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Enhanced biocatalysis mechanism under microwave irradiation in isoquercitrin production revealed by circular dichroism and surface plasmon resonance spectroscopy

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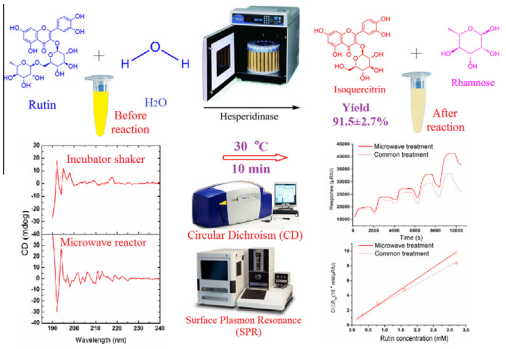
HIGHLIGHTS

- Microwave irradiation was firstly used in enzymatic synthesis of isoquercitrin.
- Reaction time (10 min) was greatly decreased to one sixty than that in a batch reactor.
- V_m/K_m under microwave irradiation was 17.49-fold higher than that in a batch reactor.
- Secondary structure data of hesperidinase proved the higher affinity to rutin by CD.
- SPR showed rutin had a higher affinity to hesperidinase under microwave irradiation.

GRAPHICAL ABSTRACT



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ABSTRACT

An efficient and rapid process for isoquercitrin production by hesperidinase-catalyzed hydrolysis of rutin was successfully developed under microwave irradiation detecting the affinity by circular dichroism (CD) and surface plasmon resonance (SPR) spectroscopy. A maximum isoquercitrin yield of 91.5 ± 2.7% was obtained in 10 min with the conditions of 10 g/L hesperidinase, 2 g/L rutin, 30 °C and microwave power density 88.9 W/L. Enzymatic reaction rate and V_m/K_m in the microwave reactor were 6.34-fold higher than in a continuous flow microreactor and 1.24-fold higher than in a biphasic system. CD and SPR analysis results also showed that hesperidinase has a better selectivity and affinity (3.3-fold than in a batch reactor) to generate isoquercitrin under microwave irradiation. Microwave irradiation greatly improved the reaction efficiency and productivity, leading to a more positive economical assessment. The binding affinity indicates the presence of strong multivalent interactions between rutin and hesperidinase under microwave irradiation.

1. Introduction

Microwave irradiation is an efficient heating source for a variety of chemical reactions, where higher yields and reaction selectivity can be achieved in a short reaction time (Yadav and Devendran, 2012). It is a clean, fast, and convenient energy source and widely

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used in organic chemistry (Nüechter et al., 2003). Microwave irradiation can produce efficient internal heating by directing microwave energy to the molecules (e.g., solvents, reagents, catalysts) of a reaction mixture and thereby accelerating the reaction (Zhou and Hawley, 2003). Because lipase-catalyzed reactions are rather sluggish in non-aqueous media, microwave-assisted lipase-catalyzed reactions have rapidly developed in the past few years. Yadav and Lathi (2004) reported that the initial activity for the transesterification of methyl acetoacetate with various alcohols in the presence of immobilized lipases increased by 2.2-fold–4.6-fold when reacting under microwave irradiation. Yu et al. used microwave reactor to enhance the activity and the enantioselectivity of Novozym 435 in the resolution of (*R*, *S*)-2-octanol in organic solvents and ionic liquids, respectively (Yu et al., 2007). The heating and driving of chemical reactions by microwave energy had become an increasingly popular tool in the scientific community and an established technique heavily used in both academia and industry (Lidström et al., 2001). The applications of microwave irradiation in enzymatic biocatalysis reactions are feasible, and microwave irradiation can be used in isoquercitrin production by rutin hydrolysis.

High-speed synthesis under microwave irradiation has also attracted a considerable amount of attentions. Microwave irradiations play a significant role in biocatalysis synthesis reactions, such as in the synthesis of biodiesel production with an increasing reaction rate and high efficiency (Motasemi and Ani, 2012). Direct microwave heating is able to reduce chemical reaction times from hours to minutes, and it is also known to reduce the occurrence of side reactions, increasing the yields of target products and improving reproducibility (Nüchter et al., 2004). Microwave-enhanced chemistry is based on the efficient heating of materials by "microwave dielectric heating" effects. This phenomenon is dependent on the ability of a specific material (solvent or reagent) to absorb microwave energy and convert it into heat (Rosana et al., 2014). The electric component of an electromagnetic field causes heating by two main mechanisms: dipolar polarization and ionic conduction. The irradiation of a sample at microwave frequencies results in the dipoles or ions aligning in the applied electric field (Stass et al., 2000). As the applied field oscillates, the dipole or ion field attempts to realign itself within the alternating electric field (Mingos and Baghurst, 1991). It is clear that conventional microwave sources cannot induce chemical reactions. Considering these unique properties of the microwave and the gaps in the synthesis of isoquercitrin, the use of microwave irradiation was investigated in the enzyme-catalyzed selective hydrolysis of rutin.

