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The Format of Title: Times New Roman 14; Boldface and capitalized

# 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 [illegible] a major challenge due to the heterogeneity of these [illegible] Taguchi approach was employed to optimi[illegible] immobilized *Candida antarctica* lipase [illegible] temperature (35-55 °C), pressure (150-250 bar), enzyme amount (10–30 mg/ml), were investigated. The maximum co[illegible]ched 63 % in 25 hours at 150 bar, 55 º C, 10 % *t*-butanol (v/v), and 20 mg/ml of lipase. The SC $CO_2$ 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.

The format of content: Times New Roman 12, 1.5 line spacing.

Abstract requires 140-170 words and only can have one

Keywords need in alphabetic order.

**Key words:** *Candida antarctica* lipase, Chlorogenic acid, lipophilization, supercritical

**The format of subtitle:** Times New Roman 12; Boldface
Do not put any punctuation mark.

The Format of content: Times New Roman 12, 1.5 line spacing.

Indent two Chinese characters (4 English characters ) at the beginning of every paragraph.

## Introduction

Chlorogenic acid (5-cafeoyl-quinic acid, 5-CGA) has been associated to a broad ge 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 primary attributed to its ca to donate hydrogen atoms of the phenolic ring to free radicals, t processes. It has been proposed that such bioactiviti natural phenolics can be enhanced by increasing the amphiphilic structured phenolic would incorporate 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).

*et al.*, : in citations, only with four or more authors use *et al.*
∗*et al*. should be italic

Citation format:
Only put the last name of author in the parentheses.
Less than 4 authors should write out all the last names. JIC requires "and" instead of "&".

## Materials and Meth

### *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 $CO_2$*

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

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## Results and Discussion

In this work, the possibility of performing a lipase-catalyzed esterification of 5-CGA and 1-heptanol in SC $CO_2$, 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 $CO_2$.

Immobilized lipase B from *Candida antarctica* (20 mg/ml) was used to successfully lipophilize 5-CGA by esterification with 1-heptanol in $SCO_2$/*t*-butanol (10 % v/v) at 150 bar, and 55 ºC. Under these conditions, reaction rates approached 2.32 µM ester/ g lipase per minute with conversions of 63 %.

The Reference format: Place “References,” alphabetically by authors.

**References**


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Only the surname of the first author is before the first name. The others are contrary.

Book reference

Journal article

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The Title of the tables: Times New Roman 12; Boldface; Capitalized.
Omits period and above a table.

**Table 1. Parameters and Levels Used in This E**

**Experimental Design**

| ameter | Level | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| e (°C) | 35 | 45 | 55 |
| re (bar) | 150 | 200 | 250 |
| BuOH (%) | 2 | 5 | 10 |
| ipase (mg/ml) | 10 | 20 | 30 |

Present tables with the minimum use of horizontal rules (usually three are sufficient) and avoiding vertical rules except in matrices.

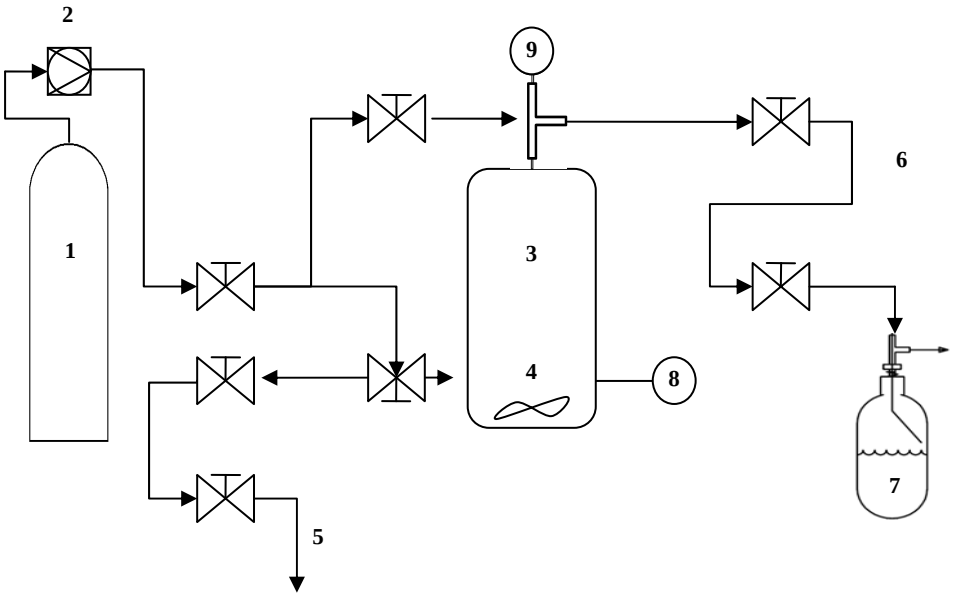


**Figure 1. Scheme of the Experimental Batch-Stirred Apparatus for Synthesis Under High Pressure: (1) $CO_2$ Tank, (2) High Pressure Pump, (3) Reactor, (4) Stirrer, (5) SC $CO_2$ Sampling Loop (From Bottom of the Reactor), (6) SC $CO_2$ Sampling Loop (From Top of the Reactor), (7) Collector, (8) Temperature Controller, (9) Pressure Gauge**

The Title of the Figures: Times New Roman 12; Boldface; Capitalized.
Omits period and below the picture.