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An innovative auto-catalytic esterification for production of phytosterol esters: experiment and kinetics

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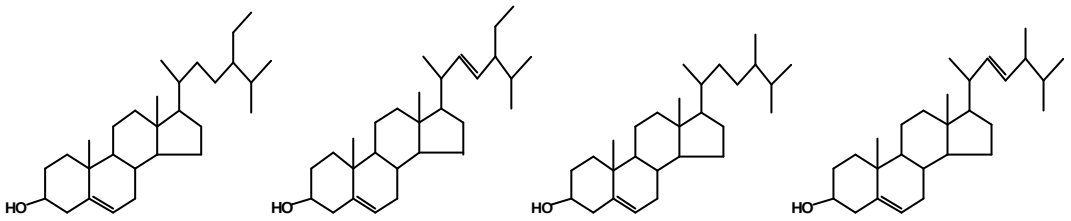
Abstract: An innovative auto-catalytic method was proposed to synthesize phytosterol esters from phytosterols and long-chain fatty acid without adding any catalyst and solvent. The effects of reaction temperature, molar ratio of oleic acid/phytosterols, reaction time and carbon-chain length of fatty acids on the reaction were investigated. Results showed that the conversion, the yield and the selectivity of the reaction were increased with the increase in reaction temperature, molar ratio and the decrease in the length of carbon chains of fatty acids. The selectivity was decreased with prolonging reaction time. A high conversion (99.1%) with a high yield (94.9%) and selectivity (95.8%) was achieved under molar ratio of oleic acid/phytosterols of 3:1, reaction temperature of 220 °C and reaction time of 4 h. The properties of the phytosterol oleic esters by autocatalysis conformed to the quality indices of phytosterol esters from China Ministry of Health and were superior to the commercial product. The kinetics suggested that the reaction order was 2 and the reaction activation energy was 58.75 KJ/mol. The auto-catalytic process, omitting the separation step of catalysts and solvents, could be considered as a promising process to synthesize phytosterol esters.

Keywords: Phytosterol esters, Phytosterols, Auto-catalytic esterifcation, Oleic acid

Introduction

Phytosterols are among the most important compounds in plant-origin foods.[1] The most well-known property is the serum cholesterol-lowering effect to decrease the risk of cardiovascular diseases when one consumes 2 - 3 g phytosterols everyday. [2-5] The chemical structures of phytosterols are similar to cholesterol although they differ in the complexity of their side chain, which is attached to the steroid ring.[6] Phytosterols are usually mixtures including ß-sitosterol, stigmasterol, campesterol, and/or brassicasterol as shown in Fig. 1.



(a) (b) (c) (d)

Fig. 1 Structural formula of four kinds of phytosterols:
(a) ß-Sitosterol, (b) Stigmasterol, (c) Campesterol, (d) Brassicasterol.

The applications of phytosterols have been limited owing to their poor solubility in fats and oils. So phytosterols must be ingested in excessively high doses to make sure that people can absorb the necessary quantity in daily life.[7] Phytosterol esters, possessing the same physiological effects as phytosterols, have higher solubility in fats and oils than free phytosterols, which contributes to practical application in foods.[8, 9] Consequently, commercial phytosterols are currently converted into phytosterol esters with long-chain fatty acid structure, allowing maximal incorporation into a limited amount of fats and oils.

The synthesis methods of phytosterol esters mainly include enzymatic catalysis [10,11] and chemical catalysis, such as esteri?cation and transesteri?cation.[12] The enzyme technology offers a good alternative for the production of phytosterol esters under mild and environmental friendly reaction conditions. But the high cost of enzymes and low productivity limited the industrial applications.[13 -16]

Currently, chemical synthesis has remained the main method for production of phytosterol esters. However, the conventional esterification reaction of phytosterols with fatty acids is carried out with homogeneous acid catalysts (such as H_2SO_4, 4-toluene sulfonic acid) which might also favor the dehydration of phytosterols to dienes or trienes.[17] In addition, sodium alkoxides (NaOMe, NaOEt *etc.*) as well as hydroxides (NaOH, KOH, $Ca(OH)_2$ *etc.*) as homogeneous base catalysts have mainly been used in transesterification reactions of phytosterols with fatty acid methyl esters or triacylglycerols.[18] Unfortunately, these bases are well known to favor the formation of soaps, difficult to separate from the products, leading to the excessive waste.[19]

Recently, many kinds of heterogeneous catalysts have been developed to product phytosterol esters to overcome the shortcomings of the homogeneous catalysts. Pouilloux *et.al.*[20] investigated some solid base catalyses, including Na_2CO_3, MgO, LiMgO and ZnO, to synthesize phytosterol ester from methyl dodecanoate and ß-sitosterol at 240 °C for 7h. A yield of 76% with 3% stigmasta-3,5-diene as side product was obtained when ZnO was used. Meng *et.al.*[21] found that ZnO exhibited a high selectivity (about 91.0% – 99.2%) and acceptable activity (the total phytosterol conversion of 75.0% – 87.3%) both for transesterification and esterification reactions in the phytosterol ester synthesis. Valange *et.al.*[22] reported that MgO/Al_2O_3 as a solid catalyst was used in the transesteri?cation of sitosterol with methyl dodecanoate in the absence of any solvent. Although the yield of phytosterol esters was 93% under 240 °C for 7 h, metal species were easily leached from the heterogeneous catalysts into the product.[22] Moreover, the side products, such as dienes (up to 91% sometimes [20]), trienes and oxides from phytosterols were easily formed at the high temperature within a long reaction time in the presence of acid or base catalysts.[20-22] These residues from poisonous catalysts and side products adversely affect the product quality of phytosterol esters as a functional food.[23, 24]

