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ORIGINAL PAPER

Synthesis of Phytosteryl Esters by Using Alumina-Supported Zinc Oxide (ZnO/Al_2O_3) from Esterification Production of Phytosterol with Fatty Acid

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Abstract The feasibility of zinc oxide-catalyzed esterification of natural phytosterols with oleic acid was investigated well by a chemical process. The influences of various reaction parameters were evaluated. Basic solid zinc oxide is the most desirable catalyst due to its high selectivity (more than 90%), reusability, activity and less corrosivity, whereas sterol selectivity with other catalysts, such as H_2SO_4, $NaHSO_4$ and NaOMe, did not exceed 80%. Further results showed that during zinc oxide-catalyzed synthesis, the nature of the acyl donor was of paramount importance with direct esterification with fatty acids, which gives better results with higher conversion rate selectivity and more mild reaction conditions than transesterification with methyl esters. The substrate molar ratio of 2:1 (oleic acid/phytosterol) was optimal. Other parameters such as optimal catalyst load (0.5%) and temperature (170 °C) showed a maximum production of steryl esters close to 98% after 8 h. It was also found that the amount of trans fatty acid formed in esterification was low, and the trans fatty acid content (%) in the phytosterol oleate ester fraction (3.26%) was much lower than that in free oleic oil (7.35%), which suggested that fatty acids in esters were more stable than free fatty acids regarding the combination with sterol. Immobilized ZnO could be a promising catalyst for replacing homogeneous and corrosive catalysts for esterification reactions of sterol.

Keywords Phytosteryl esters · ZnO · Fatty acids · Esterification · Oleic acid

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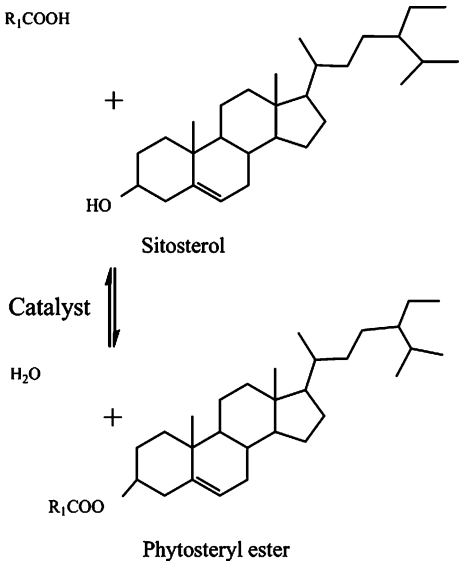
Introduction

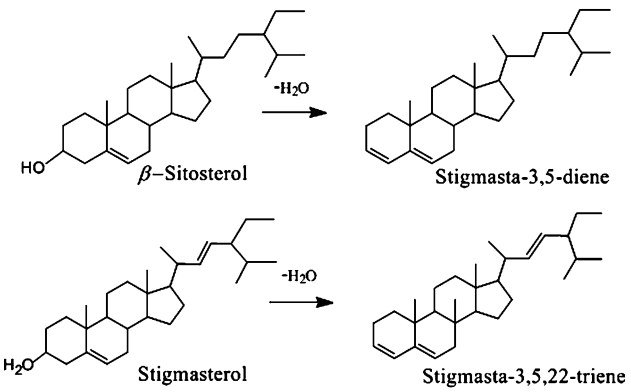
Plant sterols, stanols and their esters have been found to be effective in lowering the plasma cholesterol concentration by inhibiting the absorption of cholesterol in the small intestine [1, 2]. Clinical data indicated that cholesterol levels were reduced significantly in a population consuming a high fat diet fortified with phytosterols. However, phytosterols must be taken with excessively high doses due to their low solubility and physical reactivity. A very important goal, then, is to create a more soluble phytosterol product, which can be taken at lower dosages while maintaining a high bio-efficiency. Some methods to achieve this goal are as follows: (1) using esterified phytosterols (phytosteryl esters of fatty acids, reaction Scheme 1), which are highly soluble in the oil phase and can be incorporated in fat-based products [3, 4]; (2) microcrystalline dispersion of sterol/emulsifier complex with low melting temperature formed by co-crystallization with emulsifiers [5]; (3) suspending the insoluble free or esterified phytosterols in a liquid (oil or aqueous) medium by creating microcrystalline particles with a complex emulsifier to avoid the coarse texture of phytosterols and increasing the solubility in the medium phase [6]; and (4) entrapment of the phytosterols in micro emulsions [7].

