Journal of Molecular Catalysis B: Enzymatic 67 (2010) 60–65

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Lipase-catalyzed synthesis of phytostanyl esters in non-aqueous media

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ARTICLE INFO

Article history:
Received 15 May 2010
Received in revised form 28 June 2010
Accepted 15 July 2010
Available online 22 July 2010

Keywords:
Phytostanyl ester
Lipase-catalyzed
Plant stanol
Esterification

ABSTRACT

Stanols are more effective and safer than sterols in lowering plasma total cholesterol, while stanol esters are advantageous since this compound has a better solubility than the free stanols, which contribute to the practical application in foods. The current work focuses on the synthesis of phytostanyl esters from plant stanols and fatty acid such as lauric acid, including screening of various source lipases and selecting solvents of different Log *P* values. Among lipases from different origins, the immobilized Novozym 435 lipase from microorganism was found to be the best biocatalyst, while plant lipase from *Carica papaya* and animal lipase from Porcine pancreas are quite ineffective as biocatalyst for the esterification of plant stanols with fatty acid. The highest phytostanyl laurate esterification degree of 79.3% was obtained under the selected conditions: 25 μmol/mL plant stanols, 100 μmol/mL lauric acid, 80 mg/mL 3 Å molecular sieves and 40 mg/mL Novozym 435 at 150 rpm and 55 °C for 96 h in 10 mL of *n*-hexane. The chemical structure of sitostanyl laurate was confirmed by FT-IR, MS and NMR. The comparison of solubility of plant stanols and phytostanyl laurate in plant oil was done.

1. Introduction

Since 1900, cardiovascular disease (CVD) is the number one killer for the adults and also the number two killer for children under the age of 15 in the US. Wide epidemiological, pathological, and clinical studies have shown a significant positive correlation between increased levels of total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) and the incidence of CVD in humans [1,2]. It is well-known that hypercholesterolemia is a major risk factor for coronary heart disease (CHD). The National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) recognizes that elevated LDL-C and TC are the major risk factors for CHD and has highlighted LDL lowering as a primary therapy to reduce the risk for CHD [2,3]. The serum LDL-C level can be lowered through the reduction of cholesterol absorption [4].

Plant sterols (phytosterols), are structurally related to cholesterol, but are characterized by an extra ethyl (β-sitosterol) or methyl group (campesterol) in the side chain [5]. Plant stanols (phytostanols) are the saturated form of sterols, mainly including sitostanol and campestanol. Phytosterols and phytostanols, which are mainly present in nuts, vegetable oils, seeds, cereals and beans, have been used successfully for lowering plasma cholesterol levels by inhibiting the absorption of cholesterol from small intestine in both animals and humans and shown to be safe for half a century [6–9].

In recent years, many studies have found that phytostanols lowered serum cholesterol more effectively than phytosterols and increased fecal excretion of cholesterol to a greater extent. In contrast to plant sterols, phytostanols are minimally absorbed in vivo. In general, phytosterols are poorly absorbed in the intestine (0.4–3.5%), while phytostanols absorption (0.02–0.3%) is even lower. This contrasts with intestinal cholesterol absorption that ranges from 35% to 70%. Even if some phytostanols absorption does take place, their overall effect is to reduce serum plant sterol concentrations as well as serum cholesterol levels [1,6,10]. However, practical application of free plant stanols in foods is limited because of their poor solubility and low bioavailability. For example, the solubility of phytostanols in edible oils is very low while their melting points are rather high (about 140–150 °C) [11]. Esterification or transesterification of plant stanols with fatty acids or oils and fats can increase their lipid-solubility and thus facilitates the incorporation into a variety of foods.

Chemical synthesis of phytostanyl esters is generally performed as a high temperature esterification in the presence of an alkaline catalyst, which is accompanied by high energy consumption, browning of products and low selectivity.

However, enzyme technologies offer a good alternative for its production allowing mild and environment friendly reaction conditions.