So far, facing the economic and food safety challenge, there is an urgent need to develop a safe, practicable and environmentally friendly method for the synthesis of phytosterol esters. The esteri?cation reaction between the hydroxyl groups of phytosterols and the carboxylic group of fatty acids is feasible in thermodynamics if the

energy barrier of the reaction overcomes by increasing reaction temperature. Moreover, the phytosterols are thermostable in the range of 230 to 260 °C, [20, 21, 24] and the boiling points of long-chain fatty acids are often over 220 °C. Consequently, the phytosterol esters might be synthesized in a auto-catalytic mode from the esterification reaction between phytosterols and fatty acids under a high temperature without adding any catalyst or solvent into the reaction system.[25] In an auto-catalytic process, the general separation step of catalysts or solvents could be omitted and the product would not be polluted by catalysts or solvents, which could overcome the shortcomings of the existing processes involving homogeneous or heterogeneous catalysts and ensure the product quality of phytosterol esters. However, few efforts were made to investigate the auto-catalytic reaction of phytosterols with free fatty acids in detail up to date.

The purpose of the present work is to develop an innovative auto-catalytic method to synthesize phytosterol esters from phytosterols and fatty acids without adding any catalyst or solvent. The effects of reaction temperature, reaction time and molar ratio on the esterification reaction were investigated. The composition and the properties of phytosterol esters were analyzed. The kinetics of the auto-catalytic esterification of phytosterols with fatty acids was also studied.

Experimental

Materials

Phytosterols (containing ß-sitosterol, stigmasterol, campesterol, and brassicasterol (about 93.53%), phytosterol esters (3.88%) and others (2.59%); an average molecular weight of 399.2) and standard sample of phytosterols (with a purity of 95.30%) were kindly supplied by COFCO Tech Bioengineering (Tianjin) Co., Ltd., China. A commercial phytosterol esters produced by ADM Co., USA, was used as a reference sample. Unsaturated fatty acid (oleic acid, C18) and saturated fatty acids of different chain lengths (lauric acid (C12), myristic acid (C14), palmitic acid (C16) and stearic acid (C18)) were all analytical reagent (AR) grades and purchased from Tianjin Kermel Chemical Reagents Co., Ltd (China). The other chemicals for product analysis

(including ethanol, n-hexane, petroleum ether, pyridine, acetic anhydride, isopropyl ether) were AR grade as received without further purification.

Auto-catalytic esterification of phytosterols with oleic acid

The reaction of phytosterols with oleic acid was carried out in a three-necked flask (a total volume of 250 mL) equipped with a vacuum pump (under a pressure of 1.33 kPa) to promote the escape of water and a magnetic stirring bar to achieve better contact. The reaction was allowed to proceed under different temperatures (120 ~ 220 °C) and different molar ratio of oleic acid to phytosterols from 1:1 to 3:1 for a certain time without adding any catalyst or solvent. After the reaction completion, the product mixture was washed by ethanol to remove the unreacted phytosterols and fatty acid, then distilled under a temperature of 60 °C and a pressure of 1.33 kPa to remove ethanol in the product.

The above process was also used to synthesize phytosterol esters of other fatty acids with different carbon chain lengths (C12 – C18).

Product analysis

Gas chromatograph (GC, Agilent 6890N, USA) with a HP-5 capillary column was employed to analyze the content of phytosterols and further calculate the conversion and the yield of the reaction. Phytosterols in the samples were derived with acetic anhydride in pyridine for GC testing.[26] Cholesterol was used as an internal standard. Nitrogen was used as carrier gas at a flow rate of 1.0 mL/min and split ratio of 1:30 (v/v). The oven temperature was held at 50 °C for 1 min, raised to 250 °C at 35 °C/min and held for 1 min, then increased to 280 °C at a rate of 30 °C/min and maintained for 30 min; other conditions are injection port at 290 °C, detector at 300 °C, injection volume of 3μL. The content of phytosterols can be calculated by Eq. (1).

$$R = F\frac{A_{\text{sam}} \times m_{\text{int}}}{A_{\text{int}} \times m_{\text{sam}}} \times 100\% \quad (1)$$

where R is the content of phytosterols (wt%), F the correction factor obtained from the standard sample of phytosterols in advance, A_{sam} the total peak area of phytosterols (brassicasterols, campesterols, stigmasterols, and ß-sitosterols) in a sample, A_{int} the total

peak area of internal standard cholesterol, m_{int} the mass of the internal standard (mg), m_{sam} the mass of the sample (mg).

The conversion of phytosterols can be calculated by Eq. (2).

$$X = \left(1-\frac{R_2}{R_1}\right)\times 100\% \qquad (2)$$

where X is the conversion of phytosterols, R_1 and R_2 obtained from Eq. (1) are the content of phytosterols in the reaction mixture before and after the reaction, respectively.

The yield of phytosterol esters can be calculated by Eq. (3).

$$Y = \frac{P_2 - P_3}{P_1}\times 100\% \qquad (3)$$

where Y is the yield of phytosterol esters, P_1 the theoretical content of phytosterol esters based on the assumption that the phytosterols in the feedstock were all converted into phytosterol esters, P_2 the content of realistic phytosterol esters obtained, P_3 content of phytosterol esters in the phytosterol feedstock.

The content of phytosterol esters can be calculated according to the following method.[27] The product mixture obtained were saponified by potassium hydroxide/ethanol solution and extracted by isopropyl ether to make the phytosterol esters back to phytosterols. So it can be used the same method mentioned above to analyze the content of phytosterols and further confirm the content of phytosterol esters.

Considering the side reactions, the selectivity of reaction was used to characterize the efficiency of the reaction. The selectivity of reaction (S) can be calculated by Eq. (4).

$$S = \frac{Y}{X}\times 100\% \qquad (4)$$

where Y is the yield of phytosterol esters; X is the conversion of phytosterols.

