Direct Lipase Catalyzed Lipophilization of Chlorogenic Acid in Supercritical Carbon Dioxide

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ABSTRACT

The enzymatic lipophilization of natural antioxidants is a process of enormous pharmaceutical interest; the direct esterification of such polar compounds, however, is a major challenge due to the heterogeneity of these reactions. In the present study, the Taguchi approach was employed to optimize the esterification of 5-CGA by immobilized Candida antarctica lipase B in SC CO₂/t-butanol. The effects of temperature (35-55 °C), pressure (150-250 bar), t-butanol (2-10 % v/v), and the enzyme amount (10–30 mg/ml), were investigated. The maximum conversion reached 63 % in 25 hours at 150 bar, 55 °C, 10 % t-butanol (v/v), and 20 mg/ml of lipase. The SC CO₂ selectivity towards the esterified product was the working principle of this study, by which minimized interphase transport limitations and enhanced mass-transfer phenomena substantially improved the reaction kinetics. This investigation offers an alternative towards the functionalization of natural antioxidants which harmonizes with the use of green technologies.

Keywords: Candida antarctica lipase, Chlorogenic acid, lipophilization, supercritical
**Introduction**

Chlorogenic acid (5-cafeoyl-quinic acid, 5-CGA) has been associated to a broad range of bioactivities (Bonita et al., 2007; Clifford, 2000; Zheng et al., 2008), while its consumption from coffee water extracts determined to be safe (Watanabe et al., 2006). Biological properties of 5-CGA are primarily attributed to its capacity to donate hydrogen atoms of the phenolic ring to free radicals, thus inhibiting oxidation processes. It has been proposed that such bioactivities and the bioavailability of natural phenolics can be enhanced by increasing their amphiphilic nature, incorporating both emulsifying and antioxidant properties (Figueroa-Espinoza and Villeneuve, 2005; Jayaprakasam et al., 2006; Sabally et al., 2006; Vosmann, Weitkamp, and Weber, 2006; Weitkamp, Vosmann, and Weber, 2006).

**Materials and Methods**

**Materials**

Immobilized lipase from *Candida antarctica* (lipase B, Novozym 435) was provided by Novo Nordisk. Chlorogenic acid (5-CGA), 2-methyl-2-propanol (*t*-butanol), 1-heptanol, 1-pentanol, and geraniol were obtained from Sigma-Aldrich. All solvents used were HPLC grade and from Merck.

**Lipase Catalyzed Esterification in SC CO₂**

A batch-operated stirred-system was specially designed and built in our laboratory to carry out the enzymatic reaction. A cell volume of 50 mL is described in Figure 1.

**Results and Discussion**

In this work, the possibility of performing a lipase-catalyzed esterification of 5-CGA and 1-heptanol in SC CO₂, while simultaneously extracting the formed compound, was demonstrated.

The biotransformation of 5-CGA via esterification with 1-heptanol was used to incorporate the corresponding seven-carbon aliphatic chain, and as a result modify its polarity. As shown by HPLC analysis of samples withdrawn at specific intervals from the reaction mixture (Figure 2), lipophilization resulted in a significant increase of the acid original retention time (from 24 to 38 min) and Log P values (from -0.75 to 2).
The resulting modified lipophilicity consequently enhanced the selectivity of the reaction medium towards the formed ester.

**Conclusions**

The Taguchi experimental design provided valuable insights into the lipase-catalyzed esterification of 5-CGA in SC CO₂.

Immobilized lipase B from *Candida antarctica* (20 mg/ml) was used to successfully lipophilize 5-CGA by esterification with 1-heptanol in SC CO₂/\(\text{t}-\text{butanol}\) (10 % v/v) at 150 bar, and 55 °C. Under these conditions, reaction rates approached 2.32 μM ester/ g lipase per minute with conversions of 63 %.
References


Table 1. Parameters and Levels Used in This Experiment Based on the Taguchi Experimental Design

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature (°C)</td>
<td>35</td>
</tr>
<tr>
<td>Pressure (bar)</td>
<td>150</td>
</tr>
<tr>
<td>t-BuOH (%)</td>
<td>2</td>
</tr>
<tr>
<td>Lipase (mg/ml)</td>
<td>10</td>
</tr>
</tbody>
</table>

Figure 1. Scheme of the Experimental Batch-Stirred Apparatus for Synthesis Under High Pressure: (1) CO₂ Tank, (2) High Pressure Pump, (3) Reactor, (4) Stirrer, (5) SC CO₂ Sampling Loop (From Bottom of the Reactor), (6) SC CO₂ Sampling Loop (From Top of the Reactor), (7) Collector, (8) Temperature Controller, (9) Pressure Gauge