It has already been reported that phytosteryl esters were synthesized with different lipases [11–14]. However, little research has been performed on the preparation of phytostanyl esters by lipase-

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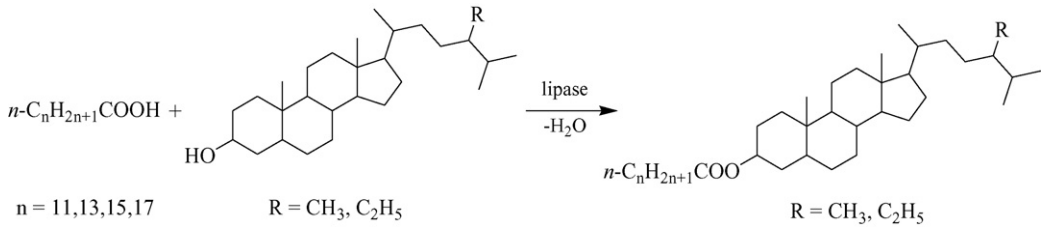
doi:10.1016/j.molcatb.2010.07.006



Scheme 1. Synthesis of phytostanyl esters in non-aqueous media.

catalyzed reactions. In this study, phytostanyl esters of different carbon chain lengths of fatty acids (C_{12}–C_{18}) were synthesized using various lipases, the prepared phytostanyl esters were isolated by column chromatography, and identification of the produced sitostanyl laurate was conducted by FT-IR, MS, NMR, and its optimal conditions using Novozym 435 lipase in non-aqueous media were explored.

2. Experimental

2.1. Materials

Lauric acid, myristic acid, palmitic acid, stearic acid, methanol, *n*-hexane, cyclohexane, ethyl acetate, and acetone were purchased from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China. The 3 Å 1/16 and 4 Å 1/16 molecular sieves were purchased from UOP Co., Ltd., Shanghai, China. Phytosterols were a generous gift from Jiangsu Spring Fruit Biological Products Co., Ltd., Taixing, China.

Novozym 435 (lipase B from *Candida antarctica*, immobilized on a macroporous acrylic resin), lipozyme RM IM (lipase from *Rhizomucor miehei*, immobilized on an anionic exchange resin), and lipozyme TL IM (lipase from *Thermomyces lanuginosus*, immobilized on silica granulation) were obtained from Novo Nordisk Co., Ltd., Shanghai, China. Porcine pancreatic lipase and *Carica papaya* lipase were obtained from Sigma–Aldrich Co., Shanghai, China.

2.2. The preparation of phytostanols

Phytostanols were prepared by catalytic hydrogenation of phytosterols under hydrogen pressure of 2 MPa, using 10%-Pd/C (w/w) as catalyst. The reaction conditions were *n*-propanol as solvent, 2% (w/w) of the catalyst, reaction temperature 65 °C and reaction time 5 h. The hydrogenation rate of phytosterols was 97.2% by determination of iodine value.

2.3. Lipase-catalyzed reaction

Phytostanols (0.1–1.5 mmol), fatty acid (0.4–1.0 mmol), lipase (50–200 mg) and solvent (5–10 mL) were added into a 25 mL screw-capped vial. The solvent had, in advance, been dehydrated with molecular sieves 4 Å for at least 24 h. The vial was placed in a water-bath at 35–65 °C and the reaction mixtures were shaken at 150 rpm (Scheme 1). Over the time course of the reactions, a portion of the reaction mixture (0.2 mL) was periodically removed from the reaction for TLC and HPLC.

2.4. Purification of phytostanyl esters by column chromatography

In a typical example, the reaction mixtures resulting from lipase-catalyzed esterification in situ of phytostanols with fatty acid were filtered under vacuum to remove molecular sieves and lipase, the filtrate was applied to a silica gel column (100–200 mesh, 12 mm × 600 mm) and then eluted with cyclohexane/ethyl acetate (4:1, v/v). The flow rate was 20 mL/h, and the eluent, 1 tube/15 min, was collected and then detected by TLC. The fractions containing the desired products were collected by rotary evaporator.

2.5. Qualitative analysis by TLC, FT-IR, MS, and NMR

Aliquots (10 μL) were withdrawn from the reaction mixtures by pipette for TLC analysis. Development was carried out in cyclohexane/ethyl acetate (4:1, v/v) and the spots were located by iodine staining for 30 min. Alternatively, the TLC plates were sprayed with sulfuric acid/ethanol (19:1, v/v) and heated in an oven kept at 110 °C for 20 min. R_f values of different substrates and products were: 0.03–0.06 (fatty acid), 0.1–0.15 (plant stanols), 0.8–0.85 (phytostanyl esters), respectively.

