JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

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Efficient Solvent-Free Synthesis of Phytostanyl Esters in the Presence of Acid-Surfactant-Combined Catalyst

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ABSTRACT: An efficient approach based on the synthesis of phytostanyl esters with an acid-surfactant-combined catalyst in a solvent-free system was developed. The effect of catalyst dose, substrate molar ratio, reaction temperature, and acyl donor was considered. The reaction conditions were further optimized by response surface methodology, and a high yield of phytostanyl laurate (>92%) was obtained under optimum conditions: 3.17:1 molar ratio of lauric acid to plant stanols, 4.01% catalyst dose (w/w), 119 °C, and 4.1 h. FT-IR, MS, and NMR were adopted to confirm the chemical structure of phytostanyl laurate. Meanwhile, the physiochemical properties of different phytostanyl esters were investigated. Compared with phytostanols, the prepared phytostanyl esters had much lower melting temperature and higher oil solubility. There was no obvious difference in melting and solidification properties between sunflower oil with phytostanyl laurate (<5%) or oleate (<10%) and the original sunflower oil, suggesting that the esterification of phytostanols greatly facilitated their corporation into oil-based foods.

KEYWORDS: *stanols, cholesterol, phytostanyl esters, esterification, sodium dodecyl sulfate, differential scanning calorimetry*

INTRODUCTION

Recently, increasing attention has been focused on the research and development of plant sterols and their derivatives because of their strong cholesterol-lowering property. Plant sterols (phytosterols), mainly including β-sitosterol, stigmasterol, campesterol, and brassicasterol, are generally extracted from the deodorizer distillates produced during vegetable oil refining and from tall oil, a byproduct of the paper pulping industry.[1,2] Plant stanols (phytostanols), consisting of sitostanol and campestanol, are the less abundant hydrogenated counterparts of plant sterols. Both phytosterols and phytostanols have been used successfully for lowering plasma cholesterol levels by inhibiting the absorption of cholesterol from the small intestine in both animals and humans and shown to be safe for half a century.[3−12]

Many studies have verified that plant stanols are more effective and safer than sterols in lowering serum total cholesterol.[13,14] In addition, plant stanols are more resistant to oxidation than plant sterols.[2] However, practical application of plant stanols is greatly restricted by their poor solubility in oil and insolubility in water. Therefore, it is beneficial to modify the chemical structure of plant stanols to improve their solubility in oil or water and retain their biological activity, finally facilitating the incorporation into a variety of food products. Esterification of plant stanols with fatty acids can significantly improve their lipid solubility.[15−19] Phytostanyl esters can be synthesized via chemical esterification, transesterification, and enzyme-catalyzed reaction.[16,20−23] Currently, a large number of studies have been carried out on the enzymatic synthesis of plant steryl/stany esters.[2,15,17,24−26] Although enzyme-catalyzed reaction has evident advantages with respect to chemical synthesis, the high cost of enzymes and low productivity greatly limit its industrial application. Up to now, the chemical path remains mainstream for commercial production of phytosteryl/phytostanyl esters. Usually, traditional esterification can be catalyzed by common acid catalysts such as H_2SO_4 and H_3PO_4, which is often accompanied by complex and unknown side reactions.[27] Recently, acid-surfactant-combined catalysts have been attracting increasing attention due to their excellent catalytic efficiency in various organic transformations.[27−29]

In the present study, an acid-surfactant-combined catalyst was first used for the highly efficient synthesis of phytostanyl fatty acid esters by direct esterification in a solvent-free system. In detail, the effect of catalyst dose, substrate molar ratio, reaction temperature, and acyl donor was considered. The reaction conditions were further optimized by response surface methodology. Fourier transform infrared spectroscopy (FT-IR), mass spectroscopy (MS), and nuclear magnetic resonance spectroscopy (NMR) were adopted to confirm the chemical structure of phytostanyl laurate. Meanwhile, the physiochemical properties of different phytostanyl fatty acid esters, including melting temperature and oil solubility, were investigated.The melting and crystallization profiles of sunflower oil with or without phytostanyl esters were also explored.

MATERIALS AND METHODS

Materials. Phytosterols (purity >95%) were generous gifts from Jiangsu Spring Fruit Biological Products Co., Ltd. (Taixing, China). Fatty acids (lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid), sodium dodecyl sulfate (SDS), hydrochloric acid (HCl), and other reagents used were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Received: July 9, 2012
Revised: August 25, 2012
Accepted: August 25, 2012
Published: August 26, 2012

Methanol and *n*-hexane used for HPLC analysis were of spectral grade and also purchased from Sinopharm Chemical Reagent Co., Ltd.