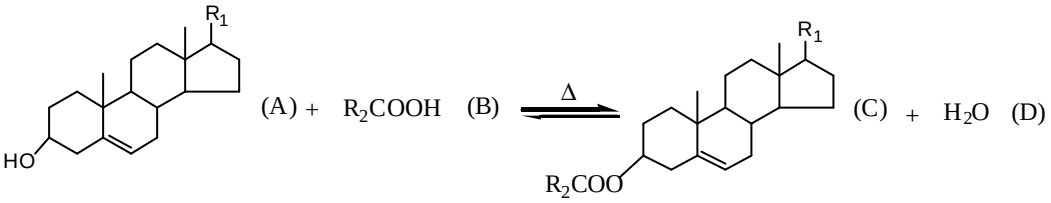
The compositions of purified product were analyzed by using a gas chromatography–mass spectrometry (GC–MS, Agilent 7890–5975, USA) equipped with a HP-5MS capillary column (30 m × 0.25 mm; Agilent, USA), a flame ionizing detector, and a mass detector with a split/splitless injector, working in electron

ionization mode.[28] The column temperature was held at 150 °C for 1 min, then heated to 200 °C at 20 °C/min to hold for 1 min, and finally increased to 280 °C at a rate of 10 °C/min to hold for 15 min. The injector temperature was set to 300 °C. The carrier gas was helium (>99.996%), at a constant flow of 1 mL/min. The MS interface temperature was 250 °C and the ion source 230 °C. Electron ionization energy was 70 eV. A volume of 1 μL solution was injected.

Peroxide value and acid value of samples were determined according to China Standard GB/T 5538-2005 and GB/T 5530-2005, respectively.

Kinetic study

The esteri?cation reaction of phytosterols with oleic acid to yield phytosterol oleic esters and water is given as follows:



The reaction rate can be described as:

$$-\frac{dC_A}{dt} = kC_A^a C_B^b - k'C_C^c C_D^d \qquad (5)$$

where C_A, C_B, C_C and C_D denote the concentrations of phytosterols, oleic acid, phytosterol oleic esters and water at the time of *t*, respectively; *a*, *b*, *c*, *d* refer to their reaction orders, respectively; *k* and *k'* are the rate constants for the forward and reverse reactions, respectively.

The kinetic model was built on the following assumptions: the whole reaction system was taken into account as an ideal solution; *k* was believed to be far larger than *k'* because the water could be instantly taken out by vacuum pump.[29] Indeed, there is no water determined in the product mixture with the Karl Fischer water tester with a sensitivity of 500 ppm. Eq. (5) can be simpli?ed as follows:

$$\frac{dC_A}{dt} = kC_A^a C_B^b \qquad (6)$$

$$C_A = C_{A0}(1 - X_A) \qquad (7)$$

$$C_B = C_{B0}(1 - X_B) \qquad (8)$$

where X and C_0 refer to the conversion at the time t and the initial concentration of the feedstock, respectively.

Eq. (6), (7) and (8) can be converted to Eq. (9)

$$\frac{\mathrm{d}X}{\mathrm{d}t} = \frac{k_1}{C_{A0}}[C_{A0}(1 - X_A)]^a[C_{B0}(1 - X_B)]^b \qquad (9)$$

when a = 1, b=1, $k_2 = k_1 C_{B0}$.

Eq. (9) can be integrated to

$$\ln\left[\frac{(1 - X)}{(1 - 0.689X)}\right] = -0.311k_2 t \qquad (10)$$

when a? 1, b? 1, Eq. (9) is integrated to

$$\int \frac{dX}{(1 - X)^a (1 - 0.689X)^b} = k_2 C_{A0}^a C_{B0}^b t \qquad (11)$$

To consider the effect of reaction temperature on the kinetic model, Arrhenius equation is listed as:

$$k = \mathrm{A}\exp(-\frac{E_\mathrm{a}}{\mathrm{R}T}) \qquad (12)$$

The plots of ln$k$ can be used as a function of the reciprocal temperature:

$$\ln k = \ln \mathrm{A} - \frac{E_\mathrm{a}}{\mathrm{R}T} \qquad (13)$$

Both the frequency factor A and the activation energy E_a were obtained from the plot of ln$k$ and 1/T.

Results and discussion

Effect of temperature

The temperature is a very important parameter in the esterification of phytosterols with fatty acids. The solubility of phytosterols and the thermodynamic equilibrium of reaction are strongly associated with the reaction temperature. It was found from the experiments that phytosterols were fully dissolved in oleic acid when the temperature was over 120 °C. And when the reaction was performed over 220 °C, byproduct levels

(such as oxide, dienes and triene from phytosterols) in product would increased.[20, 21] Hence, the esterification was firstly carried out in a range of 120 to 220 °C and the molar ratio of oleic acid to phytosterols of 1.5:1. The effect of temperature on the conversion of phytosterols was shown in Fig. 2. It was found from Fig. 2 that the conversion was significantly enhanced from 19.0% to 98.3% with increasing the temperature from 120 to 220 °C at a reaction time of 10 h. And the conversions increased rapidly with increasing reaction temperature in the period of 0-4 h. Further prolonging reaction time to 10 h, the conversions exhibited a slow rise and almost reached a plateau at 220 °C.

At the same time, the effects of temperature on the yield of phytosterol esters and reaction selectivity were investigated in the range of 180 to 220 °C as shown in Fig. 3. It can be found that the yield and the selectivity increased with the increase in the reaction temperature. For example, the yield of phytosterol esters increased from 58.7% to 89.2%, and the selectivity increased from 89.7% to 93.2% with the increase of the reaction temperature rose from 180 to 220 °C at the initial 4 h. It was suggested that increasing temperature favored the formation of phytosterol esters.[30,31] With prolonging reaction time, the yield was increased, while the selectivity all declined.[32] Herein, the temperature of 220 °C was selected in the following studies.

