D-glucopyranoside at a good quality (devoid of free quercetin) and at a reasonable price for application in nutraceuticals is of the utmost importance.

Probably the most feasible procedure for preparing quercetin-3-β-D-glucopyranoside is selective enzymatic "trimming", e. g. derhamnosylation of rutin, which is available for an affordable price at pharmaceutical quality. Rutin is typically obtained from the Brazilian tree Fava d'anta (*Dimorphandra mollis*). Enzymatic derhamnosylation of rutin has, however, numerous technical problems: (i) the selectivity of enzymatic glycoside hydrolysis thus avoiding the generation of (highly undesirable) free quercetin; (ii) the generally low water solubility of all quercetin derivatives, including glycosides; (iii) the high sensitivity of all quercetin derivatives to oxidation; (iv) the strict necessity of avoiding the use or presence of any heavy metals due to their strong complexation by quercetin (generally, by most flavonoids). The required biotechnological

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