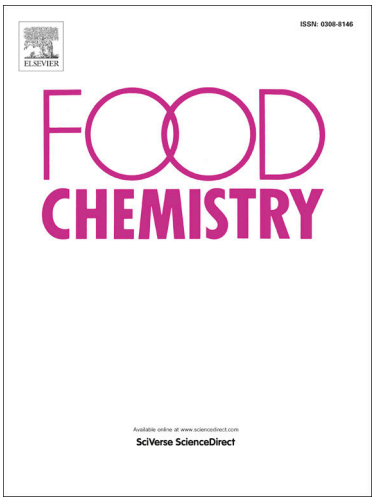
Journal Pre-proofs



Short communication

Transrutinosylation of tyrosol by flower buds of *Sophora japonica*

Elena Karnišová Potocká, Mária Mastihubová, Vladimír Mastihuba
Conceptuation

PII: S0308-8146(20)31536-3
DOI: https://doi.org/10.1016/j.foodchem.2020.127674
Reference: FOCH 127674

To appear in: *Food Chemistry*

Received Date: 14 February 2020
Revised Date: 20 July 2020
Accepted Date: 23 July 2020

Please cite this article as: Karnišová Potocká, E., Mastihubová, M., Mastihuba Conceptuation, V., Transrutinosylation of tyrosol by flower buds of *Sophora japonica*, *Food Chemistry* (2020), doi: https://doi.org/10.1016/j.foodchem.2020.127674

Transrutinosylation of tyrosol by flower buds of *Sophora japonica*

Elena Karnišová Potocká, Mária Mastihubová, Vladimír Mastihuba*

Institute of Chemistry, Slovak Academy of Sciences,

Dúbravská cesta 9, SK-845 38 Bratislava, Slovakia, *vladimir.mastihuba@savba.sk*

Abstract

Dried flower buds of Japanese sophora (*Sophora japonica*) comprising rutinosidase activity were tested in rutinosylation of tyrosol via transglycosylation process from rutin. Optimal conditions for transrutinosylation of tyrosol were 49 mM rutin and 290 mM tyrosol, giving maximum conversion up to 66.4 % and 24 % yield of isolated and purified rutinoside. The rutinosylation proceeded exclusively on the primary hydroxyl of tyrosol, thus forming rhamnosylated derivative of salidroside. This strict regioselectivity differentiates the sophora biocatalyst from microbial rutinosidases.

Keywords: rutinosidase; rutin, *Sophora japonica*; tyrosol rutinoside; transglycosylation

1. Introduction

Tyrosol and hydroxytyrosol are low-molecular phenolic antioxidants occurring in olive oil and in by-products from olive production and processing (Wani *et al.*, 2018; Conde, Cara, Moure, Ruiz, Castro & Domínguez, 2009). Their esters may find use in stabilization of lipids and emulsions (Sun, Zhou & Shahidi, 2018; Akanbi & Barrow, 2018), while glycosides of tyrosol and hydroxytyrosol display wide spectra of pharmacological activities including anticancer (Sun, Wang, Zheng & Zhang, 2012), antiviral (Wang, Ding, Zhou, Sun & Wang, 2009), antiinflammatory (Guan *et al*., 2011), antidiabetic (Li, Ge, Zheng & Zhang,

2008), neuroprotective (Nieto-Dominguez *et al.*, 2017), cardioprotective (Liang *et al.* 2010; Zhang *et al*. 2009) and hepatoprotective (Wu, Lian, Jiang & Nan, 2009).

Enzymatic glycosylations catalyzed by glycosidases offer an effective, straightforward and environmentally friendly alternative to rather complicated and oftentimes harmful chemical procedures. Generally, two methods may be employed in one-step enzymatic preparation of glycosides catalyzed by glycosidases – transglycosylation and reversed hydrolysis. Reversed hydrolysis is a thermodynamic process leading to equilibrium between the target glycoside and starting monosaccharide while transglycosylation employs partial transferase activity of glycosidases. Glycosidase thus catalyzes the transfer of monosaccharide from the nonreducing end of the substrate (polysaccharide, oligosaccharide, or glycoside) to a nucleophil (an alcohol or another molecule of the saccharide) present in the reaction mixture (van Rantwijk, Woudenberg-van Oosterom & Sheldon 1999). The presence of transferase activity is limited to retaining glycosidases and its displayed level in particular enzyme varies depending on its origin and reaction conditions.

Within our research on enzymatic syntheses of natural tyrosol glycosides and their new derivatives, we have recently prepared salidroside (tyrosol β-D-glucopyranoside) and its α- and β-D-galacto- and β-fructofurano- analogues by use of microbial enzymes (Potocká, Mastihubová & Mastihuba, 2015; Karnišová Potocká, Mastihubová & Mastihuba, 2019).

Enzymatic synthesis of salidroside by reversed hydrolysis is a well studied process, employing usually plant β-glucosidases (Bi *et al.*, 2012) Recently published enzymatic preparations of pure glycosides of tyrosol and hydroxytyrosol such as β-glucosides (Méndez-Líter *et. al.*, 2019), β-galactosides (Qi, Gu, Xu, Xiao & Lu, 2017), β-fructofuranosides (Míguez *et. al.*, 2018) and β-xylopyranosides (Nieto-Dominguez *et al.*, 2017) rely however on the more advantageous transglycosylation process.

