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Enzymatic preparation of novel caffeoyl structured lipids using monoacylglycerols as caffeoyl acceptor and transesterification mechanism

Shangde Sun*, Bingxue Hu

Lipid Technology and Engineering, School of Food Science and Engineering, Henan University of Technology, Lianhua Road 100, Zhengzhou 450001, Henan Province, PR China

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ABSTRACT

Caffeoyl structured lipids (CSLs), including hydrophilic glyceryl caffeates (GCs) and lipophilic caffeoylated acylglycerols (CAGs), are novel derivatives of caffeic acid. In the work, CSLs were successfully synthesized by enzymatic transesterification using different monoacylglycerols as caffeoyl acceptors. The effect of monoacylglycerols on reaction selectivities (for hydrophilic GCs and lipophilic CAGs) was compared. The thermodynamics, kinetic analyses, and reaction mechanism of the transesterification were also evaluated. The apparent activation energies for the transesterification, GCs and CAGs formation were 23.10, 12.03, 28.32 kJ/mol for monooleate, and 25.85, 19.19, 32.02 kJ/mol for monostearate, respectively. The values of V_{max}, K_m' and K_{IA} for the reaction using monooleate as caffeoyl acceptors were 1.19×10^{-3} mol/(L min), 0.82 mol/L, and 2.31 mol/L, respectively. High ethyl caffeate conversion and CSLs yields (98.2 ± 1.8%, 94.7 ± 2.8% for monooleate; and 96.8 ± 2.3%, 93.9 ± 2.7% for monostearate) were achieved under the optimized conditions.

1. Introduction

Caffeic acid (CA) is a natural phenolic acid, which is widely distributed in some agricultural products, beverages and Chinese medicinal herbs [1,2]. Recently, CA has attracted much attention in many industries [3], due to its biological activities, such as, antioxidant, free radical-scavenging, and anti-cancer, etc [4,5]. However, the application of phenolic acids, especially for CA, was limited due to its unsatisfactory solubility in hydrophilic and lipophilic media [6–10], which made CA modification attract considerable attention [11–15].

According to the previous reports, many groups have been used to modify CA, for examples, alkyl [16–19], and phytosteryl [20], glycerides [21–23]. Caffeoyl structured lipids (CSLs), including hydrophilic glyceryl caffeates (GCs) and lipophilic caffeoyl acylglycerols (CAGs), are novel derivatives of caffeic acid (CA), which have antioxidant and UV absorption capacities. These made these CSLs potentially use as antioxidants and UV-absorbers in food and cosmetics fields.

Monoacylglycerols, with available —OH groups and fatty acyls in their structures, can be used in the preparation of some lipophilized phenolic acids [24–26]. Compared with triacylglycerols, monoacylglycerols can enhance the modification of phenolic acids. Monooleate (MOG) and monostearate (MStG), two kinds of monoacylglycerols, with different fatty acid composition and structures, have different melting points, morphological characteristics, and steric hindrance. However, no available information of CSLs preparation using monoacylglycerols (MAGs) as caffeoyl acceptors can be found.

The specific objective of the work was to investigate an enzymatic method for novel caffeoyl structured lipids (CSLs) preparation by lipase-catalyzed transesterification using different MAGs as caffeoyl acceptors (Fig. 1). Effects of MAGs (MOG and MStG) on reaction selectivities for hydrophilic GCs and lipophilic CAGs were

Abbreviations: CA, caffeic acid; EC, ethyl caffeate; CSLs, caffeoylated structured lipids; GCs, glyceryl caffeates; GMC, glyceryl monocaffeate; GDC, glyceryl dicaffeate; CAGs, caffeoylated acylglycerols; CMAGs, caffeoylated monoacylglycerols; CDAGs, caffeoylated diacylglycerols; MAGs, monoacylglycerols; MOG, monooleate; MStG, monostearate; HPLC-ESI–MS, high performance liquid chromatography-electro spray ionization-mass spectroscopy; HTGC-FID, high temperature gas chromatography-flame ionization detector.

* Corresponding author.
E-mail addresses: sunshangde@hotmail.com (S. Sun), hbx15136172867@126.com (B. Hu).

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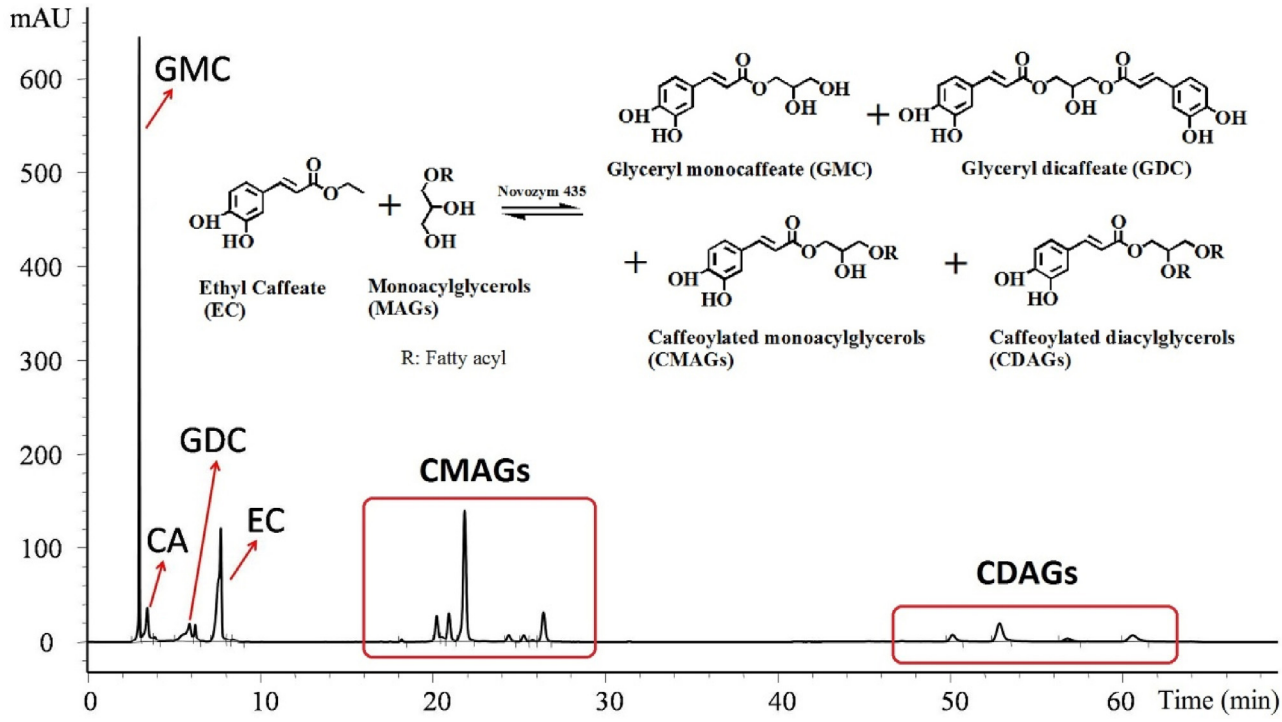