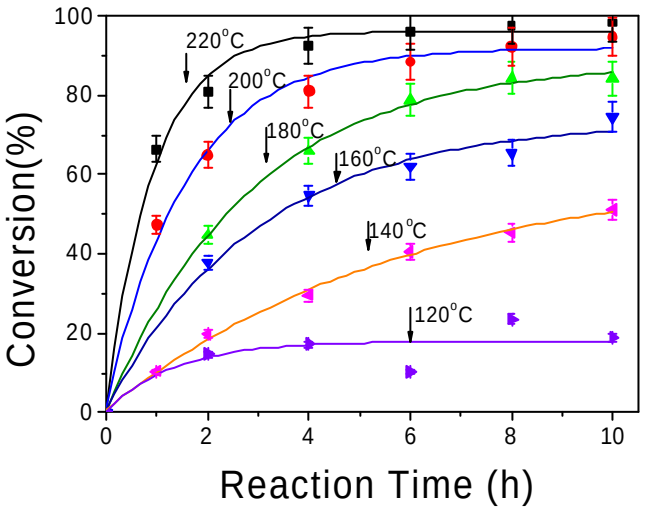


Fig. 2 Effect of temperature on phytosterol conversion at a molar ratio of oleic acid/phytosterols 1.5:1.

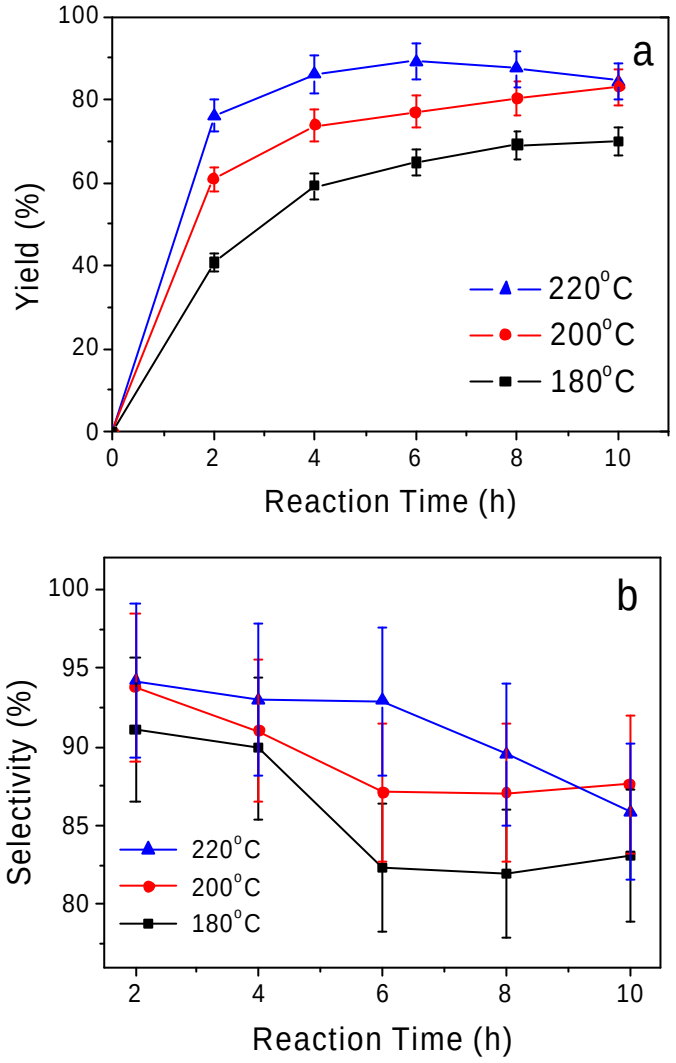


Fig. 3 Effect of temperature on the yield of phytosterol esters (a) and the reaction selectivity (b) at a molar ratio of oleic acid/phytosterols 1.5:1.

Effect of the molar ratio of oleic acid to phytosterols

Molar ratio is one of the important factors in the esterification. Theoretically, one mole of the phytosterol esters needs only 1 mole oleic acid and 1 mole phytosterol in the esterification reaction. In order to drive the reaction towards ester production, the molar ratio of oleic acid to phytosterols is often larger than 1. In addition, the excessive oleic acid functioned as a solvent was beneficial to improving the solubility of phytosterols and in turn promoting mass transfer in the esteri?cation reaction. The relationship between phytosterol conversion and oleic acid/phytosterols molar ratio (1:1, 1.5:1, 2:1 or 3:1) at 220 ℃ was demonstrated in Fig. 4. The conversion increased

considerably with an increase in the ratio. When the ratios of oleic acid to phytosterols were 1:1, 1.5:1, 2:1 and 3:1, the phytosterol conversions obtained at a reaction time 4 h were 73.5%, 92.5%, 97.4% and 99.1%, respectively. Further increasing reaction time, the conversion almost kept constant.

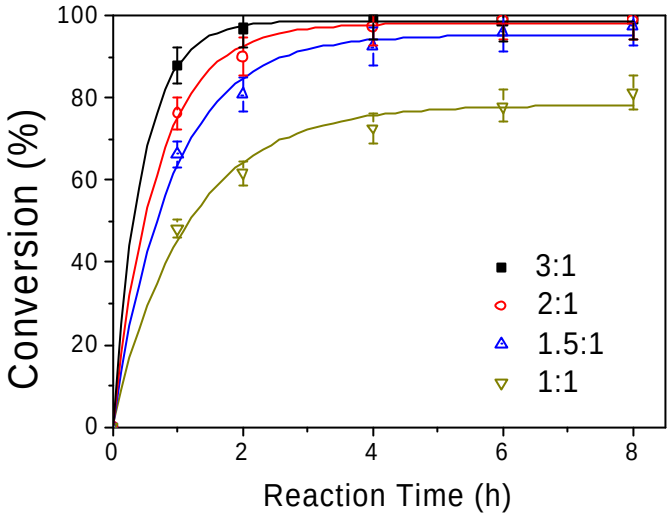


Fig. 4 Effect of the molar ratio of oleic acid/phytosterols on phytosterol conversion at 220 °C.