The isolated phytostanyl laurate was analyzed by FT-IR and the purified sitostanyl laurate was analyzed by MS, NMR. FT-IR measurement was performed on FT-IR spectrophotometer (Nicolet Nexus 470, USA) with Attenuated Total Reflectance (ATR), number of scans: 32, resolution: 4 cm^{-1}. A mass spectrum was obtained by Mass Spectrometry (Waters Maldi Synapt Q-TOF, USA) with positive ESI mode. ^1H and ^{13}C NMR spectra were measured in $CDCl_3$ as solvent using tetramethyl silane (TMS) as the internal standard with a Bruker NMR spectrometer (Avance III 400 MHz, Switzerland, operating at 400 and 100 MHz for ^1H and ^{13}C, respectively.

2.6. Quantitative analysis by HPLC

Aliquot fractions removed periodically from the reaction mixtures were diluted in 10 mL of *n*-hexane/methanol (1:9, v/v) for quantitative analysis. The area (%) of the produced phytostanyl esters was calculated as the esterification rate by HPLC with symmetry-C_{18} column (5 μm, 4.6 mm × 150 mm, Waters) and Evaporative Light Scattering Detector (ELSD) 2420 (Waters, USA). The column temperature was 35 °C and the ELSD was operated at 80 °C as drift tube temperature and used nitrogen as a nebulizing gas at pressure of 172.2 kPa. The mobile phase was *n*-hexane/methanol (1:9, v/v) and the flow rate was 1.0 mL min^{-1}. The calibration curve was prepared using the purified phytostanyl laurate.

2.7. Determination of solubility of plant stanols and phytostanyl laurate

To compare the solubility of plant stanols and phytostanyl esters in plant oil, 0.5 g of plant stanols or phytostanyl laurate was dissolved in 5 mL of sunflower oil. These mixtures were prepared at 30 °C for 5 h with the help of ultrasonicator and 0.1 mL upper solution of each were withdrawn by pipette and then diluted in 10 mL of *n*-hexane/isopropanol (1:1, v/v). 10 μL of each sample was analyzed by HPLC with *n*-hexane/isopropanol (1:1, v/v) as the mobile phase and detected with ELSD (Waters 2420, USA) at 35 °C with nitrogen gas pressure of 172.4 kPa. The amount of plant stanols or phytostanyl laurate was determined by comparison with peak areas of standard plant stanols and their esters of known concentration.

3. Results and discussion

3.1. Product identification

Plant stanols mainly include sitostanol and campestanol, and their lauric acid esters contain sitostanyl and campestanyl laurate. Sitostanyl laurate was synthesized according to the condition in Section 2.3 for the identification of the end product. The isolated phytostanyl laurate was analyzed by FT-IR and isolated sitostanyl laurate was analyzed by MS and NMR.

The absorption maximums of the IR spectrum of the produced phytostanyl laurate were observed at the following position: 1739 cm^{-1} (C=O), 1462 cm^{-1} and 1178 cm^{-1} (C–O). The absorbance of hydroxyl group was observed in plant stanols and lauric acid, but not in the synthesized product. The absorbance of carbonyl group in the product shifted to a higher wave numbers by 39 cm^{-1}, indicating the formation of ester bond.

The protonated molecular ion $[M+Na]^+$ of the product from sitostanol and lauric acid was at m/z 621, and the product was identified to be sitostanyl laurate.

