# Transesterification of Phytosterol and Edible Oil by Lipase Powder at High Temperature

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**ABSTRACT:** Phytosterol, which is hardly soluble in edible oil, was solubilized at a high concentration by converting it to FA esters by lipase-catalyzed transesterification at temperatures higher than 100°C using powdered Lipase QLM (Meito Sangyo Co., Ltd., Nagoya, Japan). Transesterification was conducted in sunflower oil containing 10% phytosterol, without adding water or solvent, at 100°C. The conversion rate was 97.1% after 7 h of reaction. The effect of temperature on the conversion rate was also examined. Maximum enzyme activity occurred in the 100–120°C range, and 20% of the maximum activity was retained even at 130°C. When the lipase was recovered by filtration and recycled for repeated reactions at 90°C, the half-life of lipase activity was 260 h. Thus, edible oils with nutritional value could be produced by blending the phytosterol-containing sunflower oil into other edible oils.

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**KEY WORDS:** Edible oil, high temperature, lipase, phytosterol, phytosteryl ester, β-sitosterol, β-sitosteryl ester, thermostable, transesterification.

Because of the nutritional advantages of phytosterols and the FA esters of phytosterols in the suppression of cholesterol absorption (1), various attempts have been made to develop phytosterol-containing edible oils. However, the m.p. of sterols are higher than 100°C, and thus they precipitate in edible oil. This is a critical drawback that prevents the commercialization of phytosterol on its own. Therefore, conversion to a corresponding FA ester, such as oleate, is considered desirable. Although FA esters of phytosterols can be synthesized by chemical reaction, the chemical method involves problems such as the formation of a 3,5-diene derivative as a side product and staining. To avoid these problems, an enzymatic method that uses lipase was developed (2).

To synthesize FA esters of sterols using lipase, the reaction was carried out in organic solvent or in an oil/water two-phase medium to increase the solubility of the sterol in the oil substrate. Osanai (3) reported the synthesis of an oleic acid ester of cholesterol using lipase and organic hydrocarbon solvents such as cyclohexane, benzene, and toluene, whereas Shimada *et al.* (4) reported the synthesis of a DHA ester of cholesterol in a two-phase medium containing 30% water. Furthermore, Weber *et al.* (5–7) efficiently synthesized steryl esters by carrying out the enzymatic reaction under reduced pressure to remove the water and alcohol from the reaction system.

However, in the future, the construction of a continuous reaction system will be required, as edible oils are manufactured in production line systems in practice. For that reason, it is desirable to construct a reaction system that requires no solvents and that contains procedures for loading and unloading reactants during the reaction. Therefore, it is preferable to construct a reaction system as simply as possible, which is advantageous from the safety standpoint as well.

We previously reported on the practical production of transesterified edible oils using powdered lipase that can be activated by water and dissolved in the oil substrate (8). On the basis of our findings, it was considered that phytosterol esters could be synthesized in a simple reaction medium consisting of phytosterol and edible oil by lipase-catalyzed transesterification at high temperatures. The present study was undertaken to investigate the production of phytosterol esters by high-temperature lipase-catalyzed transesterification of phytosterol in neat edible oil.

## MATERIALS AND METHODS

*Substrates and lipases.* Sunflower oil from Settsu Oil Mill Co., Ltd. (Osaka, Japan) was used in this study. The main FA was oleic acid (85%). Phytosterol was supplied by the Tama Biochemical Co., Ltd. (Tokyo, Japan). The main component of the sterol preparation was β-sitosterol (48%). As for the lipase, Lipase QLM (*Alcaligenes* sp.) was from Meito Sangyo Co., Ltd. (Nagoya, Japan). Other reagents used in this study were commercially available and of chemically pure grade.

*Analysis.* The composition of the reaction mixture was determined by GLC using a DB-1ht (0.32 mm × 0.1 μm × 5 m) column (Agilent Technologies, Palo Alto, CA), under the following analytical conditions: The injection temperature was 370°C, the detector temperature was 370°C, the column temperature was raised from 150 to 370°C at a rate of 15°C/min, the split ratio was 80:1, and helium was used as the carrier gas at a constant flow rate of 7.0 mL/min.