Phytosterol esters are most commonly produced in industry, while methods of phytosteryl ester production are



Scheme 1 Synthesis of phytosteryl esters

various, including chemical and enzymatic catalysis. Enzymatic reactions have many advantages, such as high specificity, mild reaction conditions and being environmentally friendly. However, the broad application of enzymatic methods is still limited by high costs of lipases, lower productivity and longer reaction time (as long as 24 h) [8, 9]. Chemical homogeneous catalyzed reactions require acid catalysts (H_2SO_4, H_3PO_4, p-TSA), which may favor the dehydration of sterols to stigmastadienes(Scheme 2) or alkaline catalysts (NaOEt, NaOH, KOH, NaOMe), which are well known to favor the formation of soaps. Moreover, homogeneous catalysts are corrosive, difficult to separate from the products and lead to excessive waste [10]. Heterogeneous catalysts, e.g., tungstosilicic acid, tungstophosphoric acid, etc., were used to synthesize phytosteryl ester in our previous study [11]. Although they are characteristic of high activity, stability and easy recovery, their lower selectivity and safety from metal residues also need to be considered. Greener chemical methods based on solid reusable catalysts (lanthanum oxides, zinc oxide) are



Scheme 2 Dehydration of phytosterols to dienes

described by Pouilloux et al. and Valange et al. [10, 12]. Lanthanum oxides and zinc oxide are active and selective catalysts for the synthesis of phytosteryl esters by transesterification. However, the reaction temperature was as high as 240 °C, and saturated methyl dodecanoate was used in their experiments. When transesterification reactions are performed at varied temperatures of 230–250 °C, diene contents in products range from 2.0 to 6.3%, and other byproduct levels (mainly oxide sterols) are from 3 to 8.5%. Limited information is available on the biological effects of dehydrated sterols and oxidized phytosterols as well as their levels in foods and in human plasma [13, 14]. The relationship between the long-term consumption of lipid oxidation products and human health is not clear, but it is generally recognized that over-used and abused oils undoubtedly contain oxidized material that, if chronically consumed in large amounts, could increase the human health risk [15]. Hence, there is no doubt that methods of phytosteryl ester synthesis with high specificity and high process control are of great advantage. In addition, functional unsaturated fatty acids were desired to synthesize nutraceutical sterol esters embodying synergistic effects of unsaturated fatty acids and phytosterols. For unsaturated fatty acids, cis/trans isomerization should be specially considered.

In this study, we aim to compare zinc oxide-catalyzed phytosterol esterfication from free fatty acids in the absence of solvent with conventional transesterification. In addition, the effect of zinc oxide immobilization on esterification was explored to facilitate the separation and reuse of catalysts.

Materials and Methods

Materials

Phytosterols (95% purity) were purchased from Spring Fruit Biological Products Co., Ltd. (Taizhou, Jiangsu, China), and the main composition was as follows: β-sitosterol 45%, stigmasterol 24%, campesterol 21% and brassicasterol 5%. Stigmasterol (≥95%) was obtained from Zhejiang Medicine Co., Ltd., Xinchang Pharmaceutical Factory (Zhejiang, China). Fatty acids, sitosterol and stigmasta-3,5-diene standards were obtained from Sigma (St. Lous, Mo). Zinc oxide and zinc nitrate (≥99%) were from Hangzhou Xiaoshan Chemical Factory, China. Alumina oxide (≥99%, surface area of 146 m^2 and a pore volume of 1.1 cm^3/g) was obtained from Chongqing Dingtai Tuoyuan Alumina Development Co., Ltd. (Chongqing, China). Oleic acid, methyl oleate and NaOMe (≥50%) were of chemical grade (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China). All other reagents and solvents used were of analytical grade.

