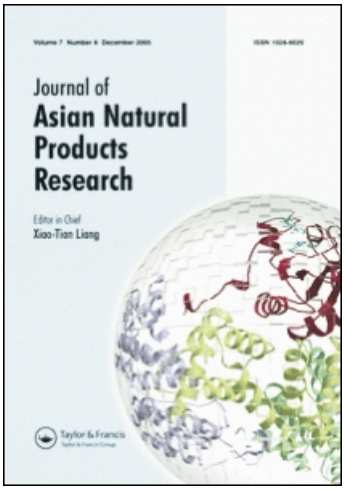

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ORIGINAL ARTICLE

The effect of ultrasound on lipase-catalyzed regioselective acylation of mangiferin in non-aqueous solvents

Zhi Wang[a], Ren Wang[a], Jin Tian[bc], Bo Zhao[a], Xiao-Fei Wei[a], Ya-Lun Su[b], Chun-Yuan Li[a], Shu-Gui Cao[a]*, Teng-Fei Ji[b]* and Lei Wang[a]*

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A simple and efficient method for regioselective acylation of mangiferin catalyzed by lipase under ultrasound irradiation is reported. Compared with the conventional methods, its main advantages are shorter reaction time and higher yields. The optimum conditions were screened out. Under the optimal conditions (lipase: PCL, acyl donor: vinyl acetate; reaction solvent: DMSO, reaction temperature: 45°C, ultrasonic power: 200 W; substrate ratio: acyl donor/mangiferin 6/1, enzyme loading: 6 mg/ml), the regioselective acylation yield was up to 84%.

Keywords: ultrasound; lipase; regioselective; acylation; mangiferin

1. Introduction

Mangiferin, which was isolated from the dry roots and stems of *Anemarrhena asphodeloides* Bge, has been reported to possess anti-inflammation, diuretic, choleretic, and cardiotonic activities and displays a high anti-bacterial activity against gram-positive bacteria [1,2]. However, mangiferin was difficult to cross the blood–brain barrier (BBB) due to its low liposolubility. With a view to improve the liposolubility, derivatization of mangiferin by acylation could improve the infiltration ability across the BBB [3].

Because of the numerous reactive hydroxy groups in mangiferin, the enzymatic route, which is more regioselective, was described as promising [4–6]. However, it is necessary to find a proper method to increase the reaction rate, while one of the major limitations of enzymatic synthesis in non-aqueous media is its low reaction rate [7].

The application of the ultrasound irradiation is one of the most promising experimental techniques introduced into the tools of organic transformation [8,9]. A large number of ultrasonic reactions can be carried out in higher yields, shorter reaction time, or milder conditions [10–12]. Ultrasonic actions in liquids can cause effects of cavitation [13,14]. When cavitation bubbles collapse near the phase boundary of two immiscible liquids, the resultant shock wave can provide a very efficient stirring/mixing of the layers.

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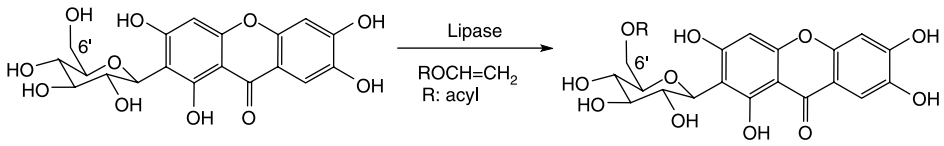

Scheme 1. Lipase-catalyzed regioselective acylation of mangiferin.

Because the effects of cavitation can enhance heterogeneous reactions and readily form transient reactive species, ultrasound irradiation is an alternative method to reduce mass transfer limitations and increase the rate of enzymatic reaction [15–17]. Furthermore, ultrasound irradiation could perturb weak interactions of protein and induce its conformational change, which may improve some enzymatic reactions [18,19].

Herein, we report a facile lipase-catalyzed regioselective acylation of mangiferin under ultrasound irradiation (Scheme 1), and the reaction conditions for the acylation have been optimized.

2. Results and discussion

2.1 Enzyme screening for acylation

Lipase catalytic acylation depended mainly on the type and origin of the enzyme [20]. In this study, five kinds of lipase from different resources [*Porcine pancreas* lipase (PPL); *Pseudomonas cepacia* (PCL); *Candida antarctica* lipase B (CALB); *Candida* sp. 415 lipase (CSL); and *Bacillus subtilis* lipase (BSL)] were selected to carry out the acylation with/without ultrasonic irradiation (Table 1). It was observed that the enzyme activity varied markedly with the type of enzyme. Furthermore, all the acylations in this work occurred at 6′-OH of mangiferin according to the analysis of NMR spectra. The conversion was enhanced from 44 to 84% under ultrasound using PCL as the biocatalyst. Thus, PCL was selected for further study because of its highest activity.