To extend the scale of glycosides of tyrosol for studies of their acylations toward natural pharmacologically active substances, we focused on preparation of a structured oligoglycoside of tyrosol, namely 2-(4-hydroxyphenyl)ethyl β-rutinoside (TR1), which would be free of its structural analogue TR2 (Figure 1).

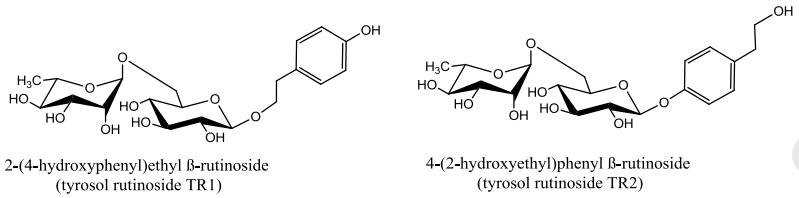


Figure 1. Structure of isomers of tyrosol β-rutinosides.

Kinetically controlled transrhamnosylation of the corresponding glucoside, salidroside, is not possible due to the inverting mechanism of α-L-rhamnosidases and these enzymes can therefore produce rutinosides only through reversed rhamnosylation. In addition to the obvious problems of the reversed hydrolysis of glycosides (long reaction times, low chemical yields), the price of rhamnose used in high starting concentration makes the reversed rhamnosylation rather unpractical. A more convenient way how to obtain TR1 may be use of rutinosidase for direct rutinosylation of tyrosol. Rutinosidases (EC 3.2.1.168) belong to a special group of β-glycosidases, so-called diglycosidases, which are specific to hydrolysis of diglycosides comprising β-D-glucose on the reducing end of the glycone. Diglycosidases (rutinosidases, acuminosidases, vicianosidases, primeverosidases) occur more typically in plant tissues, although they can be produced by microorganisms as well. Their main role in the plant kingdom is an immediate hydrolysis of diglycosides (such as cyanogenic or terpenyl glycosides) and fast liberation of toxic or semiotic aglycones in one-step mechanism (Mizutani *et al.*, 2002). Rutinosidases can be found among plants and some filamentous fungi. They hydrolyze rutinosides like rutin and hesperidin into rutinose and the corresponding aglycone. Some rutinosidases were found to be able to perform also the transrutinosylation reactions, which means that they do not

transfer monosaccharide from the non-reducing end of present rutinoside, but whole disaccharide (rutinose) is attached to the present aglycone, thus forming a new type of rutinoside. Wide variety of β-rutinosides possessing arylalkyl and linear or branched aliphatic aglycones has been so far prepared with rutin or hesperidin as sources of rutinose (Šimčíková *et al.*, 2015; Zhou *et al*., 2009; Mazzaferro & Breccia, 2012; Mazzaferro, Pinuel, Minig & Breccia, 2010; Minig, Mazzaferro, Erra-Balsells, Petroselli & Breccia, 2011; Katayama *et al*., 2013). Various terpenyl rutinosides and phenolic glycosides were also synthetized with use of rutinosidase from tartary buckwheat (Zhou et al., 2009), *Acremonium* sp. (Mazzaferro & Breccia, 2012; Mazzaferro, Pinuel, Minig & Breccia, 2010; Minig, Mazzaferro, Erra-Balsells, Petroselli & Breccia, 2011) or *Aspergillus niger* (Šimčíková *et al.*, 2015). Recently, Bassanini reported enzymatic transglycosylations of a set of arylalkyl alcohols, including tyrosol (Bassanini *et al*., 2017). The reactions were catalyzed by rutinosidase from *Aspergillus niger* and rutin was used as the rutinose donor. Rutinosylation of tyrosol provided however a hardly separable mixtures of isomers TR1 and TR2, since the rutinosylation proceeded either on the primary aliphatic or on the phenolic hydroxyls of tyrosol in ratios ranging from 1:1 to 3:1.

High levels of rutin degrading enzymes were found also in flowers and flower buds of *Sophora japonica* (Kijima, Takahashi, Itozawa & Izumi, 2005; Bahchevanska & Koleva, 1996). Although the sophora enzyme possesses high hydrolytic activity towards rutin, it has never been tested in transglycosylations. In this Short communication, we present use of dry flower buds (DFBs) of *Sophora japonica* in transrutinosylations of tyrosol from rutin as a regioselective alternative to microbial rutinosidase.

2. Materials and methods

Flower buds of *Sophora japonica* were collected from local sources, dried at laboratory temperature and homogenized to powder. The homogenized material was extracted for 1

flour by methanol in ratio 1:30 (w/v) to remove most of flavonoids and chlorophyll, filtered and dried at laboratory temperature.

Tyrosol (97%) was purchased from Maybridge, and rutin 97 % from Acros Organics. 2-(4-hydroxyphenyl)ethyl acetate is product of the Institute of Chemistry, SAS in Bratislava. The standard of tyrosol rutinoside TR1 used in HPLC analyses was prepared by non-optimized enzymatic transglycosylation.

