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Production of Calcium-Stearate by Lipase Using Hydrogenated Beef Tallow

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Abstract Calcium-stearate has been traditionally produced by chemical methods, producing wastes and requiring high energy because of high temperature operation. To achieve enzymatic production of calcium-stearate at unfavorable conditions, i.e., pH 10 and 60 °C, suitable lipase was selected and reaction conditions were optimized using calcium hydroxide and hydrogenated beef tallow as substrates. Under optimum conditions, 95% of beef tallow, in 2.5 h, was converted into calcium-stearate by using commercial lipase SDL 451. Investigation of the time-course reaction revealed that fatty acid was initially produced by lipase, followed by conversion into calcium-stearate. The fatty acid production rate was faster than that of the conversion into calcium-stearate at the beginning of the reaction. Alkaline pH, originating from the addition of calcium hydroxide, increased the converting reaction. This is the first report demonstrating that chemical production of calcium-stearate can be replaced by enzymatic reaction, thereby creating a cleaner process.

Keywords Calcium-stearate · Lipase SDL 451 · Hydrogenated beef tallow

Introduction

Calcium-stearate is commercially used as a lubricant, stabilizer, catalyst, coating material, cement additive, and animals feed additive [1]. Two industrial processes are currently employed for converting fatty acids and calcium to calcium-stearate: double decomposition process and fusion process [1–2]. However, the former has disadvantages such as high cost of equipment and occurrence of a byproduct (sodium chloride) [2–3]. In the case of the latter, problems including high viscosity and heat generation causing discoloration of the product should be overcome [4].

An enzymatic conversion process consumes less energy and produces less byproduct [5–7]. Lipase is used for various reactions such as hydrolysis, esterification, and inter-esterification [8–12]. During hydrolysis of oil, glycerol and fatty acids are produced [13].

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Addition of calcium during this hydrolysis reaction, therefore, can produce calcium-stearate (Fig. 1). Hydrogenated beef tallow containing more than 60% stearate is a good candidate for calcium state production. However, because of the high melting temperature of hydrogenated beef tallow and high pH reaction due to calcium hydroxide addition, the selection of an effective lipase as well as establishment of optimum reaction conditions for successful calcium-stearate production are critical.

In this study, we selected a high-temperature and pH tolerant-lipase and successfully produced calcium-stearate using hydrogenated beef tallow and calcium hydroxide. This novel enzymatic process can replace traditional chemical reactions, thus creating a cleaner process.

Materials and Methods

Materials and Reagents

Lipases were obtained from commercial sources: Lipolase 100 T from *Aspergillus oryzae* (Novo Nordisk, Denmark), Lipase-OF 360000 from *Candida cylindracea* and Lipase PL from *Alcaligenes* sp. (Meito Sangyo, Japan), Lipase SDL 451 from *Pseudomonas* sp. (Show Denko, Japan), Lumafast 2000G from *Pseudomonas* sp. (Genencor, Finland), Lipomax CXT 1000 (Gist-Brocades, The Netherlands), Lipase A 'Amano 6' from *Aspergillus niger*, and Lipase F-AP15 from *Rhizopus* sp. (Amano Seiyaku, Japan). Olive oil (SV 198.6), $Ca(OH)_2$ (Junsei Chemical), and hydrogenated beef tallow (Peace Oil, Korea) were used as substrates for the reaction.

Calcium-Stearate Production by Lipase

The reaction mixture contained 50 g water, 30 g of hydrogenated beef tallow, 4.4 g of $Ca(OH)_2$, and 0.075 g of lipase SDL. The reaction mixture was put into a 500-ml beaker in a water bath and stirred by an agitator equipped with an impeller (480 rpm). The impeller