Table 1. Comparing the activity of different enzymes in acylation of mangiferin.

Enzyme	Conversion without ultrasound (%)	Conversion with ultrasound (%)
PPL	15	37
PCL	44	84
CALB	42	69
CSL	26	53
BSL	11	42

Notes: Reactions were carried out in DMSO (5 ml) with mangiferin (1 mmol), vinyl acetate (6 mmol), and enzyme (30 mg) at 45°C for 6 h. The ultrasonic power was 200 W when the reaction occurred under ultrasound.

2.2 Acyl donor

The effect of the chain length (C-2 to C-12) of the acyl donor was examined (Figure 1). As shown in Figure 1, PCL exhibited the highest acylation activity toward vinyl acetate, and the reaction rate decreased with the elongation of the chain. It is well known that PCL lipase has a funnel-like binding site [21]. Therefore, a longer acyl donor might be more difficult to enter the active site to form the first tetrahedron intermediate (generally considered as the rate-limiting step) due to the steric strain.

2.3 Effect of the solvent

A suitable solvent for biocatalysis should not only maintain the enzyme activity but also dissolve substrates as well [22,23]. In this study, because of the poor solubility of mangiferin in hydrophobic solvents, several polar solvents [tetrahydrofuran (THF), acetonitrile (CH_3CN), pyridine (PY), *N*,*N*-dimethylformamide (DMF), and dimethyl sulfoxide (DMSO)] were selected to investigate their effects on the enzymatic regioselective acylation under ultrasound. As shown in Table 2, the highest enzyme activity was obtained when DMSO was used as the reaction media.

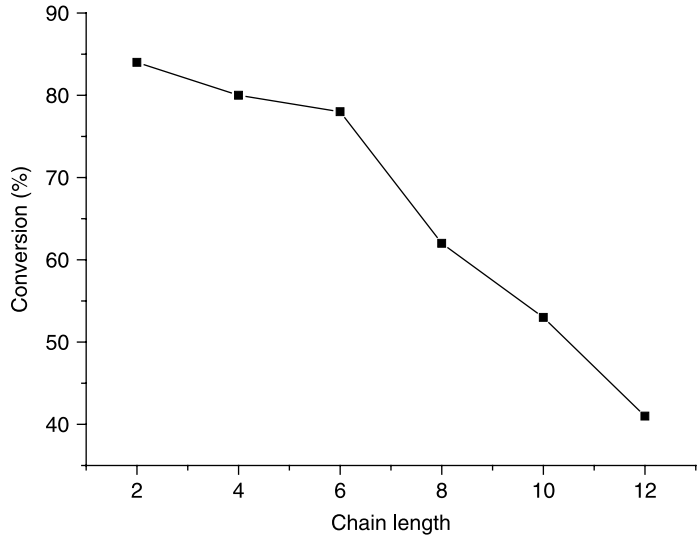

Figure 1. Effect of the acyl donor on the acylation of mangiferin. Reactions were carried out in DMSO (5 ml) with mangiferin (1 mmol), acyl donor (6 mmol), and PCL (30 mg) at 45°C for 6 h under ultrasonic power (200 W).

The results indicate that a high conversion yield was favored in a solvent with a low log *P* (defined as the logarithm of partition coefficient of a given solvent between water and 1-octanol) and a high dielectric constant. These observations are in accordance with those reported in the literature [24,25]. The effect of organic solvents on the enzymatic reaction is complicated. In addition to the log *P* and solubility of substrates, there are probably some other important parameters affecting the reaction, including other chemical or physical properties of the solvents. Further studies about the influence of organic solvents are in progress.

Table 2. Effect of the solvent on the acylation of mangiferin.

Solvent	Log *P*	Dielectric constant	Conversion
THF	0.49	7.5	57
PY	0.65	11.8	59
CH_3CN	−0.33	37.5	61
DMF	−1.0	38.3	79
DMSO	−1.4	47.2	84

Notes: Reactions were carried out in a solvent (5 ml) with mangiferin (1 mmol), vinyl acetate (6 mmol), and PCL (30 mg) at 45°C for 6 h under ultrasonic power (200 W).

