Synthesis of enzymatically modified isoquercitrin by double enzyme cascade catalysed conversion of mulberry flavonoid glycosides

Yu-Fan Zhan[1], Zhuo-Hao Meng[1], Shuai You[1, 2], Lu-Chan Gong[1, 2], Cheng-Hai Yan[1, *], Jun Wang[1, 2, *]
1 School of Biotechnology, Jiangsu University of Science and Technology, 212018 Zhenjiang, China;
2 Sericultural Research Institute, Chinese Academy of Agricultural Sciences, 212018 Zhenjiang, China;
Key words: Enzymatically modified isoquercitrin; biocatalysis; rutin; cascade reaction.
Presenting author email: biojustych@163.com; wangjun@just.edu.cn

The mulberry leaf is a plant source for the silkworm to feed on, and has long been used as a medicinal plant in East Asian countries. Modern botanical theories and biomedical research have shown that the mulberry tree contains a variety of active ingredients, which are either singly or in combination, making it highly valuable for medicinal purposes (Wen et al., 2019, Raman et al., 2016). Rutin and quercetin are functional flavonoids with important applications in the prevention and treatment of diabetes, cardiovascular diseases, tumours and other diseases (Pawlowska et al., 2008). Enzymatically modified isoquercitrin (EMIQ) is a mixture of enzymatically modified flavonol glycosides derived from isoquercitrin, with numerous pharmacological effects including antioxidant, antitumour, antidepressant, antihypertensive and hypolipidemic (Wang et al., 2012). Because EMIQ is highly water soluble and metabolized to quercetin, its bioavailability is about 17 times higher than that of quercetin (Gasparotto Junior et al., 2012, Arung et al., 2011), making it an internationally competing advanced food additive, adjuvant or active ingredient in drugs, but its content is extremely low and difficult to isolate and extract from nature (Rha et al., 2020). Most of the current studies have used quercetin and isoquercitrin as precursors, and there are few studies on the preparation of EMIQ using rutin, which is present in greater quantities in nature, as a precursor.

Multi-enzyme cascade systems usually do not require the separation of intermediates; at the same time, through the synergistic action of multiple enzymes, the equilibrium of the reaction is promoted, overcoming the problem of unstable intermediates and improving reaction activity and selectivity, allowing for efficient production of products (Kroutil and Rueping, 2014, Quin et al., 2017). The one-pot method involves combining multiple catalysts and reagents in a single reaction vessel, followed by a series of precise catalytic cascades, which not only reduces the waste of raw materials, but also increases the efficiency of the reaction.

This experiment aims to construct a one-pot dual enzyme cascade reaction system to catalyse rutin, providing a new method and new ideas for the preparation of EMIQ by bioenzymatic methods. RhaB1 and DGAS were obtained by heterologous expression, followed by an investigation of their enzymatic properties and cascade catalytic mechanism. As the result of temperature stability, RhaB1 and DGAS maintained high enzyme activity of about 80% in 2 hours at 40℃. Under different pH conditions, RhaB1 did not perform as well as DGAS at pH 7.0 and pH 8.0. However, both DGAS and RhaB1 performed better at pH 6, with enzyme activities reaching about 85%, as shown in pH stability. Therefore, the conditions of 40°C and pH 6.0 were chosen for the catalytic reaction.

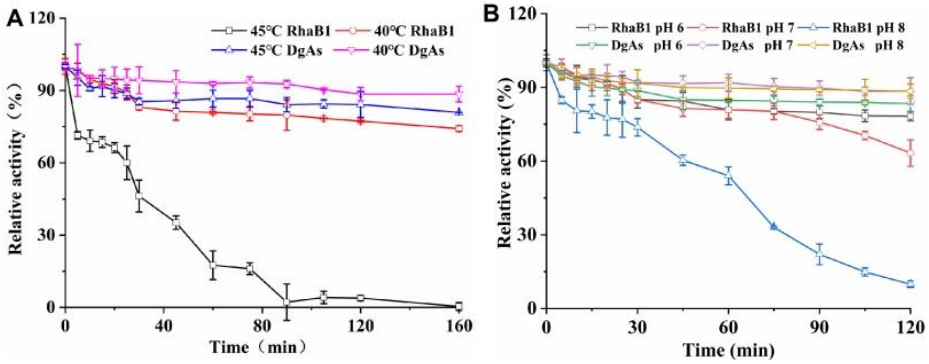

Figure.1 (A) Temperature stability in 40℃ and 45℃ of RhaB1 and DGAS; (B) pH stability of RhaB1 and DGAS.

In Figure 2, the conversion efficiency of rutin varies depending on the substrate content, and when the sucrose concentration is low, the conversion efficiency of the reactants can reach 55.6%. When the concentration of rutin was 0.05 mg/ml, the conversion efficiency of the reactants was up to about 75%. The most suitable sucrose and rutin concentrations were selected and the conversion of the reactants was up to 88.6% at pH 8.0 and 40°C for 5h. The results indicate that under suitable reaction conditions, the conversion of the reactants of the RhaB1 and DGAS cascade reaction can reach a high level.