^1H and ^{13}C NMR spectral data of sitostanyl laurate were as follows: ^1H NMR (400 MHz, $CDCl_3$): δ = 0.58 (3H, s), 0.73–0.84 (20H, m), 0.88–0.99 (4H, m), 1.01–1.14 (5H, m), 1.16–1.31 (26H, m), 1.35–1.44 (2H, m), 1.46–1.68 (8H, m), 1.69–1.78 (2H, m), 1.88–1.91 (1H, m), 2.18 (2H, t, J = 7.6 Hz), 4.59–4.65 (1H, m, 3-H). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.99 (29-CH_3), 12.07 (19-CH_3), 12.24 (18-CH_3), 14.14 (12′-CH_3), 18.74 (21-CH_3), 19.04 (26-CH_3), 19.83 (27-CH_3), 21.21 (CH_2), 22.70 (CH_2), 23.06 (CH_2), 24.23 (CH_2), 25.12 (CH_2), 26.06 (CH_2), 27.53 (CH_2), 28.28 (CH_2), 28.63 (CH_2), 29.10 (CH_2), 29.13 (25-CH), 29.27 (CH_2), 29.36 (CH_2), 29.47 (CH_2), 29.61 (2 × CH_2), 31.93 (CH_2), 32.01 (CH_2), 33.92 (CH_2), 34.08 (CH_2), 34.80 (CH_2), 35.48 (8-CH), 36.18 (20-CH), 36.77 (CH_2), 39.98 (CH_2), 42.59 (10-C, 13-C), 44.67 (5-CH), 45.83 (24-CH), 54.22 (9-CH), 56.16 (17-CH), 56.42 (14-CH), 73.46 (3-CH), 173.5 (C=O).

3.2. Determination of condition parameters

3.2.1. Effect of lipase and solvent

Different lipases were firstly selected based on the previous report about the biosynthesis of phytosteryl esters [11,14]. Furthermore, plant and animal lipases were also selected to explore the activity on the synthesis of phytostanyl esters due to their potential activity on some esterification reactions. Hence, lipases from *C. papaya* and Porcine pancreas were adopted and compared to classic microbial lipases.

In general, the hydrophobicity of organic solvent is a key factor for esterification reaction, for it could affect the activity and stability of lipases and the solubility of substrates. The hydrophobicity is expressed by the value of Log P which is defined as the logarithm of the partition coefficient of a given compound in the standard two-phase system of octanol/water. The higher the Log P is, the stronger the hydrophobicity of solvents. *n*-Hexane and acetone, which show different Log P values, are frequently used in organic synthesis fields. Taking the whole into consideration, we choose *n*-hexane, acetone, and the mixture of *n*-hexane/acetone (1:1, v/v) as solvent. The Log P value of different solvents is shown in Table 1 by the previous report [15]. Five lipases from different origins and three solvents were assayed for synthesizing phytostanyl laurate as shown in Table 1.

It was obvious that lipases had different activities for the same esterification reaction from Table 1. With the same solvent, lipases from plant and animal origins appeared to give lower conversion than microbial lipases. For example, the esterification degree achieved 33.3% in *n*-hexane employing Novozym 435 lipase B from *C. antarctica* as biocatalyst, while only 4.7% and 2.7% of esterification degree were obtained from plant and animal, respectively.

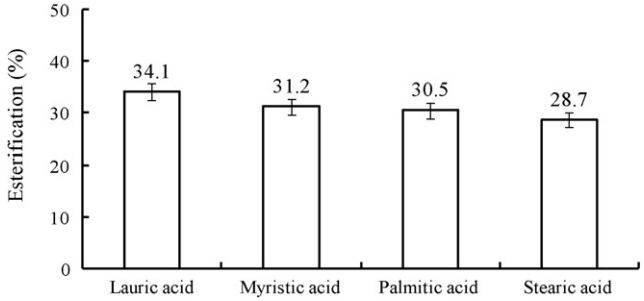


Fig. 1. Effect of carbon chain length of fatty acid on esterification of plant stanols in lipase-catalyzed reaction. Reaction conditions: 50 μmol/mL plant stanols, 50 μmol/mL fatty acid, 20 mg/mL lipase and 60 mg/mL 4 Å molecular sieves in 10 mL *n*-hexane at 200 rpm and 45 °C for 72 h.