High-performance liquid chromatography was performed on an Agilent 1200 Series apparatus (Agilent technologies, Inc., Santa Clara, California, USA). Flash chromatography was performed on Isolera One from Biotage (Uppsala, Sweden), with UV detection. ^{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.

Production of tyrosol rutinoside was monitored by HPLC on Zorbax Eclipse XBD-C18 column (3 x 100 mm, 3 µm) equilibrated and eluted at 30 °C with gradient of methanol in H_2O at flow rate of 0,4 mL/min. Tyrosol and tyrosol rutinoside were detected at 275 nm, while rutin and quercetin were detected at 254 nm.

Optimization of transglycosylation yielding to tyrosol rutinoside was performed according to concentration of rutin (16 – 49 mM) and concentration of tyrosol (72 – 434 mM) in 1 mL reaction volume at 1000 rpm and 40 °C. Reactions were monitored in 50 µL aliquots of reaction mixture withdrawn in predefined time intervals after centrifugation ca. 2 min., mixed with 200 µL of methanol to quench the reaction and filtered through 0.22 µm syringe filter. 100 µL of the diluted filtrate was mixed with 100 µL of distilled water and used for analysis by HPLC. Each reaction was performed in triplicates.

Reactions in preparative scale were performed at 40 °C, 175 rpm in volume of 50 mL. Concentrations of tyrosol and rutin were 290 mM and 49 mM, respectively. The reaction was started by addition of 1 g of pre-extracted DFBs. After 30 hours, a sample for HPLC was withdrawn and the reaction was stopped by boiling in water bath for 10 minutes,

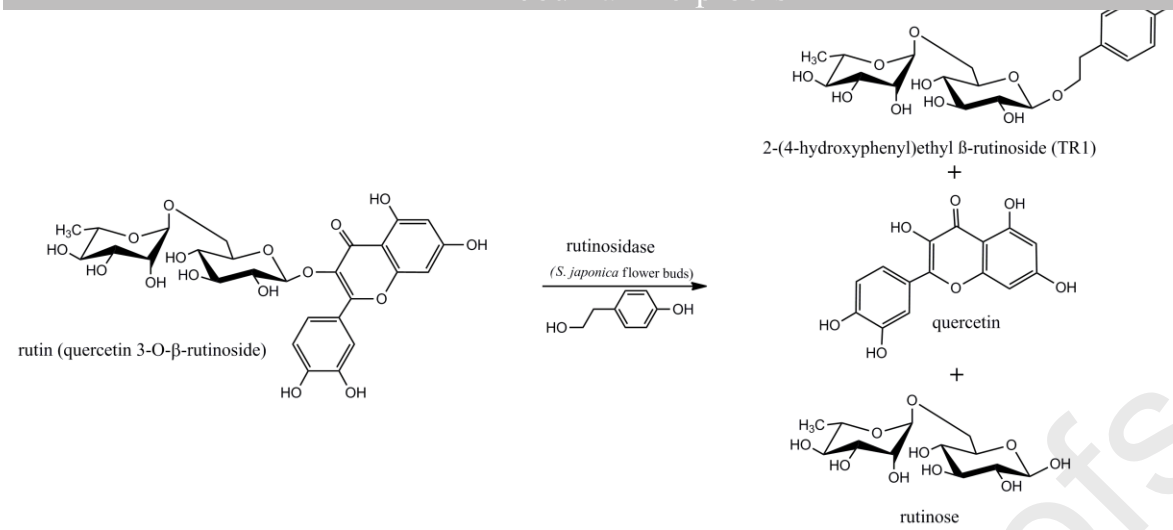
followed by filtration under reduced pressure. Filtration cake was washed with ca 10 mL of water and the filtrate was evaporated *in vacuo*. Dried filtrate was resolubilised in 15 mL of distilled water and applied on Diaion HP-20 column. Sample was separated in gradient of methanol in water. Fractions containing product were collected and evaporated *in vacuo*. Dry material was solubilised in methanol, adsorbed to ca 5 g of silica gel, dried *in vacuo* and applied on 50 g silica gel in chloroform. The column was eluted by gradient of methanol in chloroform by flash chromatography. Fractions containing the product were checked by TLC for purity, collected, evaporated and dried from methanol:toluene solution to give 0.26 grams (24 %) of pale yellow amorphous powder. Structure of the purified product was examined by NMR spectroscopy.

^{1}H NMR (400 MHz, CD_3OD) δ 7.07 (d, *J* = 8.5 Hz, 2H, H-Ph), 6.70 (d, *J* = 8.4 Hz, 2H, H-Ph), 4.75 (d, *J* = 1.6 Hz, 1H, H-1'), 4.28 (d, *J* = 7.8 Hz, 1H, H-1), 4.02 – 3.93 (m, 2H, 0.5xCH_2, H-6a), 3.84 (dd, *J* = 3.4, 1.7 Hz, 1H, H-2'), 3.75 – 3.65 (m, 3H, 0.5xCH_2, H-5', H-3'), 3.61 (dd, *J* = 11.2, 6.0 Hz, 1H, H-6b), 3.42 – 3.32 (m, 3H, H-5, H-4'), 3.30 – 3.25 (m, 1H, H-4), 3.17 (dd, *J* = 9.0, 7.8 Hz, 1H, H-2), 2.84 (td, *J* = 7.4, 2.4 Hz, 2H, CH_2), 1.26 (d, *J* = 6.3 Hz, 3H, CH_3).