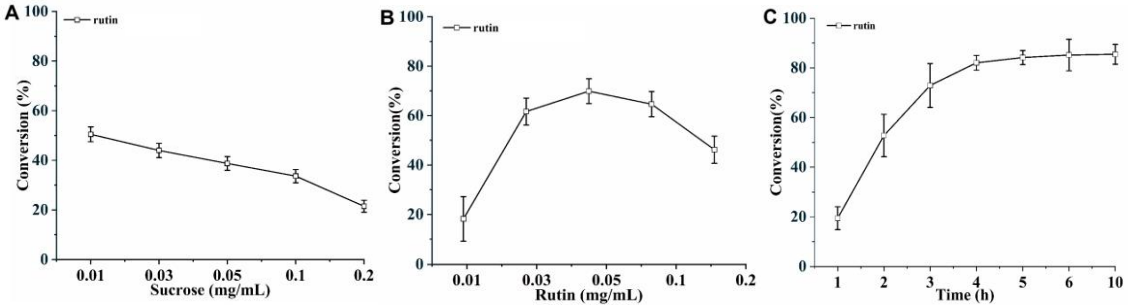

Figure.2 Effects of sucrose, rutin substrate and time concentration on the catalytic process. (A) Sucrose (0.01mg/mL-0.2mg/mL) concentration effect the conversion of reactants; (B) Rutin (0.01 mg/mL-0.2 mg/mL) concentration effect conversion rate of reactants; (C) Different time effect conversion rate of reactants.

In summary, rhamnosidase and sucrose amylase achieved efficient biotransformation of natural flavonoid glycosides in a one-pot cascade catalysis, which is conducive to improving the biotransformation and exploitation of natural flavonoid glycosides.

Acknowledgements: This work was supported by the National Natural Science Foundation of China (22278196).

References:

[1] Arung, E. T., Furuta, S., Ishikawa, H., Kusuma, I. W., Shimizu, K., Kondo, R., 2011. 'Anti-melanogenesis properties of quercetin- and its derivative-rich extract from Allium cepa'. *FOOD CHEMISTRY*, 124 (3): 1024-1028.

[2] Gasparotto Junior, A., Lima Prando, T. B., Vilhena Leme, T. D. S., Gasparotto, F. M., Botelho Lourenco, E. L., Rattmann, Y. D., Da Silva-Santos, J. E., Leite Kassuya, C. A., Andrade Marques, M. C., 2012. 'Mechanisms underlying the diuretic effects of Tropaeolum majus L. extracts and its main component isoquercitrin'. *JOURNAL OF ETHNOPHARMACOLOGY*, 141 (1): 501-509.

[3] Kroutil, W., Rueping, M., 2014. 'Introduction to ACS Catalysis Virtual Special Issue on Cascade Catalysis'. *ACS CATALYSIS*, 4 (6): 2086-2087.

[4] Pawlowska, A. M., Oleszek, W. and Braca, A., 2008. 'Quali-quantitative analyses of flavonoids of Morus nigra L. and Morus alba . (Moraceae) fruits', *JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY*, 56 (9): 3377-3380.

[5] Quin, M. B., Wallin, K. K., Zhang, G., Schmidt-Dannert, C., 2017. 'Spatial organization of multi-enzyme biocatalytic cascades', *ORGANIC & BIOMOLECULAR CHEMISTRY*, 15 (20): 4260-4271.

[6] Raman, S. T., Ganeshan, A. K. P. G., Chen, C., Jin, C., Li, S., Chen, H., Gui, Z., 2016., 'In vitro and In vivo Antioxidant Activity of Flavonoid Extracted from Mulberry Fruit (Morus alba L.)', *PHARMACOGNOSY MAGAZINE*, 12 (46): 128-133.

[7] Rha, C., Kim, H. G., Baek, N., Kim, D., Park, C., 2020. 'Amylosucrase from Deinococcus geothermalis can be modulated under different reaction conditions to produce novel quercetin 4 '-O-alpha-d-isomaltoside'. *ENZYME AND MICROBIAL TECHNOLOGY*, 141: 109648.

[8] Wang, J., Ma, Y., Wu, X., Yu, L., Xia, R., Sun, G., Wu, F., 2012. 'Selective hydrolysis by commercially available hesperidinase for isoquercitrin production'. *JOURNAL OF MOLECULAR CATALYSIS B-ENZYMATIC*, 8137 (42): 37-42.

[9] Wen, P., Hu, T., Linhardt, R. J., Liao, S., Wu, H., Zou, Y., 2019., 'Mulberry: A review of bioactive compounds and advanced processing technology'. *TRENDS IN FOOD SCIENCE & TECHNOLOGY*, 83: 138-158.