Among the three immobilized lipases from microbial origins, both Novozym 435 and Lipozyme RM IM are effective in direct esterification. Lipozyme TL IM is mainly used for interesterification of bulk fats in the synthesis of frying fats [16]. As shown in Table 1, Novozym 435 showed the highest esterification. This trend was in accordance with what was previously displayed [13]. It may be ascribed to the fact that Novozym 435 has a preference for secondary alcohol. On the other hand, the esterification rate of plant stanols rises with the increasing of Log P value of solvent using the same lipase. As shown in Table 1, the reaction in *n*-hexane exhibits the highest conversion of 33.3% after 72 h among the three solvent systems. This may be attributed to the fact that the activity and stability of the lipase is usually higher in the more hydrophobic solvents. Among the three solvents, *n*-hexane has the highest Log P value, thus, there was the highest esterification rate in *n*-hexane. Based on the above research, Novozym 435 lipase was used as the biocatalyst and *n*-hexane as reaction media in the synthesis of phytostanyl laurate for the following experiments.

3.2.2. Influence of fatty acid

Four fatty acids with different carbon chain lengths (lauric acid, myristic acid, palmitic acid, stearic acid) were used at molar ratio of fatty acid/plant stanols of 1:1 in the presence of molecular sieves as dehydrator at 45 °C. Influence of fatty acid with different chain lengths as acyl donor on the degree of esterification is shown in Fig. 1. Using lauric acid and stearic acid, conversion of 34.1% and 28.7%, respectively was observed after 72 h of reaction time. At the same time, it could be seen that esterification rate decreases with the increasing of carbon chain length of fatty acid. This phenomenon may be related to steric effect. As the alkyl chain in the fatty acid increases in size, its steric effect increases as well, this can account for this trend. Liu and Lotero reported that steric constraints are governed not only by the size of molecules, but also by their preferential conformations [17]. Sasa and Maja holded that regiospecificity of lipase may be responsible for different conversions towards different chain fatty acids [18]. It had been found that the poor reaction rate might be due to fatty acid inhibitory effect [19]. Thereafter, lauric acid was selected as acyl donor for following research experiments.

3.2.3. Influence of substrate concentration

The effect of plant stanols concentration on esterification of plant stanols in lipase-catalyzed reaction was investigated (Fig. 2). The esterification rates increase at different plant stanol concentrations with the extension of reaction time. The conversion of plant stanols tends to rise sharply from 48 h to 72 h at 25 μmol/mL where the highest conversion of phytostanyl laurate was obtained after 72 h. Meantime, the esterification rate increased with increasing the plant stanols concentration till 25 μmol/mL, but decreased

Table 1
Effect of lipases and organic solvents on esterification of plant stanols in lipase-catalyzed reaction.

Solvent	Log *P*	Esterification (%)				
		Novozym 435	Lipozyme RM IM	Lipozyme TL IM	*Carica papaya* lipase	Porcine pancreas lipase
n-Hexane	3.50	33.3	19.4	15.2	4.7	2.7
n-Hexane/acetone (1:1, v/v)	1.64	20.6	17.9	13.7	3.9	2.0
Acetone	−0.23	19.5	16.5	9.5	3.6	1.8

Reaction conditions: 50 μmol/mL plant stanols, 1:1 of molar ratio of lauric acid to plant stanols, 20 mg/mL lipase and 60 mg/mL 4 Å molecular sieves in 5 mL solvent, 45 °C, 150 rpm.

with further increase of plant stanols concentration. Plant stanols dissolved in the solvent would be an effective substrate for the condensation, but the esterification rate is expressed by the molar ratio of phytostanyl laurate to all of the initial plant stanols. Most of the plant stanols were undissolved in the solvent, especially at higher concentration, which would be the main reason for the lower esterification at higher plant stanols concentration. In addition, Douglas and Charmian considered that the relative activity of the enzyme against different substrates may be dependent on the interaction between the substrate and the active site of the native enzyme. In other words, an increase in fatty acid concentration may change the catalytic environment [20]. So the best plant stanols concentration was selected as 25 μmol/mL.

3.2.4. Influence of substrate molar ratio

The influence of molar ratio of fatty acid to plant stanols on the conversion of plant stanols was evaluated in the esterification of plant stanols with lauric acid in *n*-hexane using Novozym 435 lipase in the presence of molecular sieves. Reaction results were analyzed by HPLC for quantification of phytostanyl laurate (Fig. 3).