2.4 Temperature

The effect of temperature on the activity of PCL in acylation of mangiferin was examined. As shown in Figure 2, in the shaking bath, the activity increased with increase in temperature up to 55°C, and decreased sharply with further increase in temperature up to 60°C. While under ultrasound, the optimum temperature was observed at 45°C. Inactivation of lipase under ultrasound occurred at higher temperatures (above 45°C). The results reveal that optimum temperature and inactivation temperature of the lipase were 10°C lower under ultrasound irradiation than that in the shaking bath. The combination of heat and ultrasound irradiation under moderate pressure is the so-called manothermosonication (MTS) [26]. MTS treatments allow us to inactivate the enzyme at lower temperatures and/or in shorter time than thermal treatments at the same temperatures.

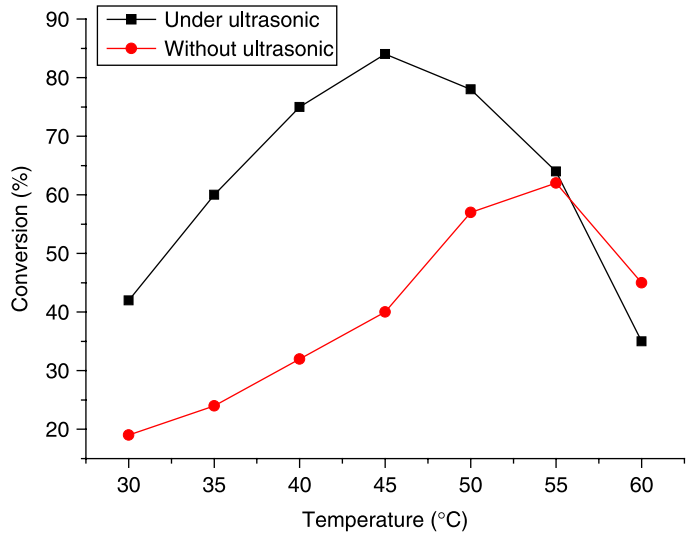

Figure 2. Effect of temperature on the acylation of mangiferin. Reactions were carried out in DMSO (5 ml) with mangiferin (1 mmol), vinyl acetate (6 mmol), and PCL (30 mg) for 6 h under ultrasonic power (200 W).

2.5 *Effect of ultrasonic power*

Ultrasonic power is an important influencing factor for reactions under ultrasound irradiation. In this work, five power values at 40 kHz ultrasound (50, 100, 150, 200, and 250 W) were selected to examine its effect on the acylation of mangiferin. Figure 3 shows that the reaction rate presented rapid increase with increasing ultrasonic power, and no significant changes were observed when the power exceeded 200 W. This can be explained by the fact that the higher intensity of the ultrasonic waves can not only increase the mass transfer of the

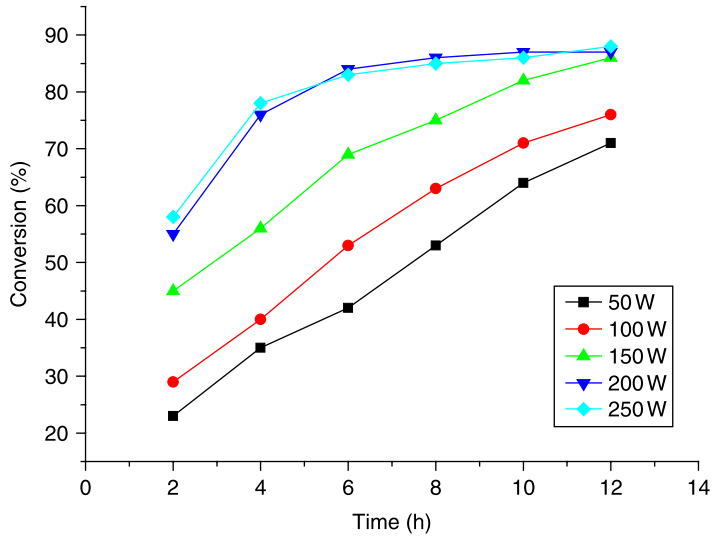

Figure 3. Effect of ultrasonic power on the acylation of mangiferin. Reactions were carried out in DMSO (5 ml) with mangiferin (1 mmol), vinyl acetate (6 mmol), and PCL (30 mg) at 45°C for 12 h.

substrate and the product, but also improve the flexible conformation of the enzyme [27]. Therefore, 200 W was selected to study the characteristics of lipase-catalyzed reactions in the following experiments. Furthermore, the reaction balance could be obtained after 6 h when the ultrasonic power exceeded 200 W. Thus, the optimum reaction time was 6 h, which could be seen from the results shown in Figure 3.