*Measurement of transesterification activity.* The transesterification activity of lipase was measured as in our previous paper (8). Sunflower oil (9 g) and phytosterol (1 g) were used as the substrates. Lipase QLM was added to the substrates in powdered form and mixed with a magnetic stirrer at 70–130°C.

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A sample was collected from each reaction after 1, 2, 3, 5, and 7 h of reaction time and then subjected to GLC to calculate the degree of conversion (%) using the following equation.

$$\text{degree of conversion (\%)} = \frac{\beta\text{-sitosteryl ester area}}{\beta\text{-sitosterol area} + \beta\text{-sitosteryl ester area}} \times 100 \quad [1]$$

The peaks of β-sitosterol and β-sitosteryl ester were identified using standard reagents. Their GLC peak areas were confirmed to be proportional to the concentrations. After the extent of the reaction was measured as a function of time, the reaction rate constant was calculated using a model for a single molecular reaction. The rate constant was used for a relative comparison of activities.

*Repeated use of lipase.* Lipase QLM (0.1 g) was added to 9 g of sunflower oil and 1 g of phytosterol, then mixed with a magnetic stirrer at 90°C. After the reaction, the lipase was separated by filtration. Ten rounds of this reaction were conducted, with each round lasting 20 h. During each round, the degree of conversion was measured after 2 h.

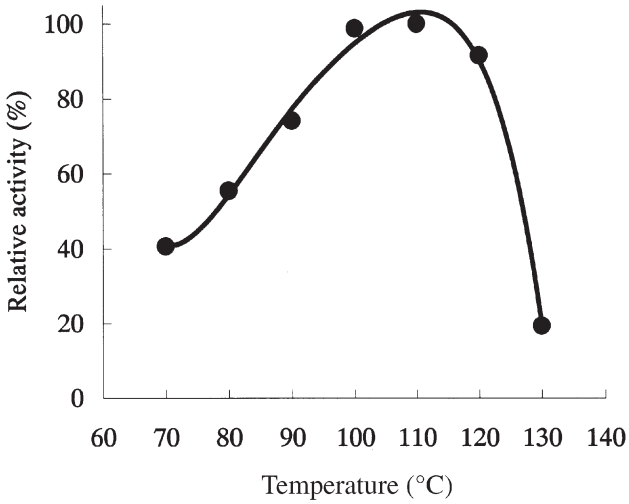


**FIG. 2.** Dependence of the relative activity of phytosterol and sunflower oil transesterification by Lipase QLM on temperature. Conditions: Lipase powder (0.1 g) was added to 9 g of sunflower oil and 1 g of phytosterol. For supplier of lipase see Figure 1.

## RESULTS AND DISCUSSION

*Transesterification of phytosterol and sunflower oil.* The transesterification of phytosterol with sunflower oil was conducted using powdered lipase QLM at 90 and 100°C (Fig. 1). Good yields were obtained (>95%). The phytosterol used contained sterols other than β-sitosterol, which did not affect the transesterification reaction, although the sterols did affect the reaction rate somewhat.

*The effect of temperature.* The effect of reaction temperature on enzyme activity is illustrated in Figure 2. The highest relative enzyme activity was observed in the 100–120°C range, and 20% of the maximum activity was retained at 130°C. Lipase activity was also retained at high temperatures in the transesterification of TAG. As reported in a previous paper (8), the enzyme has the property of being activated by water present in the oil. Thus, the reaction can be carried out under a condition with the least water content in the absence of any additional free water that may deactivate the enzyme. Enzymatic transesterification was therefore considered to take place stably at such high temperatures (8).

*The effect of phytosterol concentration.* The effect of phytosterol concentration on the relative transesterification activity is shown in Figure 3. At phytosterol concentrations greater than 20%, phytosterol could not be completely dissolved in sunflower oil, and transesterification did not occur. The enzyme activity tended to decrease when the phytosterol concentration increased, both at 90 and 100°C. This result can be explained

