Tyrosol β-rutinoside prepared by transrutinosylation using Fagopyrum tataricum seed meal

Elena Karnišová Potocká[1], Iveta Čičová[2], Mária Mastihubová[1], Vladimír Mastihuba[1]

[1]*Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovakia*

[2]*Research Institute of Plant Production, Piešťany, Slovakia*

Introduction

Therapeutic benefits of tyrosol and hydroxytyrosol and their glycosides were observed in several studies including anticancer [1], anti-inflammatory [2], antiviral [3], antidiabetic [4], neuroprotective [5], hepatoprotective [6] and cardioprotective [7]. Synthesis of these glycosides can be achieved with glycosidases. Despite their natural role of hydrolysis, glycosidases are also able to synthetise glycosidic bond through two ways, thermodynamically controlled reverse hydrolysis or kinetically controlled transglycosylation. Rutinosidase or rutin hydrolase (EC 3.2.1.168) hydrolyzes rutinosides such as rutin and hesperidin to rutinose and the respective aglycones and is able to transfer the whole rutinosyl moiety from a polysaccharide, oligosaccharide or glycoside to a nucleophile acceptor (alcohol or another molecule of saccharide) via transglycosylation in one step (in opposite of sequentional synthesis with monoglycosidases). Recently, tyrosol rutinoside was synthesized by our group [8] in isolated yield of 24 % using rutinosidase from flower buds of *Sophora japonica*. The study presented in this poster focusses on optimisation of rutinosylation process with another biocatalyst (seed meal of *Fagopyrum tataricum*) using kinetically controlled transglycosylation.

Experiment

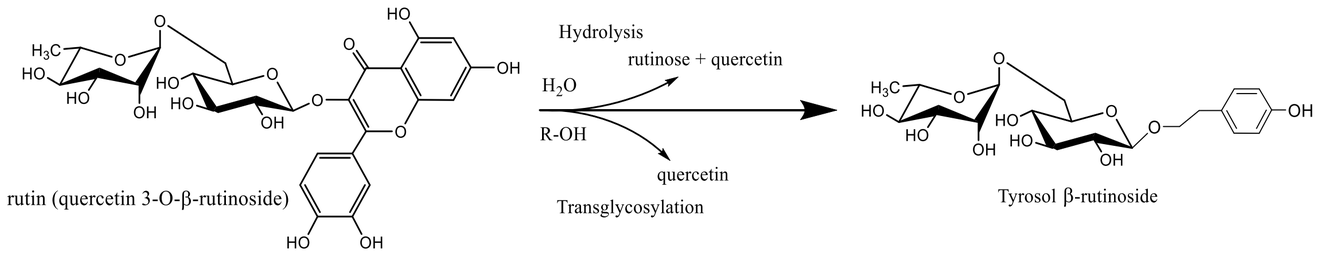
Seeds of *F. tataricum* were homogenised, sieved (≤ 0.1mm), defatted in Soxhlet extractor for 12 hours with ethanol, dried at laboratory temperature and stored in refrigerator (up to 10 °C) before use. Samples and preparative reactions were incubated in thermobox at 37 °C and 400 rpm and samples for HPLC analysis (50 μl) were quenched in boiling water bath, then diluted to 15 % ACN and filtered through 0.22 μm syringe filter. HPLC measurements were conducted on Sunshell C18 column (4.6 x 150 mm, 5μm) by ChromaNik Technologies Inc. (Osaka, Japan) equilibrated and eluted with gradient of acetonitrile (A) in water (B) at 30 °C and flow rate 0.7 mL/min. Tyrosol and glycosides were detected at 275 nm, while rutin and quercetin at 254 nm. Gradient elution was conducted in following order: 0 – 4 min 15 % A; 4 – 5 min 15 – 40 % A; 5 – 10 min 40 % A; 10 – 11 min 40 – 15 % A; 11 – 15 min 15 % A. ^{1}H NMR (400 MHz) and ^{13}C NMR (101 MHz) spectra were recorded with a 400 MHz Bruker AVANCE III HD 400 MHz equipped with a Prodigy CryoProbe and analysed with MestreNova software. Flash chromatography was performed on Isolera One from Biotage (Uppsala, Sweden), with UV detection (silicagel column - gradient of methanol in chloroform, Diaion HP-20 gradient of methanol in water, Aluminium oxide - elution with water). Elution was monitored at 275 nm and fractions were simultaneously analysed using thin layer chromatography (TLC)

Results

Transrutinosylation of tyrosol was conducted according to the Scheme 1 using defatted seed meal of tartary buckwheat as the source of β-rutinosidase. Hydrolysis of rutin to quercetin and rutinose also occured as a simultaneous reaction. Plant material has been previously extracted to ensure stability.