^{13}C NMR (101 MHz, CD_3OD) δ 156.7 (C-Ph), 130.9 (CH-Ph), 130.7(C-Ph), 116.2 (CH-Ph), 104.4 (C-1), 102.2 (C-1'), 78.0 (C-3), 76.8 (C-5), 75.1 (C-2), 74.0 (C-4'), 72.3 (C-3'), 72.2 (CH_2), 72.2 (C-2'), 71.6 (C-4), 69.8 (C-5'), 68.1 (C-6), 36.4 (CH_2), 18.0 (CH_3).

3. Results and discussion

DFBs were used for rutinosylation of tyrosol according to Scheme 1. Besides the transglycosylation, hydrolysis of rutin to rutinose and quercetin occurred in the presence of water.

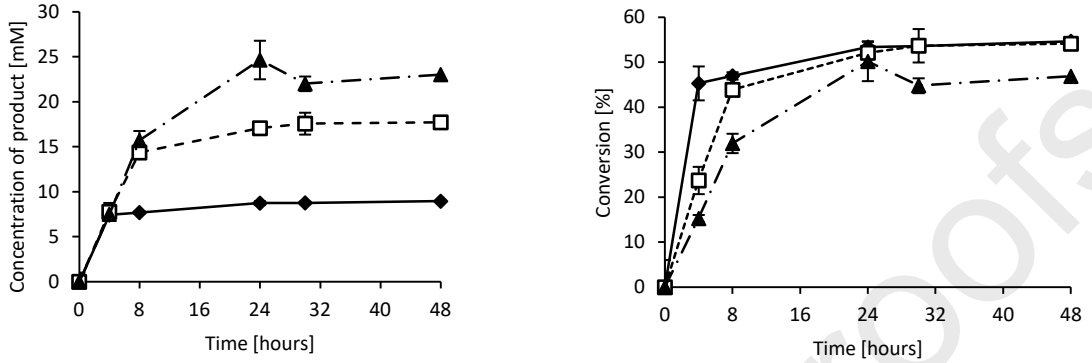


Scheme 1. Rutinosylation of tyrosol catalyzed by dry flower buds from *Sophora japonica*.

The plant material was extracted with methanol before use to remove chlorophyll, flavonoids and other substances potentially hampering purification of the product. The flower buds naturally contain also rutin in levels reaching almost 23% (Couch, Naghski & Krewson, 1952) and therefore the unextracted material was also tested for the glycosylation of tyrosol without extra added rutin. TLC analysis of reaction mixture confirmed both autohydrolysis of rutin and formation of tyrosol rutinoside. The same products were formed by extracted DFBs supplemented with rutin (Supplementary material, Figure S1). To eliminate the effect of the naturally included rutin in the buds, only extracted DFBs were used in further studies.

Initial rutin concentration positively influences formation of the target glycoside. Formation of TR1 was monitored at three different rutin concentrations (Figure 2a). The product was synthesized rapidly within 24 hours, after which time its concentration remained practically unchanged. Increase of the initial concentration of rutin resulted in higher productivity per volume, but the time of reaching the maximum product concentration was prolonged. Final conversion of rutin to the product however slightly decreased at the highest rutin concentration (Figure 2b). It is worth to note that all three rutin concentrations were above its solubility in water. The reaction mixtures were

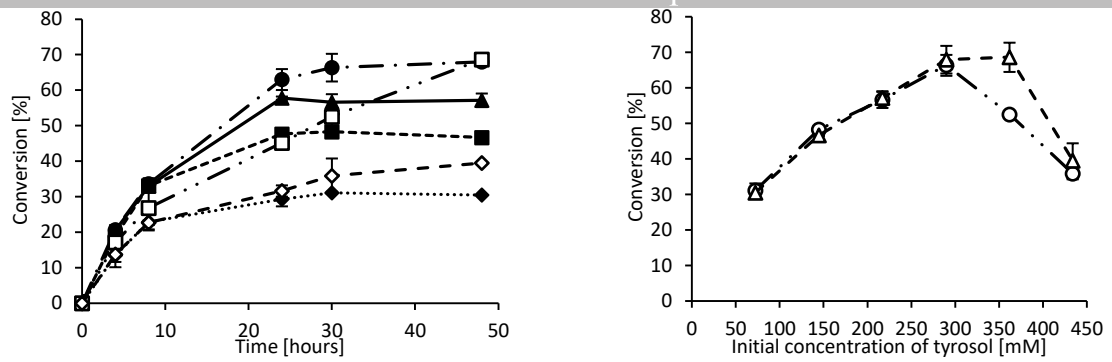
suspensions continually dissolving rutin to media as the reaction proceeded, so the actual concentration of rutin was in all cases similar and enabled to reach similar conversions. Concentration of rutin 49 mM was used in following optimizations.