Methods

Esterification/Transesterification Method

A phytosteryl ester mixture was prepared in a 125-ml three-neck glass vessel. Prior to the reaction, the phytosterols were dried in an oven overnight at 60 °C. The fatty acids/methyl esters were dried with 3A molecular sieves. The esterification/transesterification was carried out as follows: a mixture of phytosterol and fatty acid/methyl ester in a certain molar ratio was added to a three-neck vessel filled with nitrogen, heated to the desired temperature and mixed homogeneously. Then the catalyst was added. The reaction continued for 2–12 h. Aliquots were sampled at the desired time, and the reaction proceeding was monitored by TLCs.

Thin Layer Chromatography Analysis (TLC)

The TLCs of 0.3-mm silica gel layers were developed with hexane/diethyl ether/acetic acid (90/10/1 v/v/v) and the eluent spots by iodine staining.

Alternatively, the TLC plates were sprayed with a freshly prepared solution consisting of ferric chloride (50 mg), water (90 ml), acetic acid (5 ml) and sulfuric acid (5 ml), then heated and kept at 100 °C in an oven. The sterol, dehydrated sterol and steryl ester appeared as red-violet spots. The spots of dehydrated sterol, sterol ester, free sterol, free oleic acid or methyl oleate were scraped for GC analysis.

Fatty Acid Composition Analysis by Gas Chromatography

The free fatty acids and sterol ester were converted to methyl esters, respectively, as the AOCS standard procedure [16]. The oleic acid cis/trans isomerization [17] was analyzed with Agilent technologies (Wilmington, DE) series 6890 N equipment including a 7683 automated sample-injection system, and a CP-Sil 88 fused silica column (100 m × 0.25 mm, i.d. of 0.2 μm) was used. The oven temperature was programmed from 60 °C (held for 5 min) to 165 °C (held for 1 min) at 15 °C/min, then at 2 °C/min to 225 °C (for 17 min). The carrier gas was nitrogen at a flow rate of 3.0 ml/min. The split ratio, injector temperature and detector (FID) temperature were set as 1:50 (v/v), 250 and 300 °C, respectively.

Sterol and Dehydrated Sterol Analysis

Sterols were analyzed by gas chromatography (GC) method as described by Jekel et al. [18]. The chromatograph was performed with Agilent 6890 GC, a 30 m × 32 mm I.D., 0.25-μm film thickness HP-5 fuse-silica capillary column coated with 5% phenymethyl siloxane (J&W Scientific, Folsom, CA) and flame ionization detection (FID) controlled by the Agilent Chemstation software. Nitrogen was used as carrier gas with flow rate of 1.0 ml/min and split ratio of 1:20 (v/v). The oven temperature was held at 240 °C for 1 min, then raised to 280 °C at 7 °C min^{-1} and held for 25 min: injection port, 310 °C; detector, 320 °C; injection volume, 1.0 μl. The dehydrated sterols were identified by GC/MS performed on a Varian Saturn 2000 (ion trap detector), equipped with a HP-5 capillary column as described by Gutierrez et al. [19]. Sterols and dehydrated sterols were quantified using sitosterol and stigmasta-3,5-diene curves, respectively. The conversion of sterol was estimated with respect to the initial and the final sterol in the solution.

Immobilized Catalyst Preparation

Alumina (Al_2O_3) balls were chosen as support. A certain amount of zinc nitrate was dissolved in 100 ml distilled water to obtain a saturated solution. Then, a predetermined amount of alumina was added to the solution. The mixture was thoroughly stirred for 12 h. The support, impregnated with zinc nitrate, was filtrated, air-dried for 1 h and thereafter calcined at 600 °C for 8 h. To obtain maximum load, impregnation and calcination operations were repeated three times. The amount of the supported catalyst could be simply calculated by subtracting the weight of the original support. The X-ray energy spectrums were collected by energy dispersive spectrometer (Thermo NORAN-Vantage X-ray EDS) for analysis of element composition on the surface of the immobilized catalyst analysis. The average element composition in the immobilized catalyst was measured by inductively coupled plasma-atomic emission spectroscopy (IRIS Intrepid, Thermo Elemental, Franklin, MA) as described by Chen et al. [20].