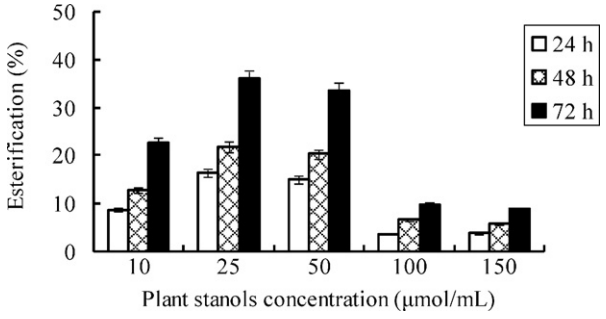


Fig. 2. Effect of plant stanols concentration on esterification of plant stanols in lipase-catalyzed reaction. Reaction conditions: 1:1 of the molar ratio of lauric acid to plant stanols, 20 mg/mL lipase and 60 mg/mL 4 Å molecular sieves in 5 mL *n*-hexane, 150 rpm and 45 °C.

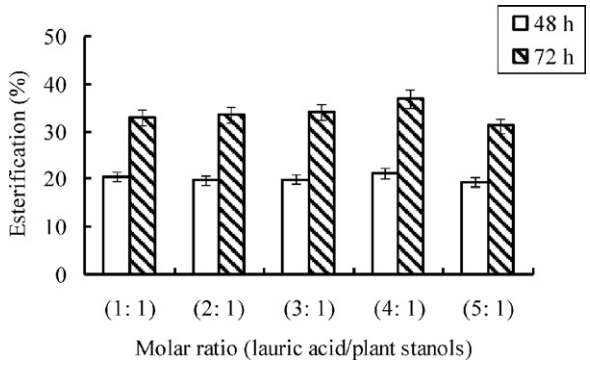


Fig. 3. Effect of molar ratio of lauric acid to plant stanols on esterification of plant stanols in lipase-catalyzed reaction. Reaction conditions: 25 μmol/mL plant stanols, 20 mg/mL lipase and 60 mg/mL 4 Å molecular sieves in 5 mL *n*-hexane, 150 rpm and 45 °C.

As expected, although equimolar ratio of both substrates can appear as ideal in terms of economical aspect of the process and further purification of the end products, it was observed that such ratio was not advantageous for phytostanyl laurate synthesis. Indeed, with equivalent molar amounts of plant stanols and lauric acid, no displacement of the equilibrium is possible and phytostanyl laurate formation only reached 20.5% and 33.4% after 48 h and 72 h, respectively. It was observed that increasing the molar ratio of lauric acid to plant stanols from 1:1 to 3:1 and finally to 4:1 led to a gradual increase in the extent of esterification. At a fixed plant stanols concentration of 25 μmol/mL, the highest conversion is achieved when the molar ratio was 4:1. The conversion decreases when the molar ratio surpasses 4:1, the reason of which may be that the amount of undissolved plant stanols rises with the increase of lauric acid amount.

3.2.5. Influence of molecular sieves type and its concentration

It is well-known that a minimal amount of water is necessary for the enzyme to ensure its optimal conformation and then to become optimally active. However, an excess of water decreases the enzyme's activity both from kinetic and thermodynamic points of view. Therefore, the removal of water from the reaction system can shift the reaction to improve the conversion. One effective method for the removal is the addition of a desiccant such as molecular sieves, which has the advantages that the cost is low and it is easy to be separated and regenerated [21].

The effect of the amount of molecular sieves 3 Å and 4 Å on esterification of plant stanols in lipase-catalyzed reaction was examined (Fig. 4). With the addition of molecular sieves 3 Å and 4 Å, the esterification rate of plant stanols raises gradually. The highest conversion of phytostanyl laurate was obtained at 80 mg/mL for both 3 Å and 4 Å molecular sieves, while the conversion of phytostanyl laurate using 3 Å molecular sieves was slightly higher than that with 4 Å molecular sieves at 80 mg/mL in *n*-hexane. At higher concentrations of molecular sieves, the esterification degree lowered.