2.6 *Substrate ratio*

The rate of an enzyme catalytic reaction depends on the concentrations of the enzyme and the substrate, so the variation of the substrate ratio may have a great effect on the reaction. In this study, the substrate ratio (vinyl acetate to mangiferin) was changed from 2 to 10 while keeping the moles of mangiferin constant at 1 mmol. It has been shown experimentally that, when using higher ratio of vinyl acetate over mangiferin, the conversion increased from 59 to 84% and a ratio of 6:1 turned out to be sufficient (Figure 4). Further increasing the substrate ratio could not increase the enzyme activity. It might be that the enzyme/substrate complex has to be dissociated before the active sites were free to accommodate more substrates.

2.7 *Enzyme loading*

The effect of enzyme loading on the acylation of mangiferin was studied under ultrasound. The mole ratios of the reactants were kept constant while changing the amount of enzyme from 2 to 10 mg/ml. With the increase in the loading of PCL, the conversion was also increased, but not obviously when the loading exceeded 6 mg/ml (Figure 5).

3. Conclusions

We analyzed the enzymatic regioselective acylation of mangiferin by PCL under ultrasound irradiation. The use of ultrasound irradiation can significantly reduce the reaction time in enzymatic acylation. The optimum temperature and inactivation were 10°C lower than that under the shaking condition, and increasing ultrasonic power was found to enhance the degree of lipase-catalyzed acylation without essential damage to the lipase activity. Therefore, this methodology is of significance in synthetic applications. Other glycoside

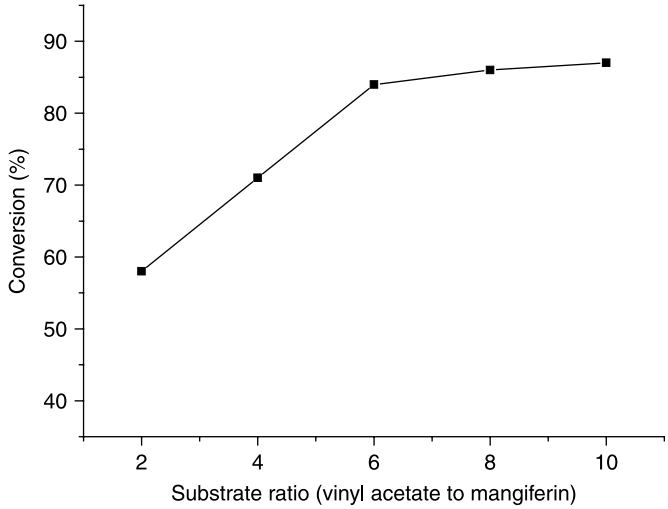

Figure 4. Effect of the substrate ratio on the acylation of mangiferin. Reactions were carried out in DMSO (5 ml) with mangiferin (1 mmol) and PCL (30 mg) for 6 h under ultrasonic power (200 W).

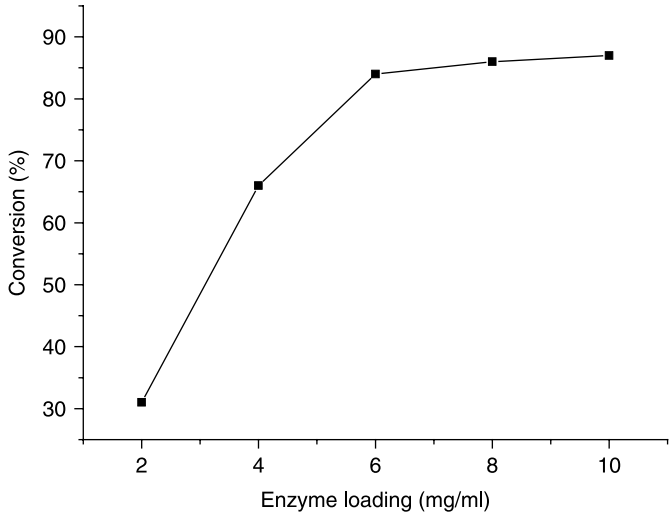

Figure 5. Effect of enzyme loading on the acylation of mangiferin. Reactions were carried out in DMSO (5 ml) with mangiferin (1 mmol) and vinyl acetate (6 mmol) at 45°C for 6 h under ultrasonic power (200 W).

substrates are under investigations in order to extend the generality of this methodology.