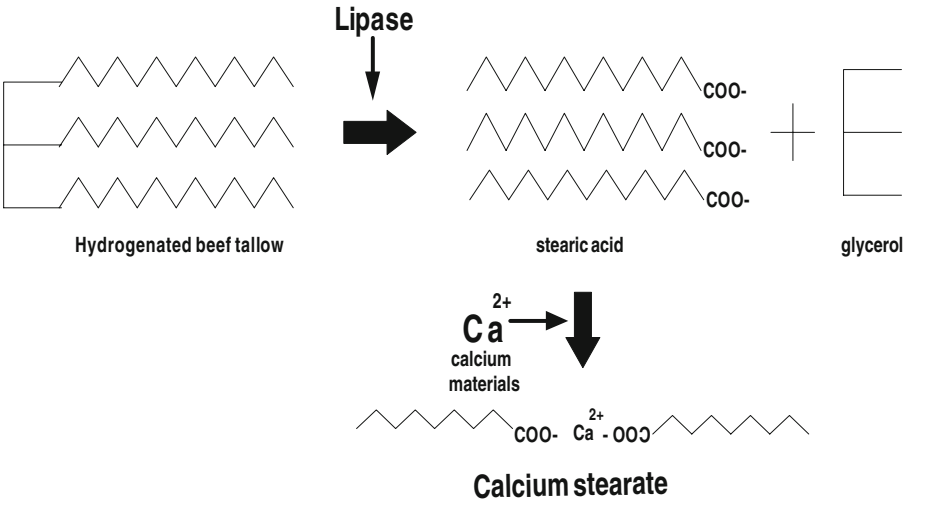

Fig. 1 Schematics of ca-stearate production by lipase

Table 1 Composition of beef tallow.

Fatty acid	Beef tallow (%)	Hydrogenated beef tallow (%)
C14	3.3	3.4
C15	0.9	0.5
C16	23.5	28.0
C17	1.3	2.5
C18	17.0	63.1
C18:1	38.2	0.5
C18:2	5.4	0.6
C20	0	1.4

consists of two paddles (5×20 mm). After 2.5 h of reaction at 60 °C at 480 rpm, powder-like calcium-stearate was filtered.

Analysis

Fatty acid composition Gas chromatography (Shimadzu GC-14B, Japan) was used to analyze the beef tallow [14]. The capillary column was Supelcowax 10 (30 M, 0.53 mm ID, 1 μm OD). The temperature of the GC injector and detector was 240 °C and 250 °C, respectively. Operating conditions were 40 psi for N_2 gas, 20 psi for H_2 gas, and 40 psi for air, respectively [15]. Initial and final temperature was 110°C and 230°C, respectively, with an increasing rate of 5°C/min.

Lipase activity Lipase activity was measured using *p*-nitrophenyl palmitate (*p*NP) as a substrate [14]. The substrate solution was made by adding 37.5 mg *p*NP in 2-propanol to a reaction buffer of 50 mM Tris–HCl containing 0.1% Triton X-100 and quickly shaken at 60 °C.

The enzyme solution contained 10,000 units of corresponding lipase in 0.2 ml distilled water. Hydrolysis reaction (0.2 ml enzyme solution and 3 ml substrate solution) was performed in a water bath at 60 °C for 5 min and the change in absorbance at 410 nm was measured with a spectrophotometer. A unit of lipase activity is described as the amount of enzyme catalyzing the release of 1 μmol of *p*NP per minute. The molar absorption coefficient of *p*NP was 15,100/M in the assay buffer (pH 8.0). 0.1 N HCl was added to adjust the pH.

Table 2 Hydrolysis activity and Ca-stearate production.

Enzyme	Hydrolysis activity (μM/unit)[a]			Ca-stearate conversion (%)
	pH 8	pH 9	pH 10	
Lumafast 2000G	0.3	0.8	0.3	ND
Lipase A'Amano6'	0.1	0.2	0.1	ND
Lipase FAP 15	0.1	0.2	0.02	ND
Lipase PL	5.1	7.1	4.7	10
Lipase-OF 360000	2.8	3.1	0.5	40
Lipase CXT 1000	13.3	18.6	12.6	50
Lipolase 100 T	2.9	8.1	5.7	60
Lipase SDL 451	16.1	19.7	14.6	75

ND not determined

[a] Lipase activity measured by *p*-nitrophenyl palmitate (*p*NP). Reaction, 60 °C