Preparation of Phytostanols. Phytostanols were prepared according to our previous report.[2] Briefly, phytostanols were prepared by catalytic hydrogenation of phytosterols under hydrogen pressure of 2 MPa, using 5%-Pd/C (w/w) as catalyst. The reaction conditions were as follows: *n*-propanol as solvent, 4% (w/w) of the catalyst, reaction temperature 65 °C, and reaction time 6 h. The hydrogenation rate of phytosterols was above 98.5% by determination of the iodine value.

Preparation of Catalyst. Aliquots of sodium dodecyl sulfate were put into a mortar, and equimolar hydrochloric acid was added to the mortar. Then the mixture was ground for 5 min and allowed to stand for 10 min. The acid-surfactant-combined catalyst (SDS+HCl) was obtained.

SDS+HCl-Catalyzed Reaction. The reaction was carried out in a reaction tube as follows: the mixture of fatty acid and catalyst was first added to a reaction tube, placed in an oil bath equipped with a magnetic stirrer, and then heated to the desirable temperature under constant nitrogen flow (2.0 mL/min). When the fatty acid melted completely, phytostanols were added to the reaction system. Over the time course of the reactions, a portion of the reaction mixture was periodically removed from the reaction tube for thin layer chromatography (TLC) and high performance liquid chromatography (HPLC).

Analysis Methods. All FT-IR spectra were recorded on an FT-IR spectrometer (Nicolet Nexus 470) with a DTGS detector. FT-IR analysis of phytostanols and lauric acid were carried out with a KBr pellet, scanning scope: 400−4000 cm^{-1}, number of scans: 32. FT-IR measurement of phytostanyl esters was performed using attenuated total reflectance (ATR), scanning scope: 650−4000 cm^{-1}, number of scans: 64.

The isolated phytostanyl laurate was examined by MS analysis. The mass spectrum was obtained by mass spectrometry (Waters Maldi Synapt Q-TOF, Milford, MA) with positive electron spray ionization (ESI) mode. The MS parameters were as follows: capillary voltage 3.0 kV, cone voltage 20 V, source block temperature 100 °C, desolvation temperature 250 °C, desolvation gas flow 500 L/h, cone gas flow 50 L/h, collision energy 6 eV, and mass scan range 50−1000 amu.

The isolated sitostanyl laurate was examined by NMR analysis. ^{1}H NMR and ^{13}C NMR spectra of sitostanyl laurate were recorded in $CDCl_3$ as solvent with a Bruker NMR spectrometer (Avance III 400 MHz, Switzerland), operating at 400 and 100 MHz for ^{1}H and ^{13}C, respectively.

Quantitative analysis was performed with HPLC using a method as previously described.[24] Aliquot fractions removed periodically from the reaction mixtures were dissolved in 10 mL of methanol/*n*-hexane/2-propanol (8/1/1, v/v/v) for HPLC analysis. The analysis was carried out with a symmetry-C_{18} column (5 μm, 4.6 × 150 mm, Waters) and evaporative light scattering detector (ELSD) 2420 (Waters). The chromatographic column temperature was 35 °C, and the ELSD was used at a drift tube temperature of 80 °C, at a sprayer temperature of 42 °C, and with a nitrogen carrier gas (172.2 kpa). The mobile phase was a mixture of methanol/2-propanol/*n*-hexane (8/1/1, v/v/v), and the flow rate was 1.0 mL/min. A standard curve was prepared using the purified phytostanyl esters. The conversion was defined as the molar ratio of the amount of plant stanyl esters to that of plant stanols at the beginning of the reaction.

Determination of Physiochemical Properties. The melting and crystallization profiles of phytostanyl saturated fatty acid esters and phytostanols were determined with a Pyris 1 differential scanning calorimeter (PerkinElmer, Waltham, MA). The baseline was obtained with an empty aluminum pan. Each sample (about 3 mg) was accurately weighed for differential scanning calorimetry (DSC) analysis. The instrument temperature was increased from 30 °C to 160 °C at 10 °C/min, and then after 5 min at this temperature, it was cooled at 10 °C/min to 30 °C. The melting and crystallization profiles of phytostanyl unsaturated fatty acid esters (oleate, linoleate) and sunflower oil with or without phytostanyl esters (laurate or oleate) were determined by a Q series differential scanning calorimeter (TA Instruments, New Castle, DE). The instrument temperature was increased from −80 °C to 60 °C at 10 °C/min, and then after 5 min at this temperature, it was cooled at 10 °C/min to −80 °C.

Solubility of phytostanols and phytostanyl esters in oil was investigated as follows. About 0.5 g of phytostanols or 1.0 g of phytostanyl fatty acid esters was weighed into a 100 mL flask and heated with water bath at 50 °C, and then sunflower oil was added dropwise until the samples were completely dissolved. Thereafter, the flask was cooled to 20 °C, allowed to stand for 24 h, and observed for turbidity or precipitation. The solubility of all samples can be respectively calculated by the amount of sunflower oil to be added and expressed as g/100 mL, 25 °C.

