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Lipase-catalyzed esterification of rutin and naringin with fatty acids of medium carbon chain

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Abstract

Flavonoids rutin and naringin were acylated with fatty acids of medium carbon chain (with 8–12 carbon atoms on their molecule) in a reaction catalyzed by immobilized lipase from *Candida antarctica* (Novozyme) in various solvent systems. The reaction parameters affecting the acylation rate and the conversion of the enzymatic process, such as the nature of the organic solvent and acyl donor used, the water activity (a_w) of the system, as well as the kinetic of the reaction have been investigated. In all cases studied, only flavonoid monoester is identified as the product, which indicates that this lipase-catalyzed esterification is regioselective. The enzymatic acylation of flavonoids seems to follow Michaelis–Menten kinetics.

Keywords: Water activity; Monoester; Acylation rate

1. Introduction

There is a great interest in natural antioxidants, which are presumable safe [1]. Such natural substances are flavonoids. However, the hydrophilic nature of these antioxidants reduces their effectiveness in stabilizing fats and oils and this has been reported as a serious disadvantage when an aqueous phase is also present [2]. Therefore, the modification of these compounds via esterification of their sugar moieties with aliphatic molecules can be used as a tool to alter their solubility in oil based formulae and emulsions (Scheme 1).

In order to change the hydrophilic/hydrophobic balance of flavonoids, various hydrolytic enzymes, such as lipases and proteases, have been used in anhydrous organic solvents as catalysts for the acylation of their sugar derivatives [3,4]. At the moment the enzymatic acylation of rutin, as well as naringin has been reported to be performed with short chain fatty acids in very toxic media, e.g. pyridine or mixtures with other solvents, since in these media their solubility is relatively high [3,5].

2. Experimental

2.1. Enzyme

The lipase used was immobilized lipase from *Candida antarctica* (Novozyme 435) from Novo-Nordisk.

2.2. Enzymatic reactions

In a typical experiment, 50 mg of lipase were added to the reaction mixture, which consisted of 16.5 mM of flavonoid, 5 ml solvent and 100 mM fatty acid or fatty

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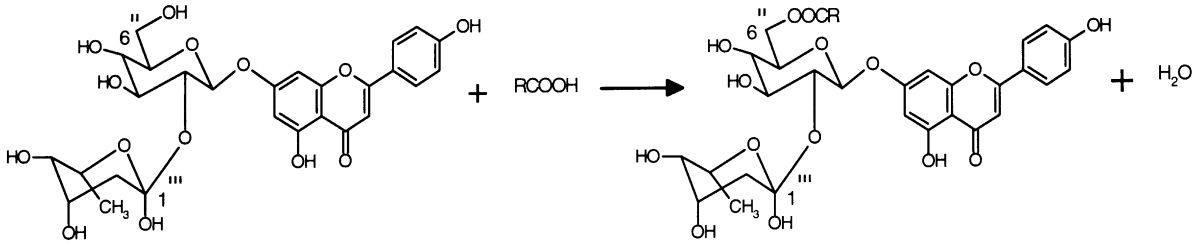

Scheme 1. Lipase-catalyzed regioselectively esterification of naringin.

acid ester. The solvent-free reaction systems consisted of 50 mg lipase, 16.5 mM flavonoid and 5 ml fatty acid or fatty acid ester (20–31 mmol). Incubation was carried out at 45 °C under magnetic stirring, in the presence of 3 Å molecular sieves (200 mg). The water activity of the organic solvents, enzyme preparation and substrates was preset by pre-equilibration for 72 h with the appropriate saturated salt solutions (LiCl, a_w = 0.11; $MgCl_2$, $a_w = 0.31$; $CoCl_2$, $a_w = 0.52$; $NaNO_3$, $a_w = 0.69$; KNO_3 $a_w = 0.87$). The water activity of the reaction system was kept constant throughout the reaction. The reaction was followed by HPLC.

2.3. Purification of flavonoid esters

Naringin esters were purified by column chromatography on silica gel (230–400 mesh, grade 60). The products were eluted with acetonitrile/methanol/water (8/2/0.3, v/v/v). Rutin esters were purified by preparative TLC.

The chemical structure of the flavonoid esters was determined by ^{13}C NMR in DMSO-d^6 using TMS as internal reference on a Varian-300 MHz spectrometer.

3. Results

3.1. Effect of reaction media

Higher conversions were achieved when using acetone or *tert*-butanol as solvent system but THF seems to be incompatible with the lipase used. The conversion of naringin seems to be higher than that of rutin in all reaction systems tested (Fig. 1).

3.2. Effect of water activity

The highest conversion (45–50%) was observed in the driest media, i.e. for water activity of 0.11 or less.