(**a**) (**b**)

Figure 2. (**a**) Time course of production of tyrosol rutinoside at varying rutin concentration; (**b**) Conversion of rutin to tyrosol rutinoside at varying rutin concentration. Reaction conditions: 16 (-◆-), 33 (-□-), 49 (-▲-) mM rutin in water, 145 mM tyrosol, 20 mg/mL of catalyst (extracted flower buds of *S. japonica*), 40 °C, 1000 rpm.

Increasing initial concentration of tyrosol had positive effect on the conversion of rutin to tyrosol rutinoside (Figure 3a) with conversions remaining unchanged after 30 hours for initial tyrosol concentrations 290 mM and lower. The highest tested acceptor concentration (434 mM) however suppressed the final conversion, while the concentration 362 mM had slowed down the transrutinosylation, reaching conversion of rutin to tyrosol rutinoside 53 % after 30 hours and 69% after 48 hours (Figure 3b). The highest conversion after 30 hours (66.4%) has been observed for starting concentration of tyrosol 290 mM and the level of product (ca. 33 mM) in the reaction mixture stayed practically unchanged within another 18 hours.



(**a**) (**b**)

Figure 3. (**a**) Effect of initial tyrosol concentration on the production tyrosol rutinoside expressed as time course of the conversion from rutin. Reaction conditions: 49 mM rutin; 72 (-◆-), 145 (-■-), 217 (-▲-), 290 (-●-), 362 (-□-), 434 mM (-◇-) tyrosol, 20 mg/mL of catalyst (flower buds of *S. japonica*), 40 °C, 1000 rpm.; (**b**) Effect of initial tyrosol concentration on the maximal conversion of rutin to tyrosol rutinoside in 30th (-○-) and 48th (-△-) hour.

Tyrosol concentration 290 mM was selected for preparative reaction in 50 mL scale to achieve the maximum conversion in shorter reaction time. The final conversion of rutin to tyrosol rutinoside within 30 hours was above 60%, separation and purification of the product were however complicated due to high content of the plant material and precipitated quercetin in the reaction mixture and yielded 260 mg (24 %) of the product. Its structure was evaluated by NMR spectroscopy. To assign all ^{1}H chemical shifts and most of ^{13}C chemical shifts in the product spectra techniques of 2D NMR (H-H COSY and HSQC) were applied.

The ^{1}H NMR spectrum of TR1 (Figure S2) showed α-anomeric L-rhamnopyranoside signal at δ 4.75 ppm (H-1´; $J_{1,2}$ 1.6 Hz) and β-anomeric D-glucopyranoside signal at δ 4.28 ppm (H-1; $J_{1,2}$ 8.1 Hz). The signals at δ 7.07 ppm and 6.70 ppm typically fit with the *ortho* and *meta* H-aromatic signals of a tyrosol residue linked to glucose on aliphatic OH and having free phenol function (Bassanini *et al*., 2017). The ^{1}H NMR spectrum of free tyrosol

in acetone-d_6 showes two triplets of hydrogens on aliphatic chain (3.69 ppm for CH_2-α and 2.66 ppm for CH_2-β). Signals splitting of the aliphatic moiety of our product is completely different and more complicated. The CH_2-β (td) are shifted at 2.84 ppm, while protons of CH_2-α are completely separated and they are parts of multiplets at 3.98 and 3.69 ppm. The ^{13}C NMR shifts (Figure S3) for C-1 at δ 104.4 ppm and C-1´ at δ 102.2 ppm were derived mostly from the HSQC. The *ortho* and *meta* aromatic CH signals of tyrosol are shifted at 116.2 ppm and 130.7 ppm. Glucose residue showed a signal for C-6 at 68.1 ppm. Two carbon signals of aliphatic residue of the tyrosol are shifted at 72.2 and 36.4 ppm respectively. The ^{13}C NMR spectrum of free tyrosol in acetone-d_6 showes signals of aliphatic carbons at 64.3 and 39.7 ppm. So, the signal of aliphatic C-α is shifted to higher magnetic field. The signal at 18.0 ppm was assigned to the methyl group of rhamnoside moiety. The structural analysis therefore definitely confirmed that tyrosol was glycosylated on the primary hydroxyl.

Rutinosylation of tyrosol by rutinosidase from *Aspergillus niger* has been recently reported by Bassanini (Bassanini *et al*., 2017). The published reaction provided high yield of a mixture of regioisomers containing 25 % of TR2 in addition to TR1. The mixture was difficult to separate and yields of purified products are not declared.

To confirm that the sole production of TR1 in our long time experiments is the result of DFBs selectivity and is not caused by re-hydrolysis of TR2 which could be produced in early stages, we compared rutinoslyation of tyrosol and its primary monoacetate, 2-(4-hydroxyphenyl)ethyl acetate (TA1). In TA1, the primary hydroxyl (target of rutinosylation in production of TR1) is blocked by acetylation, while the phenolic hydroxyl is free (Supplementary material, Figure S2). TLC analysis of reaction mixtures after 2 and 4 hours proved notable rutinosylation of tyrosol, while only products of rutin hydrolysis were formed in the reaction mixture comprising TA1 (Supplementary material, Figure S3). These results support the evidence that sophora flower buds are not able to glycosylate phenolic moiety

of the tyrosol skeleton, thus offering a more practical regioselective alternative to recently published bioproduction of TR1.