Reaction with tyrosol as the acceptor was optimised according to pH, amount of the catalyst and concentration of rutin and tyrosol.

Weakly acidic environment had only slightly affected the conversion, but increased oxidation products of rutin and quercetin have been observed at pH above 7 (data not shown), therefore, for more stable conditions, pH 6.5 was used for following experiments. Increasing of amount of catalyst had positive effect as well as increasing of the initial concentration of rutin, but higher amounts than used in optimisation had negative effect on miscibility of reaction. Rutin has much higher solubility than quercetin in water, hence hydrolysis of rutin results in production of biphasic mixture with precipitated quercetin. In contrary with previous results with rutinosidase from Sophora flower buds [8], the initial tyrosol concentration negatively influenced conversion of the product. Therefore, following conditions were used for preparative reaction: pH 6.5, 33 mM rutin, 72 mM tyrosol and catalyst in amount 3% (w/vol.). The **maximal conversion** of tyrosol rutinoside achieved more than **61 %** (respective to rutin) and the **isolated yield 35% with purity ca. 97 %**. The rutinosylation proceeds regioselectively and **results in formation of only one product** glycosylated on the primary hydroxyl of tyrosol (as confirmed by NMR).



Scheme 1: Kinetically controlled reaction leads to simultaneous production of transglycosylation product and hydrolysis of the substrate

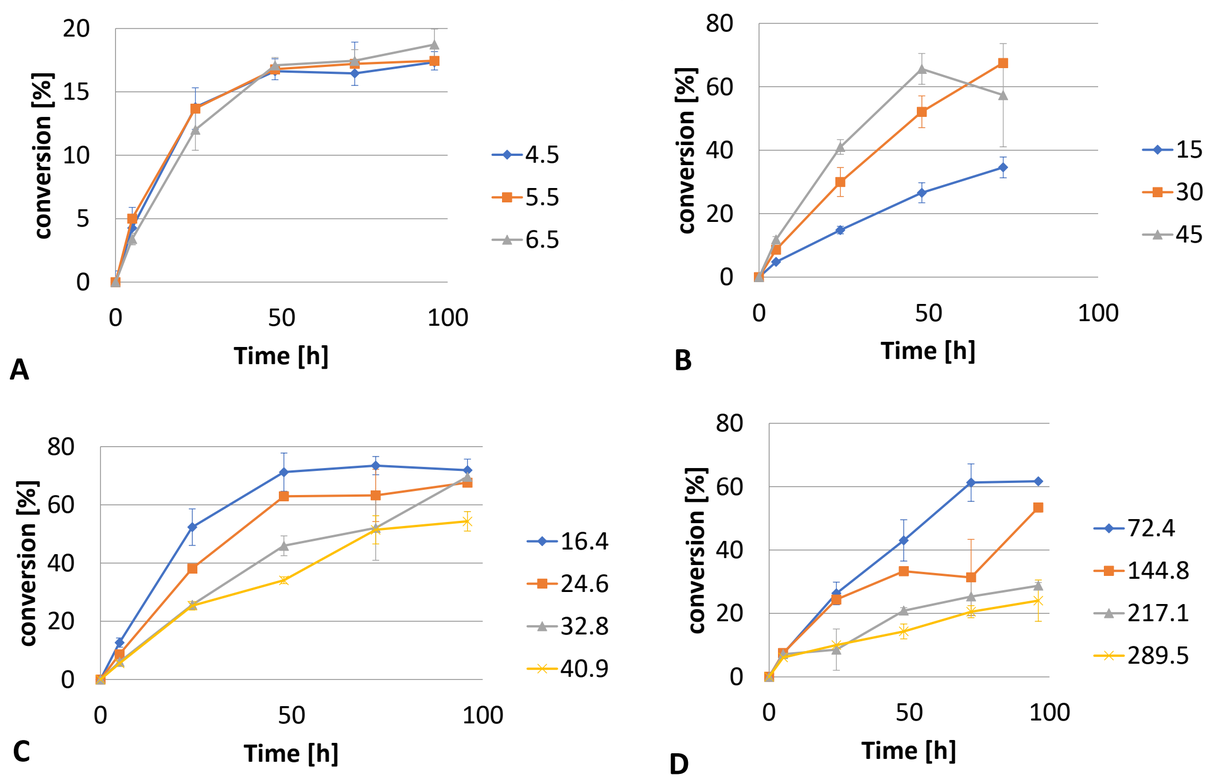


Fig. 1: Optimisation of tyrosol rutinoside synthesis according to pH (A), amount of catalyst [mg/ml] (B) and concentration of donor (C) and acceptor [mM] (D).

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