Box−Behnken Design. Response surface methodology (RSM) was used to optimize the reaction parameters on a Box−Behnken design. Factors considered important were molar ratio of lauric acid to phytostanols (X_1: 2.5:1, 3:1, 3.5:1), catalyst dose (X_2: 3%, 4%, 5%), reaction temperature (X_3: 110 °C, 120 °C, 130 °C), and reaction time (X_4: 3 h, 4 h, 5 h). The relationships and interrelationships of the variables were determined by fitting the second-order polynomial equation to data obtained.

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j$$

where Y was the predicted response variable, the conversion of phytostanols to phytostanyl laurate; β_0, β_i, β_{ii}, and β_{ij} were the constant regression coefficients of the model; and X_i and X_j (i = 1, 2, 3, 4; j = 1, 2, 3, 4; $i \neq j$) represented the independent variables.

RESULTS AND DISCUSSION

Product Analysis. Phytostanols mainly contained two components, sitostanol and campestanol, so phytostanyl esters included two esters, sitostanyl esters and campestanyl esters. The purified phytostanyl laurate was identified by FT-IR, and purified sitostanyl laurate was analyzed by MS and NMR.

HPLC Analysis. HPLC analysis was employed to determine the purity of phytostanyl fatty acid esters and calculate the conversion of plant stanols to phytostanyl esters. Lauric acid and plant stanols (campestanol and sitostanol) were eluted with relative retention times of 1.8, 3.9, and 4.8 min, respectively. Campestanyl laurate and sitostanyl laurate corresponded to 21.1 and 22.6 min. Evidently, reaction substrates and products could be clearly distinguished in terms of retention time.

FT-IR Analysis. The FT-IR spectral data of lauric acid and the potential functional groups are shown in Table 1. The broad peak between 3300 and 2500 cm^{-1} corresponded to the vibration of OH in COOH. The band at 1688 cm^{-1} corresponded to the stretch vibration of C═O in COOH. The signals between 3300 and 2500 cm^{-1} and 1688 cm^{-1} revealed the presence of a free carboxyl group. The band at 722 cm^{-1} indicated that the presence of four or more CH_2 groups in the carbon chain.

The FT-IR spectral data of phytostanols and the potential functional groups are displayed in Table 1. The strong peak at 3426 cm^{-1} was the stretch vibration signal of OH. The bands at 2960 cm^{-1} and 2863 cm^{-1} were the stretching vibration of CH_3, and the signal at 1380 cm^{-1} was the bending vibration of CH_3. The bands at 2929 cm^{-1} and 1463 cm^{-1} were the stretching and bending vibration signal of CH_2 group, respectively.

The FT-IR spectral data of the new compound and the potential functional groups are shown in Table 1. Obviously, the strong signal at 1741 cm^{-1} was the stretching vibration of C═O. The band at 1177 cm^{-1} was the stretching vibration signal of C−O, suggesting the formation of an ester bond. The band at 727 cm^{-1} indicated the presence of four or more CH_2 groups in the carbon chain. Disappearance of the carboxyl

Table 1. Fourier Transform Infrared Spectroscopy Spectra of Lauric Acid, Phytostanols, and Phytostanyl Laurate

frequency (cm^{-1})	intensity	adscription	potential functional groups
Lauric Acid			
3300−2500	strong	ν_{OH}	COOH
2950	strong	ν_{CH}	CH_3
2917	strong	ν_{CH}	CH_2
2870	strong	ν_{CH}	CH_3
2844	strong	ν_{CH}	CH_2
1688	strong	$\nu_{C=O}$	COOH
1466	middle	δ_{CH}	CH_2
1300	middle	ν_{C-O}	COOH
931	middle	δ_{OH} (out of plane)	O−H (H-bonded)
722	weak	ν_{CH}	$(CH_2)_n$, $n > 4$
Phytostanols			
3426	strong	ν_{OH}	OH
2960	strong	ν_{CH}	CH_3
2929	strong	ν_{CH}	CH_2
2863	strong	ν_{CH}	CH_3
1463	middle	δ_{CH}	CH_2
1380	middle	δ_{CH}	CH_3
1039	middle	ring vibration	polycyclic compounds
Phytostanyl Laurate			
2954	strong	ν_{CH}	CH_3
2914	strong	ν_{CH}	CH_2
2867	strong	ν_{CH}	CH_3
2849	strong	ν_{CH}	CH_2
1741	strong	$\nu_{C=O}$	R-CO-OR′
1464	middle	δ_{CH}	CH_2
1377	weak	δ_{CH}	CH_3
1177	strong	ν_{C-O}	R-CO-OR′
727	weak	ν_{CH}	$(CH_2)_n$, $n > 4$

[a] ν, stretching vibration; δ, bending vibration.

group and hydroxyl group signals and the appearance of an ester bond suggested that the new product may be phytostanyl laurate.