4. Conclusion

Synthesis of glycosylated oligosaccharides is a challenging process in which regioselectivity and anomeric specificity of glycosylation are the crucial factors. Glycosidases are regularly used with advantage in syntheses of glycosides, but demand for glycosylated disaccharides provokes wider use of more specific enzyme class – diglycosidases. Besides microbial sources, diglycosidases (such as rutinosidase) occur in various plant materials.

In our attempts to prepare rhamnosylated derivative of salidroside, we used dry flower buds from an abundant ornamental tree *Sophora japonica*. This material catalyzed direct transfer of β-rutinosyl moiety from rutin to the primary hydroxyl of tyrosol while the phenolic hydroxyl of tyrosol remained untouched. The reaction thus provides solely one of two possible product isomers, which is the main advantage of this reaction over the yet published rutinosylation of tyrosol catalyzed by microbial rutinosidase.

Acknowledgements

This work was supported by the Slovak Research and Development Agency under the contract No. APVV-18-0188. The work was also supported by the Slovak Grant Agency for Science VEGA (grant number 2/0126/19)

5. References

Akanbi, T. O., Barrow, C. J. (2018). Lipase-produced hydroxytyrosyl eicosapentaenoate is an excellent antioxidant for the stabilization of omega-3 bulk oils, emulsions and microcapsules. *Molecules*, *23*, 275-289.

261 Bancheva, S., Koleva, I. (1996). A study on the autohydrolysis of rutin to quercetin
262 from Sophora japonica blossoms. *Biotechnology & Biotechnological Equipment*, *10*,
263 56-58.
264 Bassanini, I., Krejzová, J., Panzeri, W., Monti, D., Křen, V., Riva, S. (2017) A sustainable
265 one-pot two-enzymes synthesis of naturally occurring arylalkyl glucosides.
266 *ChemSusChem*, *10*, 2040-2045.
267 Bi, Y., Wang, Z., Mao, Y., Zheng, S., Zhang, H., Shi, D. (2012). Ionic liquid effects on the
268 activity of β-glycosidase for the synthesis of salidroside in co-solvent systems. *Chinese*
269 *Journal of Catalysis, 33,* 1161-1165.
270 Conde, E., Cara, C., Moure, A., Ruiz, E., Castro, E., Domínguez H. (2009). Antioxidant
271 activity of the phenolic compounds released by hydrothermal treatments of olive tree
272 pruning. *Food Chemistry*, *114*, 806-812.
273 Couch, J. F., Naghski, J., Krewson, C.F. (1952). Rutin content of *Sophora japonica* L.
274 *Journal of American Chemical Society*, *74*, 424-425.
275 Guan, S., Feng, H., Song, B., Guo, W., Xiong, Y., Huang, G., Zhong, W., Huo, M., Chen,
276 N., Lu, J., Deng, X. (2011). Salidroside attenuates LPS-induced pro-inflammatory
277 cytokine responses and improves survival in murine endotoxemia. *International*
278 *Immunopharmacology*, *11*, 2194-2199.
279 Karnišová Potocká, E., Mastihubová, M., Mastihuba, V. (2019). Enzymatic synthesis of
280 tyrosol and hydroxytyrosol β-D-fructofuranosides. *Biocatalysis & Biotransformation*, *37*,
281 18-24.
282 Katayama, S., Ohno, F., Yamauchi, Y., Kato, M., Makabe, H., Nakamura, S. (2013).
283 Enzymatic synthesis of novel phenol acid rutinosides using rutinase and their antiviral
284 activity *in vitro*. *Journal of Agricultural and Food Chemistry, 61*, 9617-9622.

Kijima, T., Takahashi, S., Itozawa, T., Izumi, T. (2005). The activity of rutin degrading enzyme from flower buds of Japanese pagoda tree. *Journal of Food Technology*, *3*, 238-241.

Li, H.-B., Ge, Y., Zheng, X.-X., Zhang, L. (2008). Salidroside stimulated glucose uptake in skeletal muscle cells by activating AMP-activated protein kinase. *European Journal of Pharmacology*, *588*, 165-169.

Liang, X.-Q., Xie, P., Zhang, Y., Shi, T., Wang, Q.-J., Yan, T.-H. (2010). Effects of salidroside on myocardial ischemia/ reperfusion injury in rats. *Chinese Journal of Natural Medicines*, *8*, 127-131.

Mazzaferro, L. S., Breccia, J. D. (2012). Quantification of hesperidin in citrus-based foods using a fungal diglycosidase. *Food Chemistry*, *134*, 2338-2344.

Mazzaferro, L., Pinuel, L., Minig, M., Breccia, J. D. (2010). Extracellular monoenzyme deglycosylation system of 7-O-linked flavonoid beta-rutinosides and its disaccharide transglycosylation activity from *Stilbella fimetaria*. *Archives of Microbiology*, *192*, 383-393.

Méndez-Líter, J. A., Tundidor, I., Nieto-Domínguez, M., De Toro, B. F., González Santana, A., De Eugenio, L. I. (2019). Transglycosylation products generated by *Talaromyces amestolkiae* GH3 β-glucosidases: effect of hydroxytyrosol, vanillin and its glucosides on breast cancer cells. *Microbial Cell Factories 18,* 97.