Reuse of Immobilized Catalyst

The esterification reactions were performed with an oleic acid to sterol ratio of 2:1 and ZnO/Al_2O_3 load of 10 g kg^{-1} (calculated as ZnO) at 170 °C for 10 h for each run. Samples were withdrawn at fixed intervals, and the sterol ester levels were determined. Then, the esterification time courses were plotted. Initial velocity was calculated by the slope in linear range of time course and expressed as sterol ester increase min^{-1} g^{-1} immoblized ZnO. When the reaction was finished, the immobilized catalyst was separated by heat filtration, eluted by absolute ethanol, hot air dried and reused in a next batch for esterification. Seven cycles of the esterification reaction were conducted. The conversion and initial velocity were measured after each

run. Relative activity was calculated as the original catalytic activity divided by initial velocity in each run times 100%. The immobilized catalyst reuse trial of seven run reactions was carried out in triplicate.

Results and Discussion

Transesterification and Esterification of Phytosterol with Methyl Oleate or Oleic Acid Catalyzed by Different Catalysts

Due to the high reaction temperature and the free residual acidity/alkalescence, there was a competition between the esterification/transesterification reaction and the dehydration of the sterol, since the selectivity of catalyst to the phytosteryl ester and diene is varied [10]. The reaction results of phytosterol transesterification with methyl oleate and esterification with oleic acid, catalyzed by ZnO as well as other catalysts, are summarized in Table 1.

NaOMe is a commonly used transesterification catalyst for sterol ester production. In our experiments, with NaOMe as the catalyst, the total conversion could achieve 98.6% in 4 h, but the specificity for phytosteryl ester was only 70.3%. This is mainly due to the dehydration effects; stigmasta-3,5,22-triens is the predominant dehydrated sterol. The oxidation phenomenon of sterol was not serious in NaOMe-catalyzed transesterification reactions. For esterification reactions catalyzed by strong acids like H_2SO_4, oxidation and dehydration were the main adverse reactions. The color of reaction mixtures turned to dark brown immediately as soon as H_2SO_4 was added to the solution. GC analysis results (Fig. 1) showed that the dehydrated sterol level in total sterols was as high as 18.8%. Among dehydrated sterols, the stigma-3,5-diene content (48%) was the highest, followed by stigma-3, 5, 33-triene (22%); a minor amount of camesta-3,5-diene (19%) was also found. The results were in accordance with the original phytosterol composition. However, with $NaHSO_4$ as the catalyst, the side reaction of dehydration of sterol was lowered, and the phytosteryl ester specificity increased from 68.5 to 79.1%.

Compared with the traditional acid/base catalysts, ZnO exhibited higher selectivity (with the selectivity for sterol of 91–99.2%) and acceptable activity (total sterol conversion of 75–87.3%) both for transesterification and esterification reactions in the phytosterol ester synthesis. The higher specificity can be attributed to the moderate acidic and basic strength of ZnO. These results agreed with the findings in the experiment implemented by Pouilloux et al. [10] and were compared with traditional basic catalyst Na_2CO_3 and NaOH. When the reaction temperature was kept at 170 °C and the ZnO load increased from 1.5 to 30 g kg^{-1}, the dehydrated sterol content could increase from 0.4 to 1.8%. This may be due to the increase of residue basicity from zinc oxide in the reactant, which favors a dehydration reaction of sterol. Moreover, we found that ZnO-catalyzed esterification could achieve higher reactant conversion and product selectivity under milder reaction conditions than transesterification. When esterification was performed with an oleic acid to sterol ratio of 1.5:1 and ZnO load of 5 g kg^{-1} at 170 °C for 8 h reaction, 84.2%

Table 1 Comparison of transesterification and esterification for phytosteryl ester synthesis catalyzed by ZnO and other catalysts