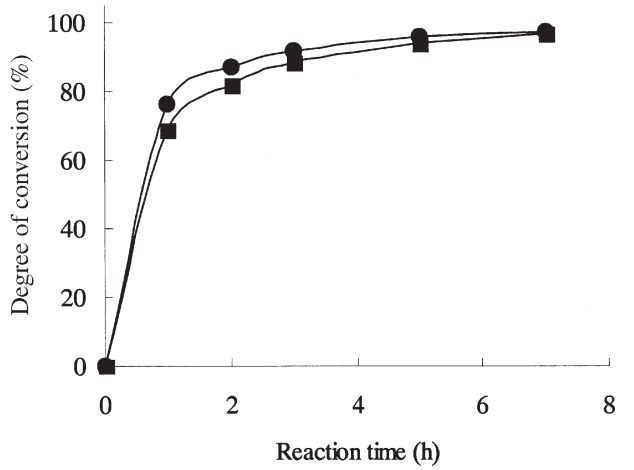


**FIG. 1.** Time course of the transesterification of phytosterol and sunflower oil at high temperatures. A mixture of 9 g sunflower oil, 1 g phytosterol, and 0.1 g Lipase QLM (*Alcaligenes* sp.; Meito Sangyo Co., Ltd., Nagoya, Japan) was stirred with a magnetic stirrer at 100 (●) and 90°C (■).

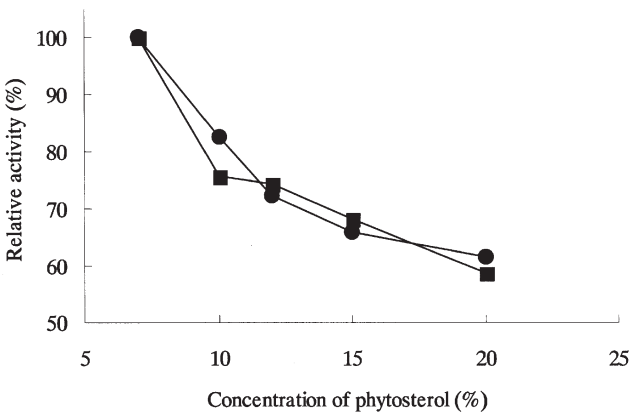


**FIG. 3.** Dependence of the relative activity of phytosterol and sunflower oil transesterification by Lipase QLM on the concentration of phytosterol at 100 (●) and 90°C (■). Conditions: Lipase powder (0.1 g) was added to 9 g of sunflower oil. For supplier of lipase see Figure 1.

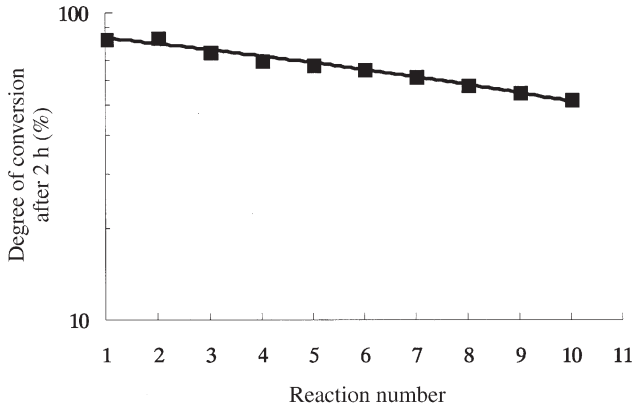


**FIG. 4.** Stability of Lipase QLM on repeated use. Conditions: Lipase powder (0.1 g) was added to 9 g of sunflower oil and 1 g of phytosterol at 90°C. For supplier of lipase see Figure 1.

by considering that the rate of esterification decreases with an increase in the rate of formation of the side product DAG when phytosterol concentration is elevated. Therefore, the phytosterol concentration chosen for investigation was 10%.

*Operational stability of lipase.* The stability of the enzyme during the high-temperature transesterification reaction was examined. In anticipation of future commercial production, we conducted the reaction repeatedly in 10 batches for 20 h at 90°C, and the recovered enzyme was recycled. The degree of conversion was determined after 2 h of reaction time. A logarithmic plot of the degree of conversion is shown in Figure 4. The half-life of enzyme activity was estimated from the slope of the graph to be 260 h. From these results, we concluded that the phytosterol ester can be manufactured on a scale of more than 100 kg per 1 kg of enzyme.

The present study demonstrated that lipase-catalyzed transesterification of phytosterol with sunflower oil could be carried out in the absence of any organic solvent and water at temperatures high enough to dissolve the phytosterol. The resulting phytosterol-containing sunflower oil can be utilized to produce edible oils containing an effective amount of phytosterol, which has the advantage of suppression of cholesterol absorption, by blending it into various kinds of edible oils.

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