Míguez, N., Ramírez-Escudero, M., Gimeno-Pérez, M., Poveda, A., Jiménez-Barbero, J., Ballesteros, A. O., Fernández-Lobato, M., Sanz-Aparicio, J., Plou, F. J. (2018). Fructosylation of hydroxytyrosol by the β-fructofuranosidase from *Xanthophyllomyces dendrorhous*: Insights into the molecular basis of the enzyme specificity. *ChemCatChem, 10*, 4892-4901.

Mihig, M., Mazzaferro, L. S., Erra-Balsells, R., Petroselli, G., Breccia, J.D. (2011). α-Rhamnosyl-β-glucosidase-catalyzed reactions for analysis and biotransformations of plant-based foods. *Journal of Agricultural and Food Chemistry*, *59*, 11238-11243.

Mizutani, M., Nakanishi, H., Ema, J., Ma, S.-J., Noguchi, E., Inohara-Ochiai, M., Fukuchi-Mizutani, M., Nakao, M., Sakata, K. (2002). Cloning of β-primeverosidase from tea leaves, a key enzyme in tea aroma formation. *Plant Physiology*, *130*, 2164-2176.

Nieto-Dominguez, M., de Eugenio, L. I., Peñalver, P., Belmonte-Reche, E., Morales, J. C., Poveda, A., Jiménez-Barbero, J., Prieto, A., Plou, F. J., Jesús Martínez, M. (2017). Enzymatic synthesis of a novel neuroprotective hydroxytyrosol glycoside. *Journal of Agricultural and Food Chemistry*, *65*, 10526-10533.

Potocká, E., Mastihubová, M., Mastihuba, V. (2015). Enzymatic synthesis of tyrosol glycosides. *Journal of Molecular Catalysis B: Enzymatic*, *113*, 23-28.

Qi, T., Gu, G., Xu, L., Xiao, M., Lu, L. (2017). Efficient synthesis of tyrosol galactosides by the β-galactosidase from *Enterobacter cloacae* B5. *Applied Microbiology and Biotechnology, 101*, 4995-5003.

van Rantwijk, F., Woudenberg-van Oosterom, M., Sheldon, R.A. (1999). Glycosidase-catalysed synthesis of alkyl glycosides. *Journal of Molecular Catalysis B: Enzymatic*, *6,* 511–532.

Šimčíková, D., Kotik, M., Weignerová, L., Halada, P., Pelantová, H., Adamcová, K., Křen, V. (2015). α-L-Rhamnosyl-β-D-glucosidase (rutinosidase) from *Aspergillus niger*: Characterization and synthetic potential of a novel diglycosidase. *Advanced Synthesis & Catalysis*, *357*, 107-117.

Sun, C., Wang, Z., Zheng, Q., Zhang, H. (2012). Salidroside inhibits migration and invasion of human fibrosarcoma HT1080 cells. *Phytomedicine*, *19*, 355-363.

Sun, Y., Zhou, D., Shahidi, F. (2018). Antioxidant properties of tyrosol and hydroxytyrosol saturated fatty acid esters. *Food Chemistry*, *245*, 1262-1268.

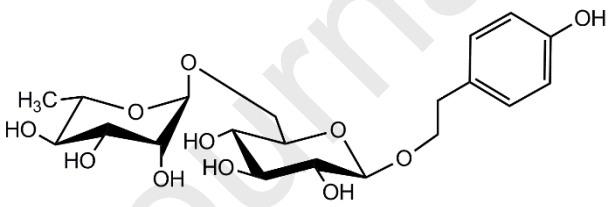
Zhang, J., Liu, A., Hou, R., Zhang, J., Jia, X., Jiang, W., Chen, J. (2009). Salidroside protects cardiomyocyte against hypoxia-induced death: A HIF-1α-activated and VEGF-mediated pathway. *European Journal of Pharmacology*, *607*, 6-14.

Zhou, L., Lu, C., Wang, G. L., Geng, H. L., Yang, J. W., Chen, P. (2009). Syntheses of R-β-rutinosides by rutin-degrading reaction. *Journal of Asian Natural Products Research*, *11*, 18-23.

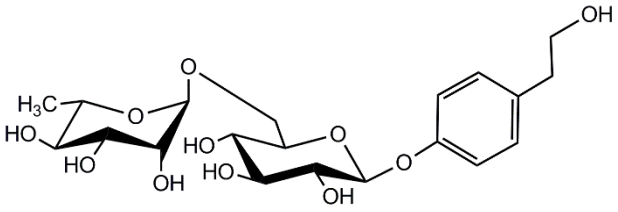
Wang, H., Ding, Y., Zhou, J., Sun, X., Wang, S. (2009). The *in vitro* and *in vivo* antiviral effects of salidroside from *Rhodiola rosea* L. against coxsackievirus B3. *Phytomedicine*, *16*, 146-155.

Wani, T. A., Masoodi, F. A., Gani, A., Baba W. N., Rahmanian, N., Akhter, R., Wani, I. A., Ahmad, M. (2018). Olive oil and its principal bioactive compound: Hydroxytyrosol–A review of the recent literature. *Trends in Food Science & Technology*, *77*, 77-90.