Fig. 1. Reaction scheme for enzymatic transesterification of ethyl caffeate (EC) with monoacylglycerols (MAGs) and the HPLC chromatogram of reaction mixture at 325 nm.

compared. The transesterification process was monitored by HPLC-ESI–MS and HPLC-UV. The effects of reaction parameters on the transesterification were evaluated. Reaction mechanism, thermodynamic and kinetics were also investigated.

2. Materials and methods

2.1. Materials

Ethyl caffeate (EC, purity >99%) was purchased from Nanjing Zelang Chemical Co., Ltd. (Nanjing, China). Commercial monooleate (MOG, water content <0.5%) and monostearate (MStG, water content <0.5%) were both purchased from Aladdin Industrial Corporation (Shanghai, China). Novozym 435 (*Candida antarctica* lipase B immobilized on polyacrylic resin by adsorption, 10,000 PLU/g solid enzymes) was purchased from Novozymes A/S (Bagsvaerd, Denmark). Oleic acid (≥99%), fatty acid methyl ester mix (C8–C22), lipid standard (mono-, di-, & triglyceride mix) were purchased from Sigma-Aldrich (Shanghai, China). Methanol (HPLC grade) and glacial acetic acid (HPLC grade) were both from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Ethanol (HPLC grade) and trichloromethane (HPLC grade) were both from Tianjin Kermel Chemical Co., Ltd. (Tianjin, China). All other reagents were of analytical grade.

2.2. Enzymatic transesterification

The transesterifications of EC with commercial monoacylglycerols (MAGs) were carried out in 25 mL round-bottom flasks. Reaction mixtures were incubated at various temperatures (50–100 °C) using an oil bath with a magnetic stirrer under normal pressure (atmospheric pressure) or reduced pressure (90 kPa), and then the biocatalyst Novozym 435 (5–30%, relative to the weight of total substrates) was added to the reaction mixtures. Samples (5 μL) were withdrawn at specific time intervals (0, 1, 2, 3, 6, 12, 24, 36, 48, 60, and 72 h).

Table 1
Lipids composition of monooleate (MOG) and monostearate (MStG).

Sample	FFAs (%)[a]	MAGs (%)[b]	DAGs (%)[c]	TAGs (%)[d]
MOG	5.55 ± 0.62	47.75 ± 2.26	41.43 ± 1.73	5.27 ± 0.64
MStG	3.34 ± 0.55	92.56 ± 2.42	4.10 ± 0.52	–

[a] FFA, free fatty acids.
[b] MAGs, monoacylglycerols.
[c] DAGs, diacylglycerols.
[d] TAGs, triacylglycerols.

2.3. HPLC-UV and HPLC-ESI–MS analysis

Samples were dissolved in the solvent mixture which consisted of 2 mL trichloromethane (HPLC grade) and 1 mL methanol (HPLC grade), and then the mixed solution was filtrated using 0.45 μm aperture filter head. Reaction progress was monitored using HPLC (Waters 1525) with a C18 column (250 mm × 4.6 mm, 5 μm) fitted with an automatic sampler (Waters 2707) and a dual absorbance detector (Waters 2489) at 325 nm. Solvent A (methanol) and solvent B (water, 0.5% v/v glacial acetic acid) were used as the mobile phases at 1 mL/min. The samples were eluted using a linear gradient from 50% B (v/v) to 90% B (v/v) in 10 min, 90% B (v/v) to 100% B (v/v) in 30 min, and 100% B maintained for 20 min, then to 50% B in 5 min, followed by 50% B for 5 min at 35 °C. Components in the sample were identified with regard to the relevant major ions detected by HPLC-ESI–MS according to our previous report [21].

2.4. Analysis of fatty acid composition of MOG and MStG

MOG and MStG were methylated according to AOCS Official Method Ce 2-66. The analysis of fatty acid methyl ester (FAME) was performed on a gas chromatography (GC) system (Agilent 6890N) equipped with a SGE BPX-70 capillary column (30.0m × 0.25 mm, 0.25 μm of film thickness) fitted with a hydrogen flame ionization detector (FID). The operating conditions were 300 °C for the FID, 210 °C for the injection port, 190 °C for the column temperature.