MS Analysis. A direct-infusion ESI-MS approach with positive-ion mode was used to detect the product. The major advantage of this approach was that it was not necessary to separate the targeted compound from other compounds prior to analysis.[30] The MS spectrum of the product is shown in Figure 1. The protonated molecular ion $[M + Na]^+$ of the product from sitostanol and lauric acid was at 621, indicating that the product was sitostanyl laurate. Fragments at m/z 415 and 414 provided strong evidence for the presence of sitostanyl laurate.

NMR Analysis. Assignment of hydrogen and carbon resonaces from the NMR spectra can be obtained from our previous publication.[24] ^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).

Determination of Reaction Parameters. *Effect of Catalyst Dose.* The influence of catalyst dose was evaluated using varying amounts of SDS+HCl, from 1% to 5% (w/w) (Figure 2). It was first observed that almost no formation of the desired phytostanyl esters occurred in the absence of catalyst (data not shown). Moreover, not surprisingly, it was also shown that the higher the catalyst dose, the better the phytostanyl ester formation. In detail, the conversion of plant stanols to phytostanyl esters tended to rise slightly from 1% to 2% and increased sharply from 2% to 3%. Finally, the conversion varied slightly with a further rise in catalyst dose from 3% to 5%. Thus, 3% SDS+HCl would be better for the synthesis of phytostanyl esters.

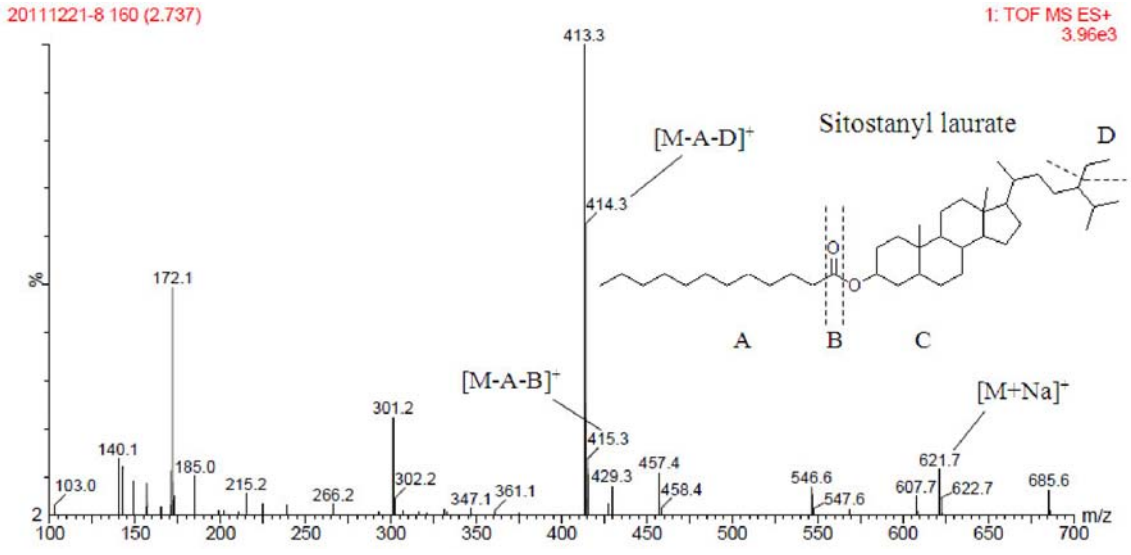

Figure 1. Electron spray ionization mass spectrum of sitostanyl laurate.

dx.doi.org/10.1021/jf302958g | J. Agric. Food Chem. 2012, 60, 9763−9769

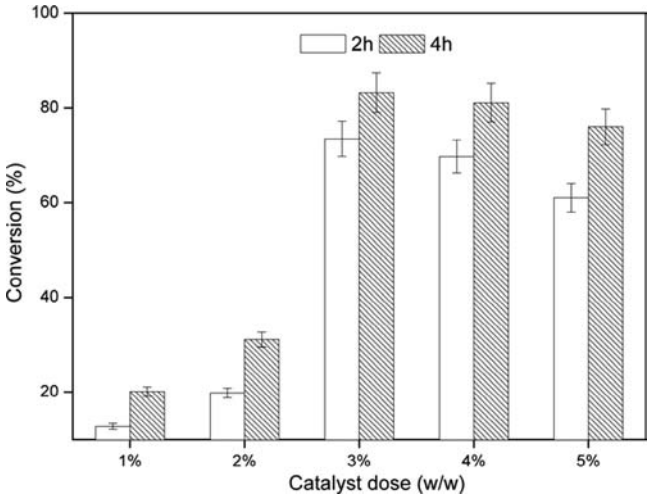

Figure 2. Effect of catalyst dose on the conversion of phytostanols to phytostanyl laurate (120 °C, 3:1 molar ratio of lauric acid to phytostanols).