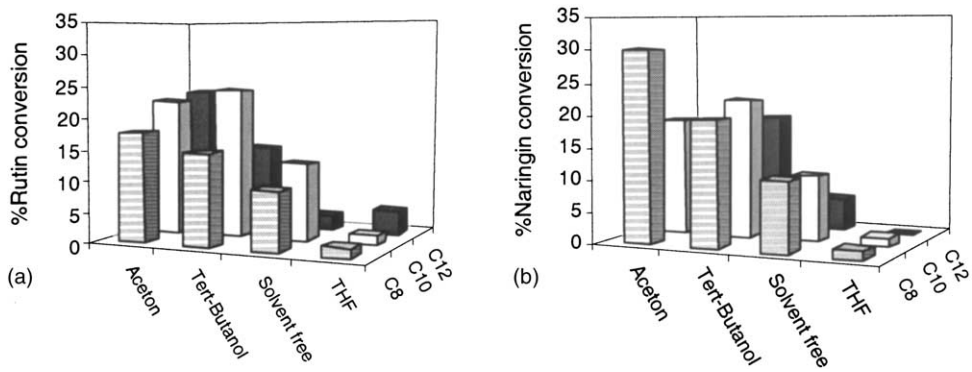

Fig. 1. Conversion of rutin (a) and naringin (b) during the esterification with octanoic, decanoic and dodecanoic acid catalyzed by Novozyme in different reaction media. All enzymatic acylations were performed without controlling the water activity of the reaction system. Reaction conditions as described in Section 2.

Table 1
Effect of water activity on the conversion of naringin, during the esterification with decanoic acid in *t*-butanol by Novozyme at 45 °C

a_w	0.87	0.75	0.53	0.31	0.11	>0.1
Conversion (%)	0	0	9.6	12.2	45.6	50

Naringin ester production is significantly enhanced when the water activity was controlled throughout the reaction course. The highest conversion (about 50%) was observed when 3 Å molecular sieves were present in the system (Table 1).

3.3. Kinetic study of naringin esterification with decanoic acid

The effect of naringin and decanoic acid concentration on the naringin acylation rate was studied. The dependence of the initial rates (Figs. 2 and 3) as a function of the substrate concentrations were used to calculate the apparent K_m values for both substrates. As it can be seen from Figs. 2 and 3 the acylation reaction follows Michaelis–Menten kinetic. The calculated apparent K_m and V_{max} values for naringin and decanoic acid acylation are: $K_{m(naringin)} = 91.76$ mM,

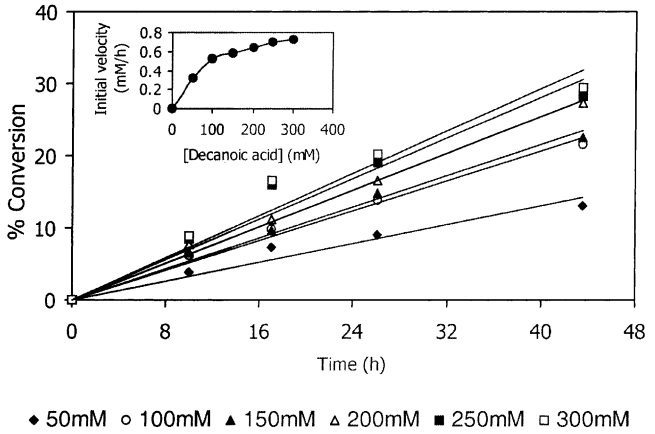

Fig. 2. Initial velocities of naringin esterification in *tert*-butanol by Novozyme at 45 °C, at different decanoic acid concentrations (initial naringin concentration 16.5 mM). Inner graph: Michaelis–Menten plot.

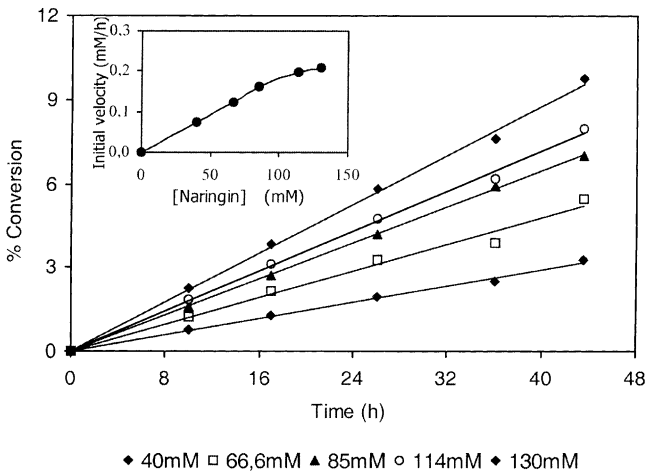

Fig. 3. Initial velocities of naringin esterification with decanoic acid (250 mM), in *tert*-butanol by Novozyme at 45 °C, at different naringin concentrations. Inner graph: Michaelis–Menten plot.

$K_{m(\text{decanoic acid})} = 93.65\,\text{mM}$, $V_{max(\text{narigin})} = 0.98$, $V_{max(\text{decanoic acid})} = 1.14$.

3.4. Characterisation of naringin decanoate by ^{13}C NMR analysis

The fatty acid esters of rutin and naringin were purified and characterized by ^{13}C NMR. The results suggest the presence of an ester bond on the C-6 of the glucose moiety of naringin molecule. On the other hand considering that rutin's sugar moiety has only secondary hydroxyl groups, it is concluded that Novozyme is selective also towards the secondary OH-groups.

Acknowledgements

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