The yield and the selectivity slightly increased with an increase in the molar ratio at the same reaction time as shown in Fig. 5. When reaction time is 4 h, the yield and the selectivity of the reaction were optimal in different ratios of oleic acid/phytosterols. For example, the yield and the selectivity of the reaction reached to 94.9% and 95.8%, respectively, under the molar ratio of 3:1, reaction temperature 220 °C and reaction time of 4 h. This is because high ratio of oleic acid to phytosterol could drive the reaction to ester production so as to improve reaction rate and equilibrium conversion of phytosterols. Phytosterols were quickly transformed into phytosterol esters, leading to a lower phytosterol concentration in the reaction mixture to impede side reaction occurring. Therefore, excess oleic acid favored the esterification. But excess oleic acid also would trigger some side reactions involving oleic acid (such as oxidation and isomerization of oleic acid [31]) and increase the cost in the downstream isolation process.[21] At the same time, the reaction selectivity was slightly decreased with prolonging reaction time.[32]

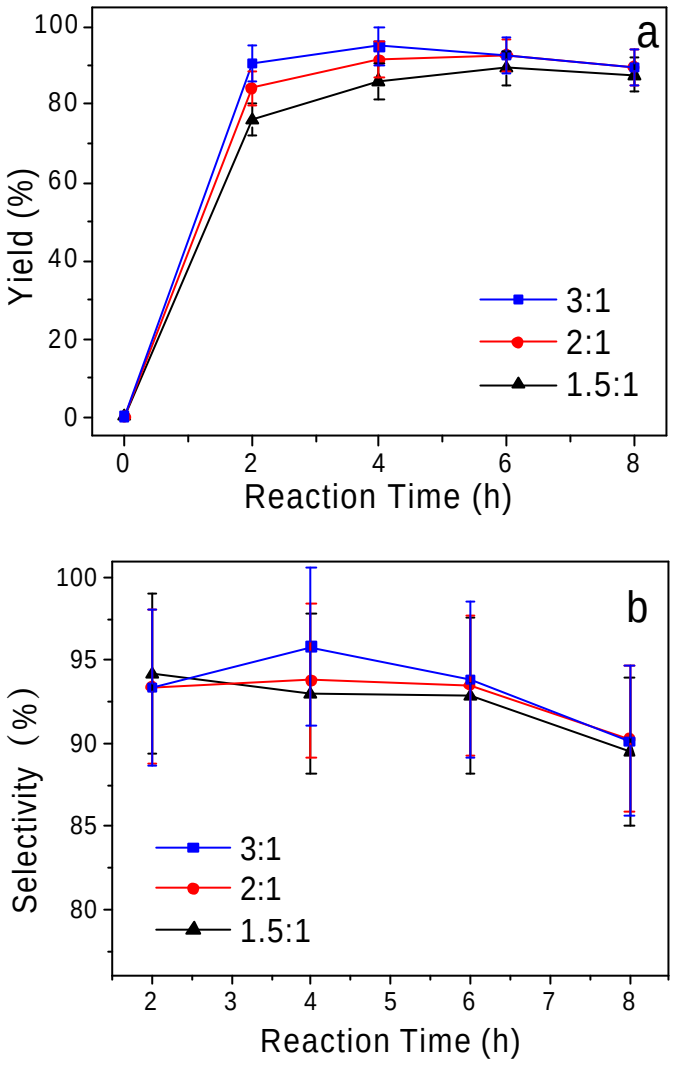


Fig. 5 Effect of oleic acid/phytosterols molar ratio on the yield of phytosterol esters (a) and reaction selectivity (b) at the reaction temperature of 220 ℃.

From the above results, a high conversion (99.1%) with a high yield (94.9%) and selectivity (95.8%) was achieved when the reaction conditions are molar ratio of oleic acid/phytosterols of 3:1, reaction temperature of 220 ℃ and reaction time of 4 h under a vacuum pressure of 1.33 kPa. At the same time, The esterification was also performed under nitrogen as an inert atmosphere. It was found that a conversion of 98.6%, a high yield of 95.1% and a selectivityof 96.4% were achieved under molar ratio of oleic acid/phytosterols of 3:1, reaction temperature of 220 ℃ and reaction time of 4 h, which

is almost identical to the result obtained under the vacuum state.

Effect of the kinds of fatty acids

Four saturated fatty acids (lauric acid, myristic acid, palmitic acid and stearic acid) and unsaturated oleic acid were selected to comparatively investigate the effect of the acids with the different carbon chain lengths (C12 - C18) on the auto-catalytic esterification at molar ratio of fatty acid/phytosterols of 1.5:1 and 220 °C for 4 h as shown in Table 1. It can be found that all the conversion, yield and selectivity of the reactions were almost over 90%, showing high reaction activity for the esterifications of five fatty acids with phytosterols. It was also found that the conversion, yield and selectivity of the reactions were slightly decreased with the increase in the length of carbon chains of the five fatty acids, which is consistent with the results reported in literature.[33] Comparing the reactivity of stearic acid (C18:0) and oleic acid (C18:1), it was found that the same conversions were obtained. But the yield and the selectivity for oleic acid were both obviously lower than those for stearic acid. This is possibly because the double bond in oleic acid would easily broken to form free radicals to trigger side reactions of phytosterols, leading to a lower yield and selectivity.[31] Anyway, the auto-catalytic method was suitable to synthesize phytosterol esters from phytosterols with different long-chain fatty acids (C12 - C18).