Effect of Molar Ratio of Lauric Acid to Phytostanols. The influence of substrate molar ratio on the conversion of phytostanols to phytostanyl laurate was evaluated using a molar ratio of lauric acid to phytostanols from 1:1 to 3.5:1 (Figure 3). Generally speaking, an equimolar ratio of both

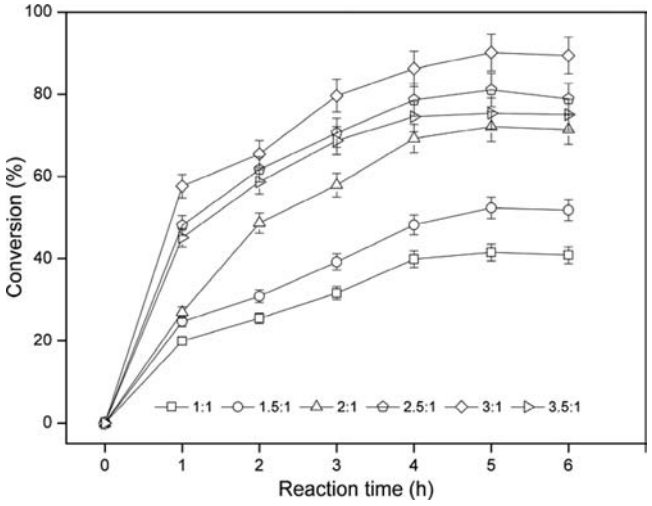

Figure 3. Effect of the molar ratio of lauric acid to phytostanols on the conversion of phytostanols to phytostanyl laurate (120 °C, catalyst dose 4%).

substrates should be ideal for esterification in terms of economic cost and further purification of the final products. However, a 1:1 molar ratio of lauric acid to phytostanols was not advantageous, and phytostanyl laurate synthesis only reached 40% after 6 h. Direct esterification was an equilibrium reaction, and an excess of one of the two substrates could shift the reaction equilibrium to the products (phytostanyl laurate). Indeed, no shift of the equilibrium was possible with equivalent molar amounts of both substrates for the direct esterification.[24] As shown in Figure 3, the change of the molar ratio of lauric acid to phytostanols from 1:1 to 3:1 led to a gradual rise of conversion of phytostanols to phytostanyl laurate, and then a decrease of conversion was observed with a further increase of the lauric acid to phytostanols ratio from 3:1 to 3.5:1, suggesting that excessive lauric acid could not enhance the conversion when the molar ratio surpassed 3:1. Therefore, a 3:1 molar ratio of lauric acid to phytostanols was considered to be optimal.

Effect of Reaction Temperature. To evaluate the influence of reaction temperature on the solvent-free esterification, a series of experiments were performed from 90 °C to 130 °C in the presence of nitrogen flow (Figure 4). The results showed

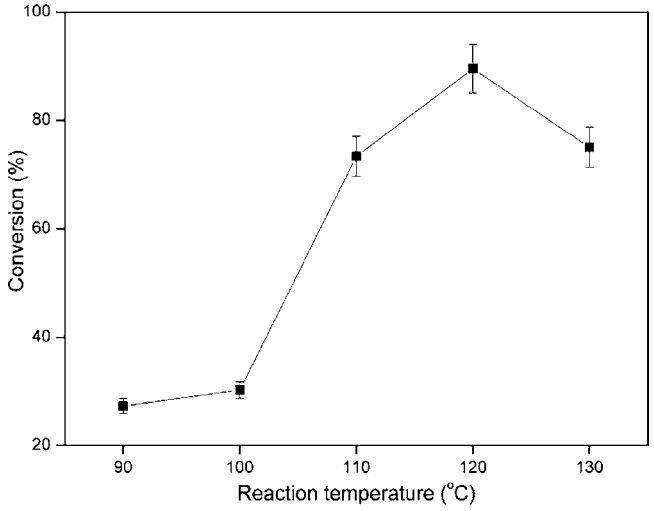

Figure 4. Effect of temperature on the conversion of phytostanols to phytostanyl laurate (catalyst dose 4%, 3:1 molar ratio of lauric acid to phytostanols, 4 h).

that the conversion of phytostanols to phytostanyl laurate increased to a varying degree as the reaction temperature increased from 90 °C to 120 °C, and then decreased with a further increase of reaction temperature from 120 °C to 130 °C. Generally speaking, as the substrates gradually melted and mixed fully while the reaction temperature increased, the reaction would be promoted at higher temperature. However, side reactions such as oxidation may also be accelerated as the reaction temperature increases.