Although enzymatic-catalyzed reactions of natural compounds are rapid, sensitive relatively and moderate methods, the synthesized products are easily oxidized. Therefore, the reaction time should be minimized to ensure the minimal oxidation. Patrícia studied the enzymatic synthesis of biodiesel from palm oil and ethanol; the reaction required 72 h to produce ethyl esters. However, under microwave irradiation, the whole reaction only required 12 h, reducing the risk of oxidation (Da Rós et al., 2013). Considering these microwave strengthening dynamics have rarely been studied, the mechanism responsible for the enhanced and strengthened mass and heating transfer are still unknown.

Isoquercitrin has many pharmacological activities, including antioxidant, antidepressant and antihypertensive, and it is a key synthetic intermediate for the production of enzymatically modified isoquercitrin (Wang et al., 2013). Among a variety of methods available, the transformation of rutin to isoquercitrin using enzymatic hydrolysis is a feasible procedure. However, problems remain exist in this method, including longer reaction times and unstable and easily oxidized isoquercitrin products. When compared with a glycine–sodium hydroxide/metal ion-based system, a 20 h shorter reaction time and a 2.3-fold increase in isoquercitrin yield were obtained (Wang et al., 2012a,b). These results indicated that ionic liquids can effectively enhance the selective synthesis of isoquercitrin and that the reaction process is simple and eco-friendly. Furthermore, ionic liquids provide stability to the isoquercitrin reaction system. However, the industrial application of hesperidinase-catalyzed transformations remains limited due to low substrate solubility, slow catalytic efficiency and long reaction time. Lee synthesized quercetin-3-*O*-glucoside (92% yield) from rutin using naringinase (produced by *Penicillium decumbens*) within 12 h (Lee et al., 2013). Vila-Real synthesized isoquercitrin using naringinase from rutin and obtained an isoquercitrin yield of 60% in 6 h (Vila-Real et al., 2011). A buffer medium containing a specific proportion of the ionic liquid (IL), [Bmim][BF_4], was developed as a co-solvent system. In this system, an isoquercitrin yield of 91.4% was obtained within 10 h (Wang et al., 2013). An isoquercitrin yield of 93.9% was obtained after 3 h in a biphasic system of [Bmim][BF_4]:glycine–sodium hydroxide (pH 9) (10:90, v/v) and glyceryl triacetate (1:1, v/v) (Wang et al., 2015b). However, an essential method for a novel and convenient reaction system that enables the ultrafast synthesis of isoquercitrin at a high efficiency remains urgent; microwave irradiation would be an ideal means to solve this issue.

The purpose of this study was to prepare a biocatalysis system for the selective and effective biotransformation of rutin to isoquercitrin using microwave irradiation. The effects of hesperidinase concentration, rutin concentration, microwave power density and reaction temperature on the isoquercitrin yield were investigated. The affinity and association saturation constant of the enzyme for the substrate was detected by CD spectra and SPR analyses. Additionally, the reaction rate V_m and Michaelis constant V_m/K_m were determined.

2. Methods

2.1. Enzyme and materials

Hesperidinase (containing α-L-rhamnosidase and β-D-glucosidase, ⩾10 units/g solid) was purchased from Sigma Chemical Co. (St. Louis, MO, USA) and produced by *Aspergillus niger*. Standard rutin and isoquercitrin were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Ionic liquid [Bmim][BF_4] was purchased from Shanghai Cheng-Jie Chemical Co., Ltd. (Shanghai, China). All reagents used were analytical grade except methanol and acetonitrile, which were HPLC grade (purchased from Tedia Co.; Fairfield, OH, USA). Water was purified using an Elga Purelab Option-Q purification system (Elga Labwater; High Wycombe, Bucks, UK) and had a resistivity of more than 18.0 MΩ cm. All other solvents and reagents were analytical grade.

2.2. Synthesis of isoquercitrin under microwave irradiation

In this study, glycine-sodium hydroxide buffer (pH 9) was used in the IL-buffer system. The system contained 720 μL of rutin solution, 100 μL of IL and 180 μL of hesperidinase solution. All enzymatic reactions were performed in the microwave reactors (Ludox LS silica source, 11-mm and 33-mm reactor, CEM MARS 5, USA) with 8 single reaction chambers under different conditions. In a typical experiment, rutin buffer solution was added to the IL on a 1.5-mL centrifuge tube. The reaction was initiated by adding hesperidinase and buffered solution, and the mixtures were incubated for different durations at various pH values, IL concentrations, substrate concentrations, and enzyme concentrations. A control experiment was set by adding the same amount of pure water. After the reaction proceeded for a specific time, the samples were handled by adding methanol to terminate the enzymatic