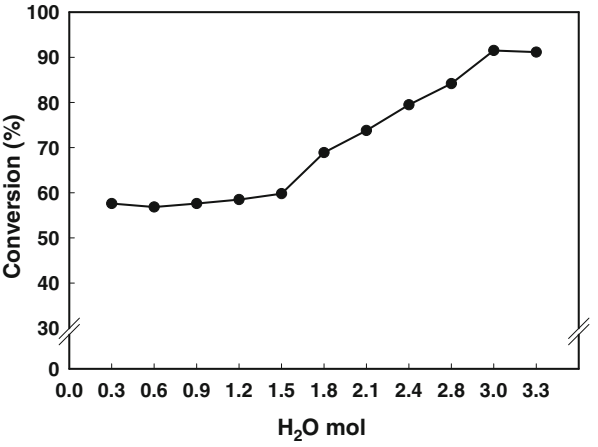

Fig. 2 Effect of water on calcium-stearate production. H_2O (11.34 ~ 57.6 g) was added to the reaction mixture containing 30 g of hydrogenated beef tallow, 4.4 g of Ca $(OH)_2$, and 0.075 g of lipase SDL

Calcium-stearate The final product was milled by a miller. To remove the glycerol and unreacted calcium hydroxide of the reactant, 240 ml of H_2O was added to 5 g of crude calcium-stearate in a separation funnel and was then evaporated completely at 85 °C for 2 h. The dried sample was extracted by chloroform. Calcium-stearate, located in the upper layer of chloroform [17] was filtered through Whatman paper #5 and then evaporated at 85 °C for 30 min. The conversion ratio is expressed as actual versus theoretical yield.

FT-IR The dehydrogenated sample (0.01 g) was mixed with 1 g of KBr powder and milled. A pellet was formulated by a pelletizer under 7 tons for 1 min and was measured by Fourier transform infrared (FT-IR) (JASCO, FT/IR-300E, Japan).

Results and Discussions

Calcium-stearate can be produced by addition of calcium ion during hydrolysis of lipid. As a source of stearate, two lipids, beef tallow and hydrogenated beef tallow, were investigated. The fatty acid composition of beef tallow and hydrogenated beef tallow were analyzed by gas chromatography. As shown in Table 1, beef tallow contained mainly oleic acid (38.2%). Hydrogenated beef tallow, however, contained more than 60% stearic acid, indicating that hydrogenated beef tallow is a good stearate source for the enzymatic production of calcium-stearate. High content of stearate in the hydrogenated beef tallow results from the efficient conversion of C:18–1 and C:18–2 fatty acids into stearate (C:18) during hydrogenation. Hydrogenated beef tallow is an economical and plentiful source of lipids and is widely used in the manufacturing of soap. Hydrogenated beef tallow is, therefore, selected as an optimum substrate.

Table 3 Effect of calcium on the Ca-stearate yield.

Calcium Compound[a]	Yield (%)
$CaCl_2$	9
$CaSO_4$	10
$Ca(NO_3)_2$	10
$Ca(OH)_2$	92

[a] Reaction mixture; 60 mM calcium compound in 50 g water, 30 g of hydrogenated beef tallow, and 0.075 g of lipase SDL at 60 °C

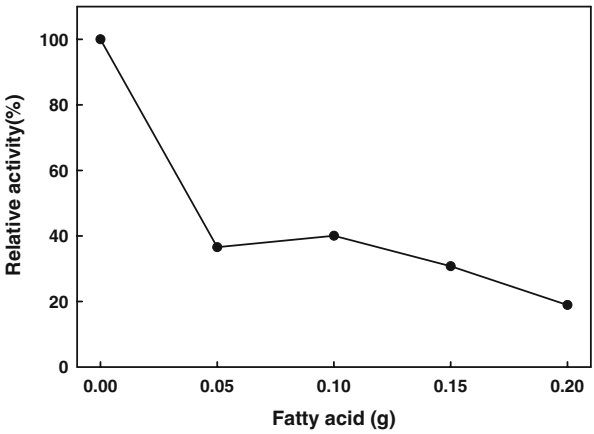

Fig. 3 Effect of stearic acid on the activity of Lipase SDL 451. The reaction mixture contained fatty acid, 50 g water, 30 g of hydrogenated beef tallow, 4.4 g of Ca $(OH)_2$, and 0.075 g of Lipase SDL 451

The melting point of beef tallow is 60 °C. Also, the pH of the reaction mixture increases up to 10.5 during the reaction because of the addition of $Ca(OH)_2$. The lipase showing maximum activity was screened from various commercial lipases. As shown in Table 2, Lipase PL, Lipase-OF 360000, Lipase CXT 1000, Lipolase 100 T, and Lipase SDL 451 were initially selected. Using these lipases, the yield of calcium-stearate at 60 °C was measured. Lipase SDL 451 showed the maximum production of calcium-stearate (Table 2). This enzyme was thus used for further optimization experiments. Authenticity of the produced calcium-stearate was examined by FT-IR. The observed FT-IR pattern corresponded with the standard, indicating that it is suitable for enzymatic production (data not shown).