Effect of Acyl Donor. Four saturated fatty acids with different carbon chain lengths (lauric acid, myristic acid, palmitic acid, and stearic acid) and two unsaturated fatty acids with different degrees of unsaturation (oleic acid and linoleic acid) were tested to investigate the effect of acyl donor on the synthesis of phytostanyl esters in the presence of SDS +HCl. The results are displayed in Figure 5. Obviously, phytostanyl esters of different fatty acids can be efficiently prepared under neat conditions, suggesting that SDS+HCl were an efficient catalyst for the synthesis of phytostanyl esters. The highest conversion was obtained when using lauric acid as acyl donor, and the conversion gradually decreased with the increase in carbon chain length of the fatty acid (C12−C18). This phenomenon can be accounted for by a steric effect. As the alkyl chain in the fatty acid increased in size, its steric effect increased as well.[24] In addition, there was no significant discrimination in terms of conversion of plant stanols to phytostanyl esters when using stearic acid, oleic acid, and linoleic acid as acyl donor.

Optimal Reaction Conditions. The Box−Behnken design for the four variables (reaction temperature, catalyst dose, molar ratio, and reaction time) was used, and analysis of variance (ANOVA) of the result was carried out (the detailed

dx.doi.org/10.1021/jf302958g | J. Agric. Food Chem. 2012, 60, 9763−9769

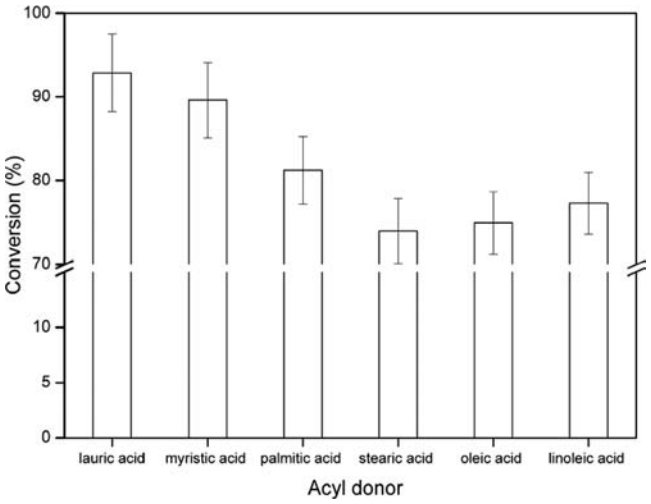

Figure 5. Effect of acyl donor on the conversion of phytostanols to phytostanyl esters (119 °C, catalyst dose 4.01%, molar ratio 3.17:1, 4.1 h).

data are not shown). Briefly, the model F value of 20.52 implied that the model was significant, and there was only 0.01% chance that the model F value could occur due to noise. The value of R^2 was 0.9535, indicating that the model was suitable to represent the experimental data. The value of Q^2 corrects the R^2 value for the sample size, and the number of terms in the model was 0.9071. A relatively lower value of the coefficient of variation (CV = 2.43%) suggested a good precision and reliability of the experiment. The yield of phytostanyl laurate could be expressed as follows:

$$Y = 92.23 + 63.50X_1 - 0.42X_2 + 0.087X_3 + 0.26X_4 + 1.62X_1X_2 - 1.21X_1X_3 + 0.9X_1X_4 + 0.96X_2X_3 + 0.015X_2X_4 - 1.28X_3X_4 - 10.19{X_1}^2 - 1.62{X_2}^2 - 2.19{X_3}^2 - 3.30{X_4}^2 \quad (1)$$

Equation 1 suggested that the maximum yield of 93.31% could be obtained with a molar ratio of 3.17:1 and a catalyst dose of 4.01% at 119 °C for 4.1 h. This prediction was verified by additional independent experiments under the above conditions, and an average conversion ratio, 92.89%, was obtained, which did not significantly differ from the predicted value. Therefore, this model would be effective to adequately predit the Y value for the synthesis of phytostanyl laurate.