[a]Method	Catalyst load	Conversion ratio (%)	Selectivity (%)		
		Sterol	Sterol ester	[b]Dehydrated sterol	Others
Transesterification	[c]ZnO, 30 g kg^{-1}	76.8 ± 1.9	91.6 ± 3.2	4.6 ± 0.2	3.8 ± 0.2
	ZnO, 30 g kg^{-1}	10.2 ± 0.6	96.8 ± 3.6	1.8 ± 0.2	1.4 ± 0.3
	[d]NaOMe, 10 g kg^{-1}	98.6 ± 2.6	70.3 ± 3.8	26.7 ± 0.8	3.0 ± 0.6
Esterification	None	28.3 ± 1.1	98.4 ± 3.2	0.6 ± 0.1	1.0 ± 0.3
	ZnO, 1.5 g kg^{-1}	75.8 ± 1.4	99.2 ± 2.8	0.4 ± 0.3	0.4 ± 0.2
	ZnO, 5 g kg^{-1}	84.2 ± 1.3	98.4 ± 2.6	0.6 ± 0.3	1.0 ± 0.2
	ZnO, 10 g kg^{-1}	87.3 ± 1.5	96.7 ± 3.0	1.6 ± 0.4	1.7 ± 0.2
	[e]H_2SO_4, 5 g kg^{-1}	98.2 ± 1.5	68.5 ± 3.5	18.8 ± 0.4	12.7 ± 1.4
	[f]$NaHSO_4$, 5 g kg^{-1}	95.4 ± 3.2	79.1 ± 3.6	12.6 ± 0.3	8.3 ± 0.7

[a] The fatty acyl group donors of the transesterification and esterification reaction were oleic acid and methyl oleate. Unless otherwise stated, reactions were performed with a fatty acyl group donor to sterol ratio of 1.5:1 at 170 °C for 8 h

[b] Dehydrated sterols were calculated as stigmasta-3,5-diene

[c] Temperature, 240 °C

[d] Time, 4 h

[e] Time, 2 h

[f] Time, 2 h

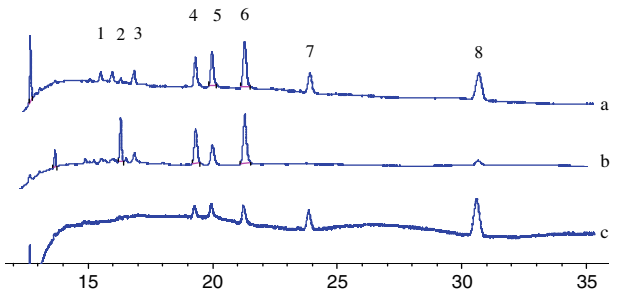


Fig. 1 Gas chromatography of products from esterification reactions catalyzed by **a** H_2SO_4; **b** $NaOCH_3$; **c** $NaHSO_3$. *1* Campesta-3,5-diene, *2* stigmastan-3,5,22-trien, *3* stigmastan-3,5-diene, *4* camsterol, *5* stigsterol, *6* sitosterol, *7* oxided sterol, *8* oxided sterol

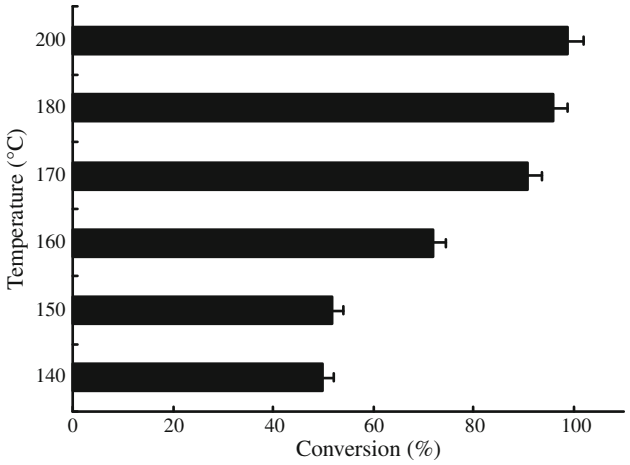


Fig. 2 Effects of temperature on phytosteryl oleate yields. Oleic acid to sterol molar ratio of 3:1, ZnO load of 5 g kg^{-1} and reaction time of 6 h. The results are the average of at least three independent esterification experiments; the *error bar* represents the standard deviation from the mean