Table 1 Effects of carbon chain lengths of fatty acids on esterifications

Entry	[a]Kinds of fatty acids	Conversion /%	Yield /%	Selectivity /%
1	Lauric acid ([b]C12:0)	95.3	94.3	99.0
2	Myristic acid (C14:0)	94.4	93.1	98.6
3	Palmitic acid (C16:0)	93.9	91.3	97.2
4	Stearic acid (C18:0)	92.9	90.1	96.9
5	Oleic acid (C18:1)	92.5	86.2	93.2

a: Other conditions were molar ratio of fatty acid to phytosterols of 1.5:1 and 220 °C for 4 h; b: the first digit is the number of carbon atom in the fatty acid, and the second

digit is the number of carbon-carbon double bond in the fatty acid.

Product analysis

The product of phytosterols esters obtained under molar ratio of oleic acid/phytosterols 3:1, the reaction temperature 220 °C for 4h was used for product analysis. In order to accurately determine the composition change of the reaction, the product mixture (as crude product) for GC measurement was not purified in advance. Table 2 shows the percentage of the compositions of the feedstock and the crude product mixture. It can be seen from Table 2 that the total conversion of four phytosterols was 99.4%. The conversion of individual phytosterol was different. The conversions were 99.5% for ß-sitosterol, 99.5% for stigmasterol, 99.1% for campesterol, and 80.9% for brassicasterol. It means that ß-sitosterol, stigmasterol and campesterol have the almost same reactive activity. They were higher than that of brassicasterol. And the yield of brassicasterol oleic ester was 80.61%, much lower than others. In addition, it was found that some dienes and trienes were formed during the esterification. But the amount of dienes and trienes was less than those reported [20, 21]. It is possibly because no strong acid catalyst existed. But the unknown compositions were obviously increased, possibly resulting from the side products of free oleic acid because free oleic acid is more unstable than oleic acid in esters,[21] most of which could be removed by ethanol washing.

Table 2 The compositions of phytosterols and crude product mixture

Compositions	Phytosterols (%)	Crude product mixture (%)	Conversion (%)	Yield (%)
brassicasterol	1.93	0.12	80.9	
campesterol	25.36	0.07	99.1	
stigmasterol	23.38	0.04	99.5	
ß-sitosterol	42.86	0.07	99.5	
brassicasterol oleic ester	1.39	1.33		80.6
campesterol oleic ester	0.22	13.85		96.0

stigmasterol oleic ester	0.62	11.86	89.2
ß-sitosterol oleic ester	1.65	23.66	96.7
brassicasta-3,5,22-triene		0.84	
campesta-3,5-diene		0.86	
stigmasta-3,5,22-triene		0.67	
ß-Sitoster-3,5-diene		2.20	
oleic acid		39.42 [a]	
unknown matter	2.59	4.93	

[a]The content of oleic acid was calculated by acid-base titration

As phytosterol esters were little dissolved in ethanol; and oleic acid and phytosterols were dissolved in ethanol at above 50 °C, the crude product mixture was purified by ethanol washing for over 3 times. After washing, a small amount of ethanol remained in phytosterol esters could be removed by distillation at 60 °C and 1.33 kPa. The ethanol containing oleic acid and phytosterols could be also reclaimed by vacuum distillation. The properties of the purified product were tested and compared with a commercial product as shown in Fig. 6 and Table 3.

It was found from Fig. 6 that the GC spectra of the resultant product and the commercial product possessed almost the identical characteristic peaks, suggesting the two samples had identical components. The first four peaks (1, 2, 3 and 5) were attributed to brassicasta-3,5,22-triene, campesta-3,5-diene, stigmasta-3,5,22-triene and ß-sitost-3,5-diene, respectively. The second four peaks (4, 6, 7 and 8) were assigned to brassicasterol oleic ester, campesterol oleic ester, stigmasterol oleic ester and ß-sitosterol oleic ester, respectively. The third four peaks (9-12) were ascribed to brassicasterol, campesterol, stigmasterol and ß-sitosterol, respectively. The peak area of phytosterols (9-12) obtained from the auto-catalytic esterification was obviously lower than that from the commercial sample. It indicated that the auto-catalytic product had a lower content of free phytosterol. And the unknown peaks obtained in the auto-catalytic product were also less and weaker than those obtained in the commercial product, far

lower than the content of side products in the phytosterol esters obtained under anion exchange resin NKC-9 as catalyst (see supplementary information).

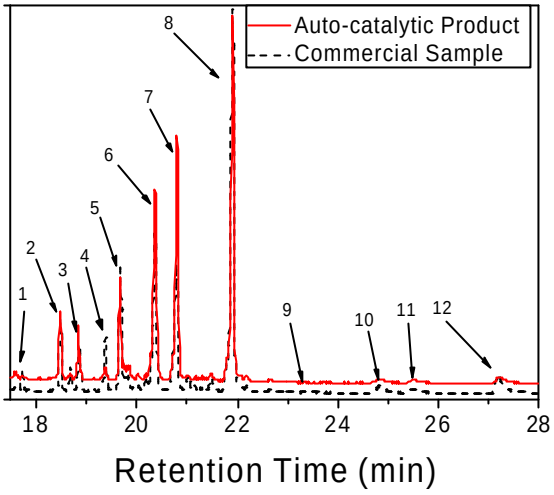


Fig.6 The GCs of auto-catalytic product and commercial sample

1. brassicasta-3,5,22-triene；2. campesta-3,5-diene；3. stigmasta-3,5,22-triene；4. brassicasterol oleic esters；5. stigmasta-3,5-diene；6.campesterol oleic esters；7. stigmasterol oleic esters；8. ß-sitosterol oleic esters；9. brassicasterol；10. campesterol；11. stigmasterol；12. ß-sitosterol.