Physiochemical Properties of Phytostanols and Phytostanyl Esters. The melting and crystallization (solidification) profiles of different phytostanyl fatty acid esters and phytostanols were investigated (Figure 6). The DSC curves of phytostanyl saturated fatty acid esters and unsaturated fatty acid esters were obtained from two kinds of DSC instruments because of differing melting properties. Obviously, the melting temperature of phytostanols was 144.6 °C. The melting temperatures of phytostanyl saturated fatty acid esters gradually increased with the increase of carbon numbers in the fatty acyl group, which was much lower than that of the phytostanols (Figure 6a). Similar trends with lower melting temperature were also perceived in the lipase-catalyzed synthesis of β-sitosteryl esters of medium chain fatty acids (C_6–C_{12}).[25] Vaikousi et al. also found that the melting temperature of soy stanyl fatty acid esters increased with the increase in carbon

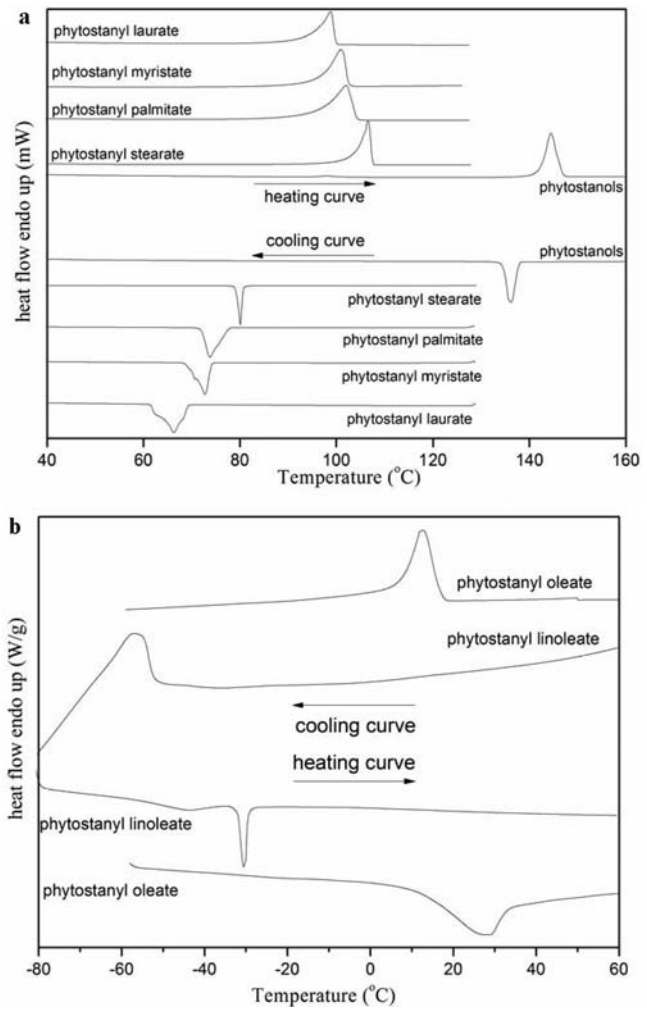

Figure 6. Differential scanning calorimetry analysis for phytostanols and phytostanyl esters (a: phyotstanols and phytostanyl saturated fatty acid esters; b: phytostanyl oleate and phytostanyl linoleate).

chain length of acyl donors (C_8–C_{12}).[31] Meanwhile, phytostanyl unsaturated fatty acid esters had a lower melting temperature compared to that of phytostanyl saturated fatty acid esters and phytostanols (Figure 6b). In detail, the melting temperatures of phytostanyl oleate and phytostanyl linoleate were 26.8 °C and −30.0 °C, respectively. The melting temperature of phytostanyl stearate was 105.6 °C, indicating that the melting temperature of phytostanyl esters increased with the decrease in degree of unsaturation in the fatty acyl group. Furthermore, the solidification property of phytostanyl fatty acid esters had a trend similar to that of their melting property. The results suggested that the melting and solidification properties of phytostanols were greatly ameliorated by direct esterification with fatty acids.

The solubility of phytostanyl fatty acid esters and fatty acids in vegetable oil at room temperature was investigated and is also displayed in Table 2. The solubility of phytostanols in vegetable oil was 1.5 g/(100 mL oil). Undoubtedly, phytostanyl unsaturated fatty acid esters had higher oil solubility compared with that for saturated fatty acid esters. The oil solubility of phytostanyl oleate and phytostanyl linoleate was 28.3 g/(100 mL oil) and 30.1 g/(100 mL oil), respectively. The oil solubility of phytostanyl saturated fatty acid esters decreased with the increase in carbon number of the fatty acyl group, which was in

Table 2. Solubility of Phytotanyl Esters and Fatty Acids

	lauryl	myristyl	palmityl	stearyl	oleoyl	linoleoyl
Phytostanyl Esters						
oil solubility (g/100 mL)	7.2 ± 0.3	6.1 ± 0.3	4.9 ± 0.3	2.7 ± 0.1	28.3 ± 1.6	30.1 ± 1.8
Fatty Acids						
oil solubility (g/100 mL)	9.9 ± 0.5	6.8 ± 0.3	1.9 ± 0.1	1.4 ± 0.1	mutually soluble	mutually soluble

agreement with that of fatty acids. Vu et al. held that a higher solidification temperature led to lower oil solubility for β-sitosteryl fatty acid esters at ambient temperature.[25] The oil solubility of phyotstanols was improved to varying degrees by direct esterification with different fatty acids.