conversion and 98.4% sterol ester selectivity were obtained, whereas at the same oleic acyl donor to sterol ratio and reaction time, ZnO load of 30 g kg^{-1}, and reaction temperature of 240 and 170 °C, conversions from transesterification were only 76.8 and 10.2%, respectively. In addition, the dehydrated sterol levels (4.6 and 1.8%) from transesterification were also apparently higher than those (0.4%) by esterification. This may be due to the fact that higher active energy was needed by transesterification, and the corresponding higher reaction temperature could easily lead to the formation of dehydrated sterols. León-Camacho et al. studied the law of stigmasta-3,5-diene formation in olive oil during deodorization and found that among all parameters (oil load, N_2 flow and temperature), the temperature was the most effective factor for stigmasta-3,5-diene content and the formation of stigmasta-3,5-diene from β-sitosterol [21]. The apparent kinetic constants (Kap) during the deodorization process in the formation of stigmasta-3,5-diene increased nearly fivefold (from 2.2×10^{-9} mol l^{-1} S^{-1} to 9.9×10^{-9} mol l^{-1} S^{-1}) when temperature varied from 240 to 260 °C. For unsaturated fatty acyl group donors, cis/trans isomerization needs to be considered. However, it was seldom discussed in the literature [9]. Therefore, it was investigated in this paper and is discussed in the following.

Temperature Effects

The effect of the reaction temperature on the production of the phytosteryl oleate ester catalyzed by ZnO is shown in Fig. 2.

Over the range of 140–200 °C, the catalyst activity increased with the reaction temperature. The highest conversion was approximately 100%, observed at 200 °C. However, loss of sterol (such as oxidation, dehydration or degradation) and deterioration of free phytosterols were more severe at high temperature [21, 22]. In addition, under such high temperatures, poly-unsaturated fatty acids are prone to oxidation and isomerization [23].

Trans-fatty acid was a harmful derivative of unsaturated fatty acid. The formation of trans fatty acid at different temperatures was investigated, and the individual fatty acid in products was identified by standards (figures not shown). The trans fatty acid level increased with the reaction temperature. Under the conditions of 140 and 150 °C, the level of trans fatty acid was lower, accounting for 1.2 and 1.4% of total fatty acids, respectively. While the temperature was set at 200 °C, the concentration of trans fatty acid rapidly increased to 4.75%. Hence, to achieve high yields and low by-product accumulation, a temperature of 170 °C was fitting.

In addition, the trans fatty acid level in total fatty acids in the phytosterol oleate ester fraction (3.26%) was much lower than that in free oleic oil (7.35%). This may be due to the longer time exposure of free fatty acid to a high temperature environment. This possibly suggested that fatty acid in esters is more stable than free fatty acid in respect to the combination with sterol or that the reaction pathway of the cis and trans fatty acid is not similar regarding the sterol; the cis fatty acid reacts more readily than does the trans fatty acid, and the resulting cis-phytosterol leads more slowly to the trans-phytosterol.

Effects of Substrate Molar Ratio

Figure 3 shows the effect of the molar ratio of oleic acid to phytosterols on the yield of phytosteryl oleate. The results showed that the conversion ratio of phytosterols was significantly higher with excessive oleic acid than that when the

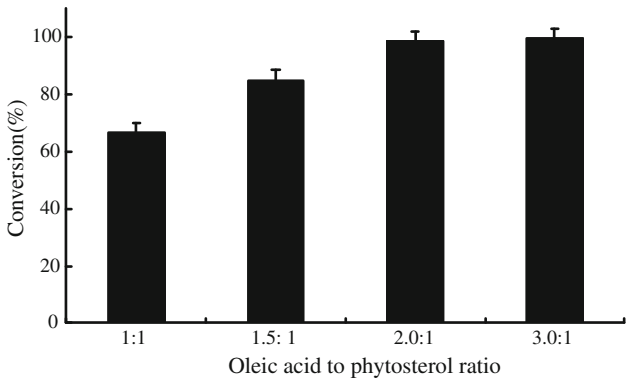


Fig. 3 Effect of the oleic oil to phytosterol molar ratio on the conversion of phytosterol. ZnO load of 5 g kg^{-1}, reaction temperature at 170 °C and reaction time of 8 h. The results are the average of at least three independent esterification experiments; *error bar* represents the standard deviation from the mean

