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Enzymatic Hydrolysis Preparation of Mono-*O*-lauroylsucrose via a Mono-*O*-lauroylraffinose Intermediate

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ABSTRACT: 1′-*O*-Lauroylsucrose and 6′-*O*-lauroylsucrose were formed through hydrolysis of the C-6″ galactose group of 1′-*O*-lauroylraffinose and 6′-*O*-lauroylraffinose, respectively, in the presence of α-galactosidase. The enzymatic hydrolysis of 1′-*O*-lauroylraffinose and 6′-*O*-lauroylraffinose is discussed in detail. Acetic acid−sodium acetate was chosen as the buffer solution of the enzymatic hydrolysis reaction. The optimum conditions for the enzymatic hydrolysis reaction were as follows: buffer solution, pH 3.8; enzymatic time, 48 h; and enzymatic temperature, 37 °C. Under the optimal process conditions, the efficiency of α-galactosidase was ca. 82.6%. The isomers were fully compared in solubility, hydrophile−lipophile balance (HLB) values, critical micelle concentration (CMC), and thermal stability. The results showed that all lauroylsucrose isomers have similar solubilities in polar solvent, HLB values, CMC values, and thermal stabilities.

KEYWORDS: *raffinose lauric acid monoesters, α-galactosidases, enzymatic hydrolysis, mono-O-lauroylsucrose*

INTRODUCTION

Sugar-based surfactants are an important category of nonionic amphiphiles constituted of carbohydrates as polar head groups conjugated with long-chain fatty acids or hydrophobic molecules. These compounds have broad applications in the cosmetic, food, and pharmaceutical industries.[1] They also have been applied as detergents in membrane protein extraction, purification, and crystallization.[2−4] Moreover, due to their biodegradability and nontoxicity, sugar esters are now considered as perfect raw materials for food emulsifiers and personal care formulations. In addition, monoesters have better solubility in water than higher substituted derivatives.[5] Sucrose has three active primary alcoholic hydroxyls and five secondary alcoholic hydroxyls. The reaction activity of free hydroxyl groups follows the order OH-6 > OH-6′ > OH-1′ > secondary-OH[6] (Figure 1). A high number of isomers are easily obtained in the chemical synthesis of sucrose esters, which limits the application of products. In contrast, Wang[6] and Riva[7,8] reported that an enzyme catalysis method has good selectivity in the presence of dimethyl sulfoxide (DMSO), dimethylformamide (DMF), or pyridine as solvent or in the absence of any solvent. In a previous study, long-chain fatty acids were selectively linked to primary alcoholic hydroxyls OH-6 and OH-6′ in enzyme-catalyzed reaction.[9−12]

α-D-Galactose-galactohydrolase (EC 3.2.1.22), which is abundant in plants, microorganisms, and animals,[13] is commonly referred to as α-galactosidase, normally catalyzing the hydrolyses of α-linked galactose residues from different substrates such as the raffinose family oligosaccharides, guar gum, locust bean gum, glycoconjugates, glycoproteins, and glycosphingolipids.[14−16] Ceramide trihexosides with higher homologues and derivatives are also cleaved by the action of α-galactosidase.[17,18] These properties make the enzyme very useful in many industrial applications, such as decreasing pollution in the paper industries[19] and increasing nutrition values in the products of soybean and other legumes (as feed products and food).[20,21] To our knowledge, the α-galactosidase hydrolysis of raffinose fatty acid monoesters under acetic acid−sodium acetate condition is rarely reported.

In this paper, raffinose and fatty acid methyl ester (methyl laurate, methyl octanoate) were chosen as acyl acceptors and acyl donors, respectively. 6′-Sucrose and 1′-sucrose fatty acid monoester were obtained by α-galactosidase hydrolyzing raffinose fatty acid ester intermediates synthesized by chemical catalysis.[5] Sucrose fatty acid monoesters were confirmed by infrared spectroscopy (IR), electrospray ionization mass spectrometry (ESI-MS), and nuclear magnetic resonance (NMR). In addition, solubility, HLB values, CMC values, and the thermal stability of monoesters obtained by chemical−enzymatic method and chemical method[22] were investigated.

MATERIALS AND METHODS

Chemicals and Reagents. Raffinose was purchased from Shaanxi Sciphar High-Tech Industry Co. Ltd. (Shaanxi, China). Methyl octanoate was purchased from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). Methyl laurate was purchased from Shanghai crystal pure industrial Co. Ltd. (Shanghai, China). Enzyme A, α-galactosidase (3000 u/g), was purchased from Hubei YuanCheng Pharmaceutical Co. Ltd. Enzyme B, α-galactosidase (2500 u/g), was purchased from Hubei KangBaoTai Fine Chemical Co. Ltd. CD_3OD was purchased from Cambridge Isotope Laboratories, Inc. Other reaction media and chemicals were purchased from local suppliers. All other reagents and chemicals were of analytical or high-performance liquid chromatography (HPLC) grade.