The main properties of the two phytosterol esters were illustrated in Table 3. In the auto-catalytic product, the content of phytosterols, phytosterol esters and total phytosterols was 0.5%, 93.01% and 59.87%, respectively. And the acid and peroxide values were 0.4 mgKOH/g and 3.87 meq/kg, respectively. Obviously, the main parameters of the auto-catalytic product were superior to those of the commercial product, all of which conform to the standard of China Ministry of Health. Furthermore, no solvent other than ethanol was used in the post-treatment in the auto-catalytic process. And the produced water in the esterification was also fully evaporated at 220 °C in a vacuum state during esterification. So solvent residuals in the product synthesized, such as water, methanol and n-hexane, should meet the product requirements.

Table 3 The main properties of auto-catalytic and commercial products

	auto-catalytic product	Commercial product	[a]Standard Requirements
Appearance	pale yellow, viscous oil paste	pale yellow, viscous oil paste	pale yellow, viscous oil paste
Phytosterol esters (%)	93.01	92.26	=90
Free phytosterols (%)	0.50	1.64	=6
Total phytosterols (%)	59.87	59.52	=59
Acid value (mgKOH/g)	0.40	0.42	=1
Peroxide value (meq/kg)	3.87	4.25	=5

[a]The quality standard from China Ministry of Health published in March 2010.

Kinetics of the reaction

The reaction order was assumed to be 2 in average and ?tted with Eq. (10) at different levels of reaction temperature and molar ratio of oleic acid/phytosterols in order to calculate the reaction rate constant. A linear relationship between $-\ln[(1-X)/(1-0.689X)]$ and t was showed in Fig. 7. As can be seen from Fig. 7 and Table 4, there were all straight lines with correlation coefficients of over 0.98. It is suggested that the proposed kinetic model and reaction order appropriated to this reaction and the assumptions could be correct.

Further, the reaction rate constant k can be obtained from the slope of each straight line. The reaction rate constants k increased with an increase in oleic acid/phytosterols molar ratio and reaction temperature as shown in Table 4.

In addition, the dependence of the reaction rate constants on reaction temperature is described by the Arrhenius equation (Eq. (12) and (13)). The plot of $\ln k$ and $1/T$ was represented by a straight line as shown in Fig. 8. The activation energy of 58.75 KJ/mol was calculated from the slope of the line in a temperature range of 120 to 220 °C,

larger than the activation energy (24.29KJ/mol) in enzyme catalytic synthesis of sterol ester.[35] And the frequency factor *A* was 1.83×10^6.

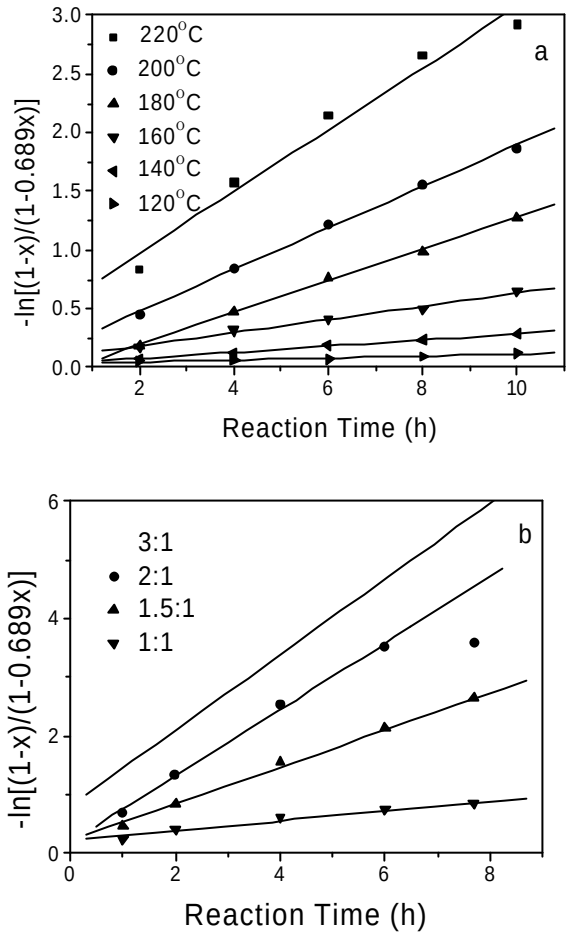


Fig. 7 The plot of -ln[(1-*X*)/(1-0.689*X*)] and *t* obtained at (a) reaction temperature (at a molar ratio of oleic acid/phytosterols of 1.5:1), (b) molar ratio of oleic acid to phytosterols (at a reaction temperature of 220 °C).

Table 4 The reaction rate constants obtained at different reaction conditions.

Reaction conditions	Molar ratio of oleic acid/phytosterols				Reaction temperature (°C)					
	1:1	1.5:1	2:1	3:1	120	140	160	180	200	220
Rate constant *k* ($Lmol^{-1}min^{-1}$)	0.084	0.313	0.566	0.646	0.021	0.084	0.175	0.414	0.569	0.845