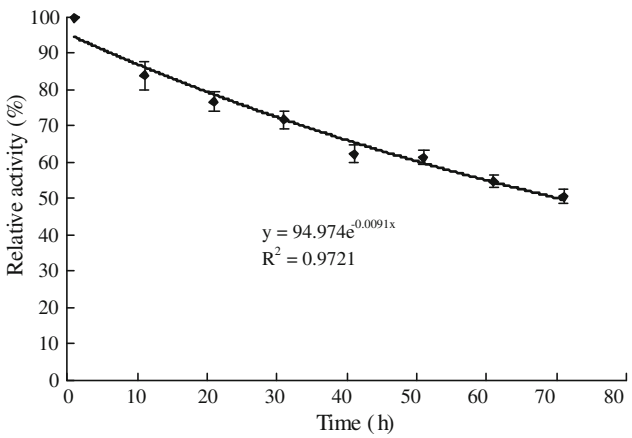


Fig. 4 Stability and kinetics of ZnO/Al_2O_3 catalyst for repeated use. The esterification reactions were performed with an oleic acid to sterol ratio of 2:1 and ZnO/Al_2O_3 load of 10 g kg^{-1} (calculated as ZnO) at 170 °C for 10 h each run. The relative activity present is the mean measurement from at least three independent trials on immobilized catalyst reuse of seven runs; *error bar* represents standard deviation from the mean

substrates were supplied at stoichiometric levels. This phenomenon agrees with the law of mass action. In addition, when oleic acid was excessively added, it could function as a solvent, which was beneficial for solubilizing the phytosterols, reducing the viscosity of the reaction solution and in turn improving the mass transfer in the esterification reaction. However, the excessive use of oleic acid could not improve the efficiency of the esterification reaction. An excess of non-reacted oleic acid would lead to a huge cost increase in the downstream isolation process. Therefore, a molar ratio (oleic acid vs. phytosterol) of 2:1 was the optimum condition, with the temperature of 170 °C and ZnO load of 5 g kg^{-1}.

Immobilization and Operational Stability of the Catalyst

In order to conveniently separate and reuse the catalyst, attempts were made to immobilize ZnO with aluminum oxide. It was determined that the raw ZnO load was about 54% of Al_2O_3, the surface element Zn to Al mass ratio of the supported ZnO was 1.52:1, and the average Zn to Al mass ratio was 0.92:1. The results indicated that the amount of ZnO absorbed on the surface is significantly higher than that entrapped into the micro cavities of alumina balls.

The stability of the immobilized catalyst during high-temperature esterification was studied, and the activity versus time curve is plotted in the Fig. 4. It can be noticed that the decrease in catalytic activity during the first run reaction was obviously higher than that during other runs (approximately twofold). This is due to the unbalanced distribution of ZnO in alumina balls. The ZnO in the outer layer desorbed more easily than that in the inner layer and micro cavities of alumina balls. This has been verified by the fact that the surface Zn to Al ratio decreased from 1.52:1 to 1.18:1, and the average Zn to Al ratio decreased from 0.92 to 0.78 after the fist run reaction. The catalytic activities and average ratios of Zn to Al kept steady from the second run reactions on. In general, the results suggested that after the first run, the immobilized ZnO could be reused several times without any dramatic loss of activity. Even after six consecutive applications, 60% of the original catalytic activity remains. The half-life of the catalyst was determined as 70 h by fitting the data with an exponential model. Of course, further optimization would be required for a technical application of the process.

Conclusion

The synthesis of phytosteryl ester catalyzed by ZnO could be obtained from esterification of fatty acids with plant sterols. Comparison with traditional acid/base catalyst was performed with respect to the selectivity and efficiency of producing phytosteryl ester in a solvent-free system. With high process control, for example, with a temperature of 160–170 °C, the fatty acid/phytosterol ratio of 2 ~ 2.5:1 and ZnO load of 0.5%, above 90% phytosteryl ester yield and more than 85% phytosterol selectivity were obtained after 8 h reaction, whereas the trans fatty acid level in products was no more than 4%. This study also demonstrated that the immobilized ZnO could be a promising catalyst for replacing homogeneous and corrosive catalysts such as sodium methylate in the process of commercially

synthesizing phytosteryl esters from fatty acids and phytosterols.