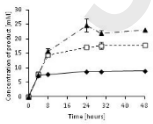
Wu, Y. L., Lian, L. H., Jiang, Y. Z., Nan, J. X. (2009). Hepatoprotective effects of salidroside on fulminant hepatic failure induced by D-galactosamine and lipopolysaccharide in mice. *Journal of Pharmacy and Pharmacology*, *61*, 1375-1382.

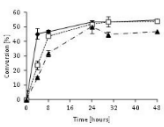


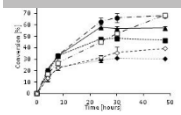
2-(4-hydroxyphenyl)ethyl ß-rutinoside
(tyrosol rutinoside TR1)

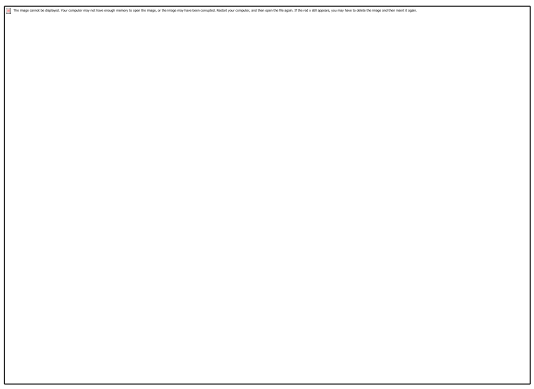


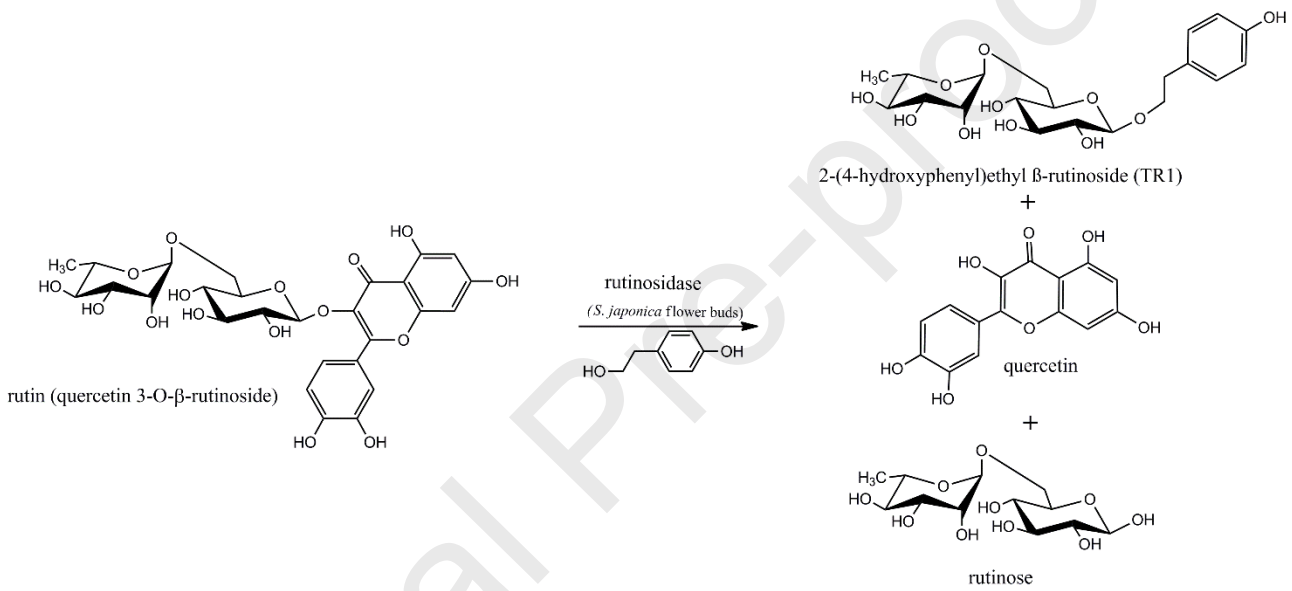
4-(2-hydroxyethyl)phenyl ß-rutinoside
(tyrosol rutinoside TR2)











- Dried flower buds from *Sophora japonica* (DFBs) comprise transglycosylating rutinosidase
- The DFBs catalyze transrutinosylation of tyrosol from rutin
- Rutinosylation of tyrosol proceeds exclusively on the primary hydroxyl

Elena Karnišová Potocká: Experimental work including chromatographic analyses, synthetic reactions, product purification, Writing – original draft preparation

Mária Mastihubová: methodology consultations, structure evaluation (NMR), Editing

Vladimír Mastihuba: Conceptuation, Supervision, Writing - manuscript reviewing and finalization,

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

xThe authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Groups of **Prof. Segio Riva**, Istituto di Chimica del Riconoscimento Molecolare (ICRM), C.N.R., via Mario Bianco 9, Milano, 20131, Italy and **Prof. Vladimír Křen**, Institute of Microbiology, Czech Academy of Sciences, Vídeňská 1083, Praha 4, CZ-14220, Czech Republic are our competitors on the field of enzymatic acylations and glycosylations, including rutinosylations of tyrosol.