R^2	0.985	0.994	0.996	0.984	0.994	0.993	0.992	0.992	0.998	0.987

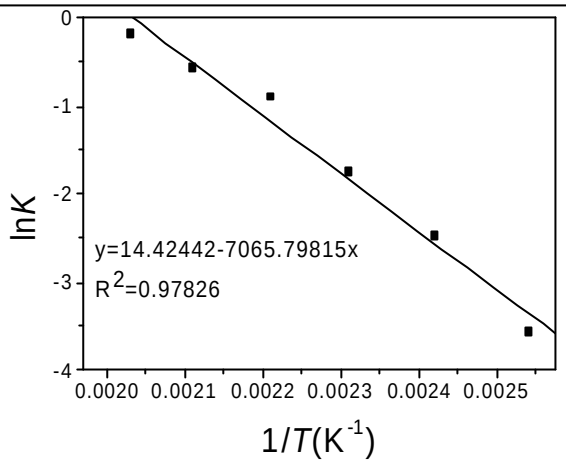


Fig. 8 The plot of ln*k* and 1/*T*

Conclusions

Phytosterol esters have been successfully prepared by auto-catalytic esteri?cation of phytosterols and long-chain fatty acid without adding any catalyst or solvent. A very high conversion (99.1%) with a high yield (94.9%) and selectivity (95.8%) was achieved under molar ratio of oleic acid/phytosterols of 3:1, reaction temperature of 220 °C and reaction time of 4 h. The conversion, the yield and the selectivity of the reaction were increased with the increase in reaction temperature, molar ratio of oleic acid/phytosterols and the decrease in the length of carbon chains of fatty acids. The selectivity was decreased with prolonging reaction time. A higher yield and selectivity would be obtained in the reaction of phytosterols with saturated stearic acid compared with unsaturated oleic acid. The properties of the phytosterol oleic esters by autocatalysis conformed to the quality indices of phytosterol esters from China Ministry of Health and were superior to the commercial product. The reaction order calculated was 2 and the reaction activation energy was 58.75 KJ/mol. The auto-catalytic process, overcoming the shortcomings of existing processes involving homogeneous and heterogeneous catalysts, could be considered as a promising process to synthesize phytosterol esters.

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References

1 X. Zhang, A. Cambrai, M. Miesch, S. Roussi, F. Raul and D. Aoude-Werner, J. Agric. *Food Chem*. 2006, 54, 1196- 1202.

2 A. O Cherif, H. Trabelsi M. B. Messaouda, B. Kâabi, I. Pellerin, S. Boukhchina, H. Kallel and C. Pepe, *J. Agric. Food Chem.*, 2010, 58, 8709- 8714.

3 *S. S Abumweis, R. Barake and P. J H Jones, J.Food Nutr.Res.,2008,5*2, 1- 17.

4 T. Bohn, Q. G. Tian, and J. Waksman, *J. Agric. Food Chem.*, 2007, 55, 267- 272.

5 L. Normén, L. Ellegård and H. Janssen, *Eur. J. Nutr., 00*6, 45, 165–170.

6 W. Panpipat, X. B. Xu and Z. Guo, *Biochem.Eng.J.*, 2013,70, 55– 62.

7 C. F. Torres, G. Torrelo, and J. Senorans, *Process Biochem.*, 2007, 42, 1335–1341.

8 A. W. Brown, J. L. Hang and P. H. Dussault, *Lipids*, 2010, 45, 855–862.

9 *A. Jozwiak, M. Ples , K. Skorupinska-Tudek , M. Kania , M. Dydak , W. Danikiewicz and E Swiezewska., Biochimica et Biophysica Acta*, 2013, 1831, 438–447.

10 M. M. Zheng, L. Dong and F. H. Huang, *J. Mol. Catal. B-Enzym.*, 2012, 74, 16–23.

11 Z. Tan and F. Shahidi, *J. Agric. Food Chem.*, 2011, 59, 12375–12383.

12 P. Villeneuve, F. Turon and Y. Caro, *Enzyme Microb. Tech.*, 2005, 37, 150–155.

13 W. He, C. S. Jia and B. Feng, *J. Mol. Catal. B-Enzym.*, 2010, 67, 60–65.

14 X. X. Pan, J. Wang and T. W. Tan, *Appl. Biochem. Biotechnol.*, 2012, 168, 68–77.

15 M. M. Zheng, L. Wang and F. H. Huang, *Ultrason. Sonochem.*, 2012, 19, 1015–1020.

16 Y. Yong, Y. X. Bai and Y. F. Li, *Process Biochem.*, 2008, 43, 1179-1185.

17 *U.S. Pat.*, 6441206, 2002.

18 *U.S. Pat.*, 6231915, 2001.

19 S. Valange, A. Beauchaud and J. Barrault , *J. Catal.*, 2007, 251, 113–122.

20 Y. Pouilloux, G. Courtois, M. Boisseau, *Green Chem.*, 2003, 5, 89-91.

21 X. Meng, Q. Pan and T. Yang, *J. Am. Oil Chem. Soc.*, 2011, 88, 143–149.

22 S. R. Manuel, J. Barrault and S. Valange, *C. R. Chimie*, 2011, 14, 656–662.

23 A. Conchillo, I. Astiasaraán and D. Ansorena, *J. Agric. Food Chem.*, 2005, 53,

7844- 7850.

24 P. Fernandes and J.M.S. Cabral, *Bioresource Technol.*, 2007, 98, 2335–2350.

25 M. T. Sanz, R. Murga, S. Beltran and J. L. Cabezas, *Ind. Eng. Chem. Res*., 2002, 41, 512-517.

26 H. J. Yang, F. Yan and J. X. Li, *Bioresource Technol.*, 2010, 101, 1471–1476.

27 S.L. Abidi, *J. Chromatogr. A*, 2001,935, 173-201.

28 M.B. Chen, Master's thesis, *Hua Zhong Agricultural University*, 2005.

29 W. Y. Shi, B. Q. He, J. X. Li, *Bioresource Technol.*, 2011,102, 5389–5393.

30 C.M. Le?n, S.M. Alvarez and C.E. Graciani, *Grasasy Aceites*, 2004, 55, 227–232.

31 C.M. Le?n, M. Ruiz and C.E. Graciani, *Eur. J. Lipid. Sci. Technol.*, 2001, 103, 85–92.

32 R. Cantrill and Y. Kawamura, the 69th JECFA, 2008.

33 Y. Yang, W. He, and B. Feng, *J.Mol. Catal. A-Chem*., 2012, 357, 39– 43.

34 N. Morinaga, A. Maeda, T. Mizuno, M. Bunya, S. Sugihara, A. *Enzyme Microb. Tech.*, 2011, 48 , 498–504.

35 A. Sengupta and M. Ghosh, *Eur. J. Lipid Sci. Technol.*, 2011, 113, 763–767.

Table Of Content

A safe, practicable and environmental friendly method was developed to synthesize phytosterol esters from phytosterols and long-chain fatty acids without adding any catalyst or solvent.