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References

1. Institute of Food Science and Technology Trust Fund Information Statement. Phytosterol Esters (Plant Sterol and Stanol Esters). http://www.ifst.org (Jan 2010)
2. Trautwein EA, Guus SM, Duchateau JE, Lin YG (2003) Proposed mechanisms of cholesterol-lowering action of plant sterols. Eur J Lipid Sci Technol 105:171–185
3. Kim BH, Akoh CC (2007) Modeling and optimization of lipase-catalyzed synthesis of phytosteryl esters of oleic acid by response surface methodology. Food Chem 102:336–342
4. Negishi S, Hidaka I, Takahashi I (2003) Transesterification of phytosterol and edible oil by lipase powder at high temperature. J Am Oil Chem Soc 80:905–907
5. Akashe A, Miller M (2001) Use of mesophase-stabilized compositions for delivery of cholesterol-reducing sterols and stanols in food products. US Patent 6,274,574
6. Burruano B, Bruce RD, Hoy MR (2000) Method for producing water dispersible sterol formulations. US Patent 6,110,502
7. Weber N, Weitkamp P, Kumar DMJ (2002) Cholesterol-lowering food additives: lipase-catalysed preparation of phytosterol and phytostanol esters. Food Res Int 35:177–181
8. Vu PL, Shin JA, Lim CH, Lee KT (2004) Lipase-catalyzed production of phytosteryl esters and their crystallization behavior in corn oil. Food Res Int 37:175–180
9. Villeneuve P, Turon F, Caro Y, Escoffier R, Barea B, Barouh B, Lago R, Piombo G, Pina M (2005) Lipase-catalyzed synthesis of canola phytosterols oleate esters as cholesterol lowering agents. Enzyme Microb Technol 37:150–155
10. Pouilloux Y, Courtois G, Boisseau M, Piccirilli A, Barrault J (2003) Solid base catalysts for the synthesis of phytosterol esters. Green Chem 5:89–91
11. Meng XH, Sun PL, Pan QY, Shi ZP, Yang K, He RJ (2006) Synthesis of plant sterol esters catalyzed by heteropolyacid in a solvent-free system. Eur J Lipid Sci Technol 108:13–18
12. Valange S, Beauchaud A, Barrault J, Gabelica Z, Daturi M, Can F (2007) Lanthanum oxides for the selective synthesis of phytosterol esters: correlation between catalytic and acid–base properties. J Catal 0:1–10
13. Dutta PC, Przybylski R, Eskin NA, and Appelqvist LÅ (2007) Formation and analysis and health effects of oxidized sterols in frying fats. In: Erickson MD (ed) deep frying: practices, chemistry and nutrition. AOCS Press, IL, pp 125–178
14. Hovenkamp E, Demonty I, Plat J, Lutjohann D, Mensink RP, Trautwein E (2008) Biological effects of oxidized phytosterols: a review of the current knowledge. Prog Lipid Res 47:37–49
15. Dobarganes C, Marquez-Ruiz G (2003) Oxidized fats in foods. Curr Opin Clin Nutr Metab Care 6:157–163
16. David F (1997) Official methods and recommended practices of the AOCS, 5th edn. American Oil Chemists' Society Champaign, Illinois, USA, Ce2-66
17. Ledoux M, Laloux L, Wolff RL (2000) analytical methods for determination of trans-C18 fatty acid isomers in milk fat. Analusis 28:402–412
18. Jekel AA, Vaessen HAMG, Schothorst RC (1998) Capillary gas-chromatographic method for determining non-derivatized sterols—some results for duplicate 24 h diet samples collected in 1994. Fresenius J Anal Chem 360:595–600
19. Gutierrez A, Romero J, del Rio JC (2001) Lipophilic extractives in process waters during manufacturing of totally chlorine free kraft pulp from eucalypt wood. Chemosphere 44:1237–1242
20. Chen M, Wang X, Yu YH, Pei ZL, Bai XD, Sun C, Huang RF, Wen LS (2000) X-ray photoelectron spectroscopy and auger electron spectroscopy studies of Al-doped ZnO films. Appl Surface Sci 158:134–140
21. León CM, Alvarez SM, Graciani CE (2004) Formation of Stigmasta-3, 5-diene in olive oil during deodorization and/or physical refining using nitrogen as stripping gas. Grasasy Aceites 55:227–232
22. Soupas L, Huikko L, Lampi A, Piironen V (2005) Esterification affects phytosterol oxidation. Eur J Lipid Sci Technol 107:107–118
23. León CM, Ruiz MMV, Graciani CMM, Graciani CE (2001) Kinetics the Cis-trans isomerization of linoleic acid in the deodorization and/or physical refining of edible fats. Eur J Lipid Sci Technol 103:85–92