Water content is important for hydrolysis, esterification, and inter-esterification. To examine the effect of water on the calcium-stearate production, various amounts of water were added to the reaction mixture. As shown in Fig. 2, calcium-stearate production increased as the amount of water increased and reached maximum conversion (91.5%) at

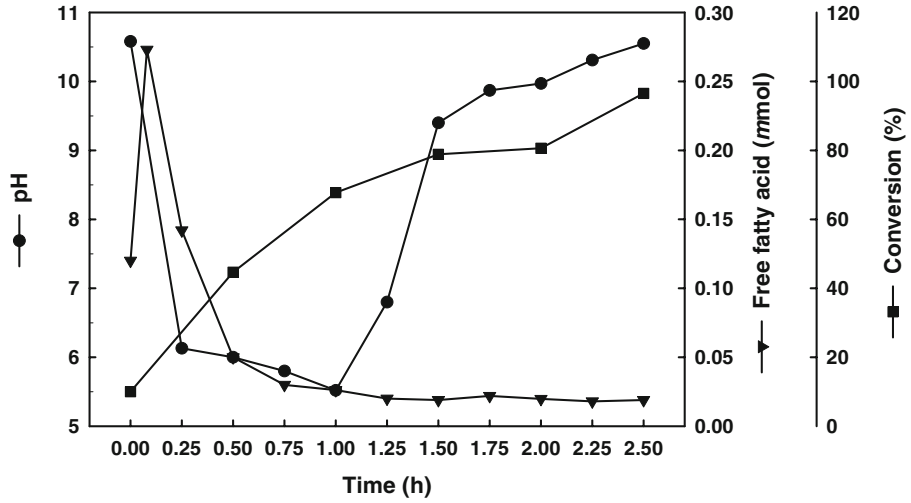

Fig. 4 Time-course production of calcium-stearate using Lipase SDL 451

3-mol water addition. Calcium is an important ingredient for calcium-stearate production. The activity of Lipase SDL 451 was examined, and no significant change was observed up to 40 mM of $CaCl_2$ (data not shown). However, calcium-stearate conversion increased by using the OH^- ion in $Ca(OH)_2$ form, thus reflecting the importance of the OH^- ion (Table 3). Calcium-stearate was produced in a powder form and attached to the reactor surface. To maintain the homogeneity of the solution, several surfactants were added to the reaction mixture. Commercial calcium-stearate is also available in a dispersed liquid form. Therefore, surfactant addition can produce dispersed calcium-stearate in situ. However, the activity of the lipase as well as the calcium-stearate production decreased significantly by the addition of various surfactants (data not shown).

Lipase-mediated hydrolysis reaction produces fatty acid, which can cause feedback inhibition. As shown in Fig. 3, significant inhibition of lipase activity was observed with the addition of free fatty acid. However, as shown in Fig. 4, there was no significant accumulation of free fatty acid, and consequently no inhibition of conversion was observed after 30 min of reaction. Lipase SDL 451 is an alkaline lipase showing maximum activity at pH 9 and little activity at pH 6 (data not shown). However, no noticeable decrease in the calcium-stearate production was observed under these reaction conditions. This phenomenon was caused by the consumption of generated fatty acid by the production of calcium-stearate. As shown in Fig. 4, pH sharply dropped from 10.5 to 5.5 and recovered to 10.5 immediately. This trend is a result of the generation of fatty acid (pH drop) and its consumption (pH rise).

More than 95% conversion was achieved in 2.5 h by screening the lipase and optimizing the reaction conditions (Fig. 4). Produced calcium-stearate could be recovered easily by simple filtering. By this enzymatic process, production cost can be reduced to 60% of the traditional process based on the cost estimation (data not shown). This simple and novel process can replace the traditional manufacturing process, which consumes high energy and produces byproducts.

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