4. Experimental

4.1 Materials

Lipase from PCL, CALB, BSL, and PPL were purchased from Sigma (Beijing, China). CSL was purchased from the Institute of Microbiology Chinese Academy of Sciences (Beijing, China). Mangiferin was purchased from Sigma. All acyl donors were purchased from Sigma. Ultrasound irradiation was carried out in a Kunshan KQ-250DE ultrasonic cleaner, with a frequency of 40 kHz and a power of 250 W. The reaction flasks were located in the maximum energy area in the cleaner, and the reaction temperature was controlled by a water bath. NMR spectra were recorded on an Inova 500 (^{1}H, 500 MHz) spectrometer. ESI-MS was performed on an Agilent 1100 LC/MSD.

4.2 Acylation of mangiferin catalyzed by lipase

Shaking experiments were carried out over a water bath under magnetic stirring, whose temperature could be maintained within ± 0.3°C of the desired temperature. Ultrasound irradiations were carried out in a water bath of an ultrasonic cleaner. A 25 ml round-bottomed flask equipped with a mechanical stirrer was placed at the center of the ultrasonic bath. The reaction mixture was then sonicated at different temperatures up to the desired time. After completion, the reaction mixture was evaporated to dryness to obtain the crude product, which was purified by silica gel chromatography (chloroform–methanol, 4:1) to give the product as a yellow solid. The reaction was monitored by HPLC analysis.

4.3 Identification

HPLC analysis of mangiferin was performed on an Agilent 1200 HPLC with a YMC C_{18} column (150 mm × 4.6 mm; Greenherbs Co. Ltd, Beijing, China) and detected at 257 nm using methanol–H_2O–H_3PO_4 (20:80:0.1) as the mobile phase at a flow rate of 1 ml/min.

All the products were characterized by ^{1}H NMR and ESI-MS experiments.

4.3.1 Vinyl acetate as the acyl donor

^{1}H NMR (DMSO-d_6, 500 MHz): δ 13.53 (1H, s, 1-OH), l0.40 (3H, s, 3, 6, 7-OH), 7.35 (1H, s, 8-H), 6.86 (1H, s, 5-H), 6.37 (1H, s, 4-H), 4.62 (1H, d, $J = 10.0$ Hz, 1′-H), 4.13 (2H, m, 6′-H), 3.75 (1H, 5′-H), 3.70 (1H, 2′-H), 3.43 (1H, 3′-H), 3.41 (1H, m, 4′-H), 2.06 (3H, s, CH_3CO_2); ESI-MS *m/z*: 465.6 $[M+H]^+$, 487.5 $[M+Na]^+$.

4.3.2 Vinyl butyrate as the acyl donor

^{1}H NMR: δ 0.91 (3H, t, $J = 7.0$ Hz, CH_3), 1.63 (2H, m, CH_2), 2.27 (2H, t, 7.0 Hz, CH_2), other NMR peaks are similar to those of mangiferin acetate; ESI-MS *m/z*: 493.5 $[M+H]^+$, 531.4 $[M+K]^+$.

4.3.3 Vinyl hexanoate as the acyl donor

^{1}H NMR: δ 0.94 (3H, t, 7.0 Hz, CH_3), 1.18 (4H, m, $2CH_2$), 1.70 (2H, m, CH_2), 2.48 (2H, t, 7.0 Hz, CH_2), other NMR peaks are similar to those of mangiferin acetate; ESI-MS *m/z*: 521.7 $[M+H]^+$.

4.3.4 Vinyl octanoate as the acyl donor

^{1}H NMR: δ 0.89 (3H, t, 7.0 Hz, CH_3), 1.29 (8H, m, $4CH_2$), 1.66 (2H, m, CH_2), 2.31 (2H, t, 7.0 Hz, CH_2), other NMR peaks are similar to those of mangiferin acetate; ESI-MS *m/z*: 549.4 $[M+H]^+$, 571.5 $[M+Na]^+$.

4.3.5 Vinyl decanoate as the acyl donor

^{1}H NMR: δ 0.86 (3H, t, 7.0 Hz, CH_3), 1.26 (12H, m, $6CH_2$), 1.68 (2H, m, CH_2), 2.27 (2H, t, 7.0 Hz, CH_2), other NMR peaks are similar to those of mangiferin acetate; ESI-MS *m/z*: 577.7 $[M+H]^+$, 599.8 $[M+Na]^+$.

4.3.6 Vinyl dodecanoate as the acyl donor

^{1}H NMR: δ 0.87 (3H, t, 7.0 Hz, CH_3), 1.29 (16H, m, $8CH_2$), 1.48 (2H, m, CH_2), 2.37 (2H, t, 7.0 Hz, CH_2), other NMR peaks are similar to those of mangiferin acetate; ESI-MS *m/z*: 605.3 $[M+H]^+$.

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