The melting and crystallization profiles of sunflower oil with or without phytostanyl esters were explored (Figure 7). Broad peaks were observed in both cooling and heating curves. This was attributed to the mixed compounds in vegetable oils, which generally showed a melting and solidification temperature instead of a single peak.[25] When phytostanyl laurate or phytostanyl oleate was present in varying amounts in sunflower oil, no obvious difference was found in heating and cooling curves, indicating that the addition of phytostanyl laurate and phytostanyl oleate had almost no effect on the sunflower oil. However, visible crystallization was found when phytostanyl laurate was present at more than 5% in sunflower oil, and the oil solution became turbid, which is not recommended for common use. In other words, phytostanyl laurate and phytostanyl oleate did not crystallize in sunflower oil at a dosage below 5% and 10%, respectively. Below those corresponding concentrations, the melting and solidification properties of sunflower oil with phytostanyl laurate or phytostanyl oleate were similar to that of the original sunflower oil.

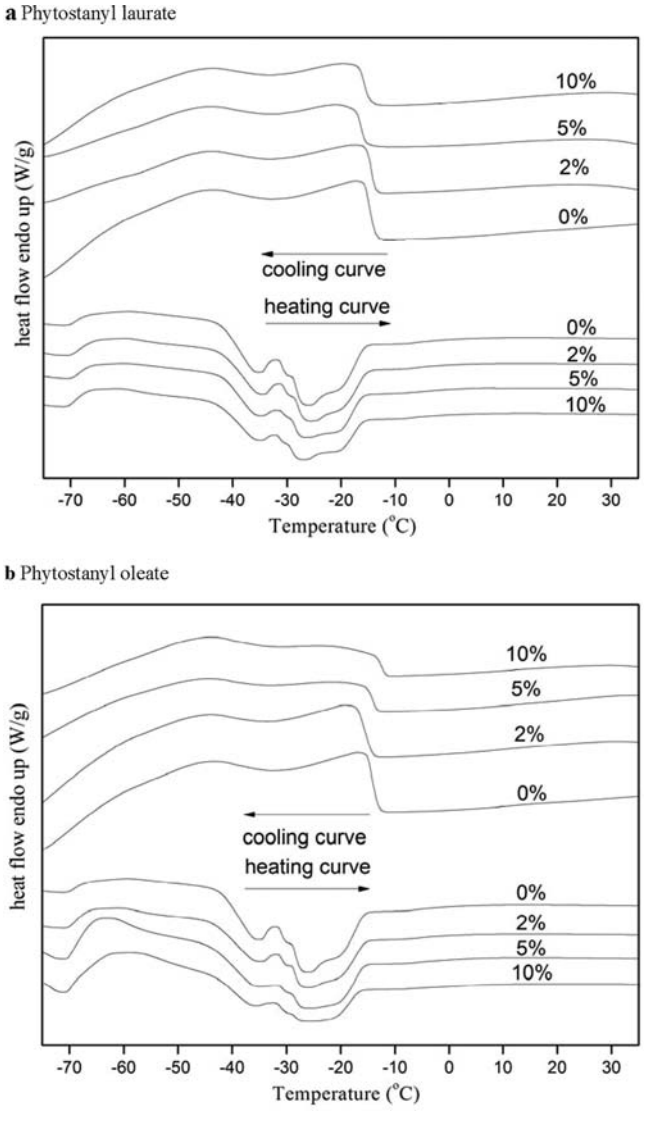

Figure 7. Differential scanning calorimetry analysis of sunflower oil with or without phytostanyl esters (a: phytostanyl laurate, 0%, 2%, 5%, 10%; b: phytostanyl oleate, 0%, 2%, 5%, 10%).

As an efficient cholesterol-lowering food component, phytostanyl fatty acid esters can be efficiently and easily synthesized by direct esterification of phytostanols with different fatty acids under a solvent-free system in the presence of SDS+HCl. After the optimization of reaction parameters, a high yield, above 92%, of phytostanyl laurate can be achieved. Compared to phytostanols, the prepared phytostanyl esters had much lower melting temperatures and higher oil solubility. There was no obvious difference in melting and solidification properties between sunflower oil with phytostanyl laurate (<5%) or phytostanyl oleate (<10%) and the original sunflower oil, suggesting that the esterification of phytostanols with fatty acids greatly facilitated their incorporation into oil-based foods.

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Funding

This study was financially supported by the NSFC (31071600), Natural Science Foundation of Jiangsu Province (BK2011155), Funding of Jiangsu Innovation Program for Graduate Education (CXZZ11-0485), Fundamental Research Funds for the Central Universities (JUDCF10054), and a project funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

Notes

The authors declare no competing financial interest.

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