

The total pages
are not over 25.

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

Direct Lipase Catalyzed Lipophilization of Chlorogenic Acid in Supercritical Carbon Dioxide

Times New Roman
12; Boldface

Carlos E. Hernandez¹, Ho-Hsien Chen², Chi-I Chang³, and Tzou-Chi Huang^{2*}

1. Department of Tropical Agriculture and International Cooperation, 2. Department of Food Science
and, 3. Graduate Institute of Biotechnology, National Pingtung University of Science and Technology,
Taiwan, R. O. C.

*: Corresponding author. Email: jic@mail.npust.edu.tw. Fax: 886-8-7740446

Times New Roman 10;
No boldface

Times New Roman 12; Boldface;
all alphabets should be capitalized

ABSTRACT

The enzymatic lipophilization of natural antioxidants is a process of enormous pharmaceutical interest; the direct esterification of such compounds has been a major challenge due to the heterogeneity of these reactants. A Taguchi approach was employed to optimize the reaction conditions. The immobilized *Candida antarctica* lipase was used. The reaction temperature (35-55 °C), pressure (150-250 bar), and enzyme amount (10-30 mg/ml), were investigated. The results showed that 63 % in 25 hours at 150 bar, 55 °C, 10 % *t*-butanol (v/v), and 10 mg of enzyme. 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.

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

Abstract requires 140-170 words
and only can have one
paragraph.

Keywords need in alphabetic order.

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

Introduction

Chlorogenic acid (5-caffeoyl-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 initiating various biological processes. It has been proposed that such bioactivities of natural phenolics can be enhanced by increasing their amphiphilic character. An 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).

Materials and Methods

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 with a 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 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.

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 "&"

Second Subtitle:
Times New Roman 12;
Boldface; Italic

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 SCO₂/t-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 %.

The Reference format: Place "References,"
alphabetically by authors.

References

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

Badaoui, E. E. and T. Rebière. 2013. *Education, Informality, and Efficiency: A Matching Model for a Developing Economy*. 2nd ed., Paris, France: Revue d'économie politique.

Book
reference

Bernier, J., G. Atlin, A. Kumar, R. Serraj, and D. Spaner. 2009. "Breeding Upland Rice for Drought Resistance," *Journal of Science Food Agriculture*, 88(6): 927-939.

Journal
article

Crosson, P. 1995. "Natural Resource and Environmental Consequences of Rice Production," in *Proceedings of the International Rice Research Conference*, February 13-17, Laguna, Philippines, pp. 83-100.

Conference
proceeding

FAO, 2004. *Rice is Life*. International Year of Rice 2004. Retrieved March 17, 2014, from the World Wide Web: <http://www.fao.org /rice2004/en/world.htm>.

Web site
article

Guerra, L. C., S. I. Bhuiyan, T. P. Tuong, and R. Barker. 1998. "Producing More Rice with Less Water from Irrigated Systems," in *SWIM Paper 5*, IWMI/IRRI, Colombo, Sri Lanka, p. 24.

Working
paper

Munroe, K. E. 2005. *Development Strategies for International Cooperation in Community Fisheries Industry in The Gambia*. PhD Dissertation, Department of Tropical Agriculture and International Cooperation, National Pingtung University of Science and Technology, Pingtung, Taiwan.

Thesis
article

Table 1. Parameters and Levels Used in This Experiment

Experimental Design

Parameter	Level		
	1	2	3
Temperature (°C)	35	45	55
Pressure (bar)	150	200	250
EtOH (%)	2	5	10
Lipase (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.

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

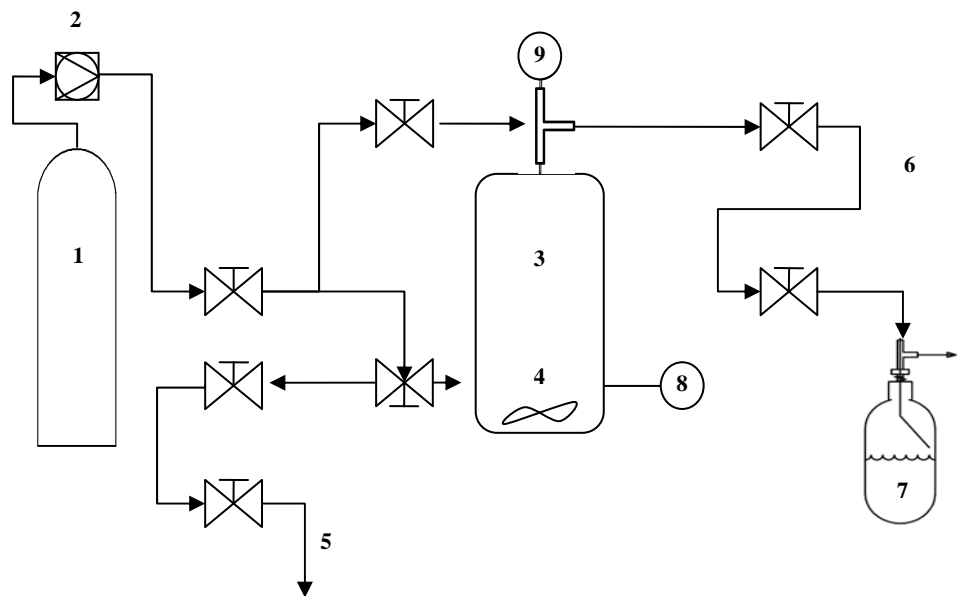


Figure 1. Scheme of the Experimental Batch-Stirred Apparatus for Synthesis Under High Pressure: (1) CO₂ Tank, (2) High Pressure Pump, (3) Reactor, (4) Stirrer, (5) SC CO₂ Sampling Loop (From Bottom of the Reactor), (6) SC CO₂ Sampling Loop (From Top of the Reactor), (7) Collector, (8) Temperature Controller,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

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