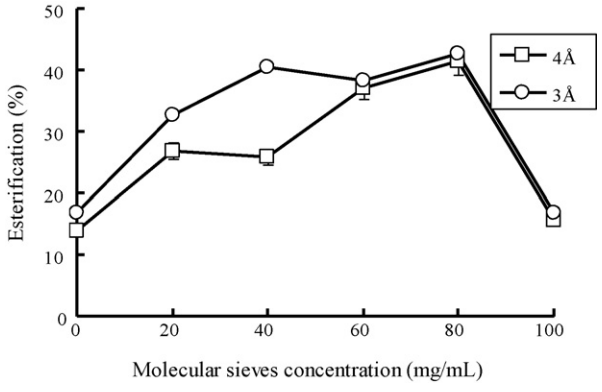


Fig. 4. Effect of molecular sieve type [(□) 4 Å; (○) 3 Å] and concentration on esterification of plant stanols in lipase-catalyzed reaction. Reaction conditions: 25 μmol/mL plant stanols, 4:1 of the molar ratio of lauric acid to plant stanols, 20 mg/mL lipase in 5 mL *n*-hexane, 150 rpm, 45 °C, 72 h.

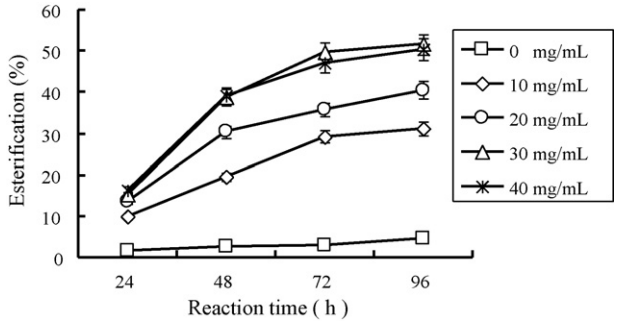


Fig. 5. Effect of enzyme load on esterification of plant stanols in lipase-catalyzed reaction. Reaction conditions: 25 μmol/mL plant stanols, 4:1 of the molar ratio of lauric acid to plant stanols, 60 mg/mL 4 Å molecular sieves in 5 mL *n*-hexane, 150 rpm and 45 °C.

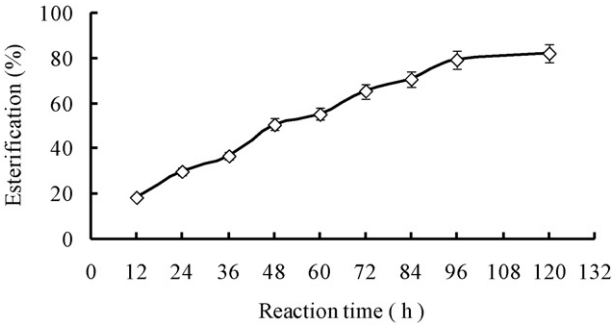


Fig. 7. Reaction time on esterification of plant stanols in lipase-catalyzed reaction. Reaction conditions: 25 μmol/mL plant stanols, 4:1 of the molar ratio of lauric acid to plant stanols, 30 mg/mL lipase and 80 mg/mL 3 Å molecular sieves in 10 mL *n*-hexane, 150 rpm, 55 °C.

This may be that the loss of excess water absorbed by molecular sieves led to the decrease in lipase activity.

3.2.6. Influence of enzyme load

The influence of the enzyme load was evaluated with a 1:4 plant stanols/fatty acid molar ratio using varying amount of Novozym 435 lipase from 0 to 40 mg/mL (Fig. 5). It is firstly observed that almost no formation of the desired phytostanyl laurate occurred in the absence of lipase. Moreover, not surprisingly, it was also showed that the higher the enzyme load, the better was the phytostanyl laurate formation. However, this experiment was useful to determine a good combination between esterification rate and economical interest of the reaction, this latter point being directly influenced by the enzyme amount used for the reaction. Herein, although the use of minimal amount of Novozym 435 lipase such as 10 mg/mL would be economically attractive, it did not result in any satisfactory production of phytostanyl laurate. After 72 h, esterification rate only reached 29.3%. Increasing of enzyme load appeared to be more satisfactory. With 20 mg/mL enzyme amount the reaction kinetic was faster but still only reached 35.8% of phytostanyl laurate formation after 72 h. Finally, the formation of the phytostanyl laurate was much faster with 30 mg/mL enzyme load and resulted in a 49.7% conversion after 72 h of reaction. It is also found that there was no apparent difference in esterification rate between enzyme load of 30 and 40 mg/mL.

3.2.7. Influence of reaction temperature

The solubility of the substrates, the stability and activity of the lipase are strongly associated to the temperature of the reactions [22]. Effect of temperature on esterification of plant stanols in lipase-catalyzed reaction was investigated at different temperatures ranging from 35 °C and 65 °C and displayed in Fig. 6. With the rise of the temperature of the reaction, the solubility of substrates and the activity of lipase increase. Thus, at 35 °C lower conversion was obtained compared to the reaction performed at 45 °C and 55 °C. When temperature increased from 55 °C to 65 °C, an apparent decrease in esterification rate was observed. This might be related to the loss of lipase activity at this temperature. When the reactions were conducted at 55 °C, we observed the highest conversion of 62.9%.

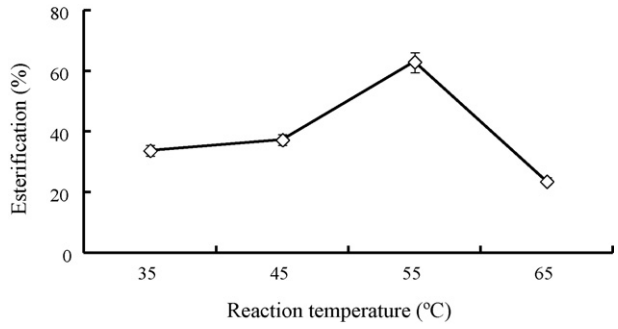


Fig. 6. Effect of reaction temperature on esterification of plant stanols in lipase-catalyzed reaction. Reaction conditions: 25 μmol/mL plant stanols, 4:1 of the molar ratio of lauric acid to plant stanols, 20 mg/mL lipase and 60 mg/mL 4 Å molecular sieves in 5 mL *n*-hexane, 150 rpm, 72 h.

3.2.8. Time course on synthesis of phytostanyl laurate

The time course of product formation for the esterification of plant stanols with fatty acid catalyzed by the Novozym 435 is shown in Fig. 7. In this experiment, all parameters used have been selected by the previous works. From Fig. 7, the reaction tended to gradual increase at first, with a conversion into phytostanyl laurate of 79.3% at 96 h, which has a difference to Li Rui's study [23]. Li Rui reported that the conversion of conjugated linoleyl β-sitosterol was rapidly increased with the first 24 h, and then tended to gradual increase. After 96 h, the product conversion was increased little, which means that prolonging reaction time beyond 96 h will not lead to a significant increase in phytostanyl laurate formation, indicating the reaction was nearly reached equilibrium at 96 h.

3.3. The comparison of solubility

The solubility of phytostanyl laurate reached 9.9% (w/w) in sunflower oil at 30 °C, while phytostanols only 0.8% under the same conditions. Direct esterification of plant stanols with fatty acid in the presence of Novozym 435 can significantly improve its solubility in plant oil and thus facilitate the incorporation into a variety of fatty foods.

4. Conclusions

In conclusion, phytostanyl esters are high value added products due to their strong cholesterol-lowering properties and their easier incorporation in food ingredients formulation than free stanols. Phytostanyl esters have recently been used as functional food ingredients. Enzyme technologies appeared to be very appropriate to obtain the desired phytostanyl esters in good yields. Our studies displayed one such method for the synthesis of phytostanyl esters using lipase-catalyzed reaction in organic solvent in the presence of molecular sieves as dehydrator, and the highest yields of phytostanyl esters were achieved under the selected reaction conditions. Also, the structure of the target product was characterized by IR, MS and NMR.

Acknowledgements

This study was financially supported by the Fundamental Research Funds for the Central Universities (JUSRP30903), Free Exploring Program of State Key Laboratory of Food Science and Technology (Jiangnan University, SKLF-TS200905), National High-Tech Research and Development Program of China (2006AA10Z312) and Natural Science Foundation of Jiangsu Province (BK2008100).

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