Synthesis of Raffinose Lauric Acid Monoester.[5] Raffinose and anhydrous K_2CO_3 were added in a dry three-branch round-bottom flask equipped with a stopper filled with DMSO. After raffinose was completely dissolved in DMSO, methyl laurate was added to the mixture with reduced pressure reaction at 55−75 °C under ultrasonic

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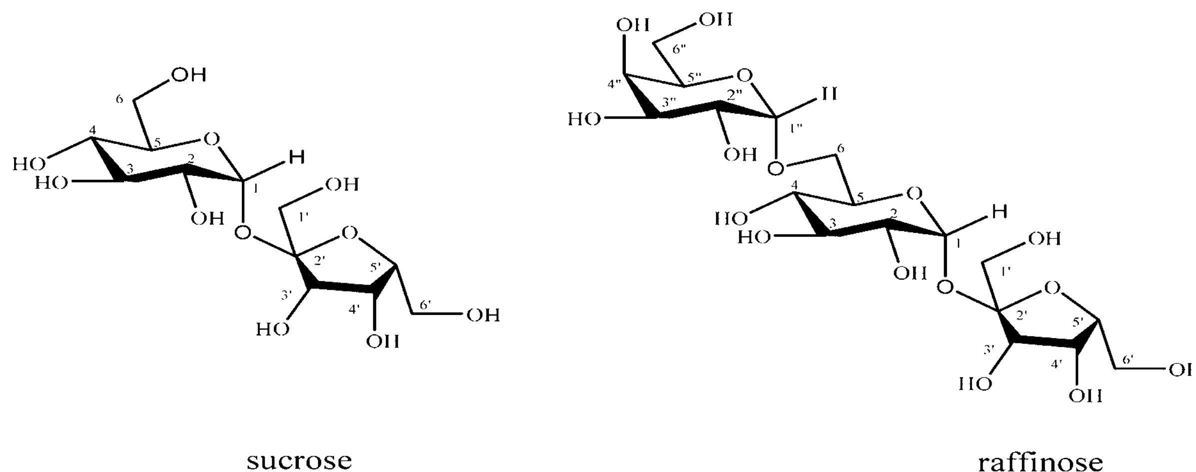

Figure 1. Carbon-bit numbers of sucrose and raffinose.

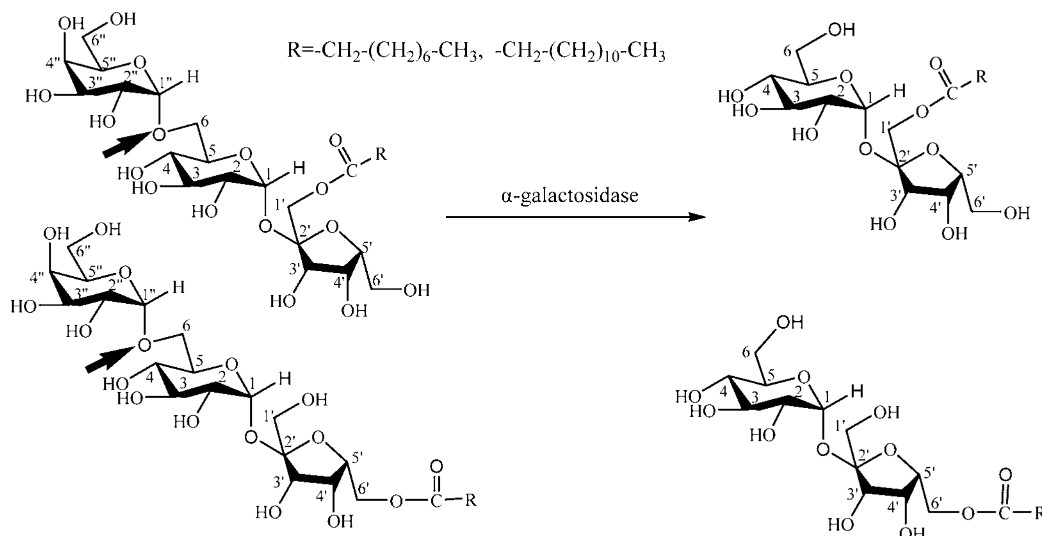

Figure 2. Enzymatic hydrolysis of raffinose derivatives.

irradiation (SB25-12DTDN, ultrasonic cleaner operating system at a frequency of 40 kHz, Scientz Biotechnology Stock Co. Ltd., Ningbo, China). After the preset time, the reaction was stopped and the solution was transferred to a round-bottom flask. DMSO was evaporated to obtain solid fractions of raffinose monoesters and diesters. The residues were completely dissolved in 1-butanol/20% aqueous sodium chloride solution, and the mixture was separated into two phases (water phase was discarded). The extraction process was repeated twice. Then, the organic phase was washed twice with 10% aqueous NaCl to further eliminate the nonreacted raffinose. After that, the organic phase was dried with anhydrous Mg_2SO_4. The filtered solution was evaporated off and raffinose esters were obtained. Monoesters were isolated by silica gel column chromatography.

Enzymatic Hydrolysis of Raffinose Lauric Acid Monoesters. Raffinose lauric acid monoester (0.15 g, 0.22 mmol) was added in a dry 50 mL one-neck round-bottom flask equipped with a stopper filled with 20 mL of buffer solution. After the raffinose lauric acid monoester was completely dissolved in the buffer solution, α-galactosidase (0.3 g, 15 mg/mL) was added to the mixture at 27−47 °C. By setting the electric sets, the desired reaction temperature was controlled and maintained. After the preset time, reaction was stopped and the solution was transferred to a 50 mL centrifuge tube and centrifuged for 15 min at 3500 rpm (KDC-1044, low-speed centrifuge, University of Science and Technology of China Chuangxin Co. Ltd. Zonkia Branch, Hefei, China). The supernatant was filtered through a 0.45 μm microporous membrane, and the buffer was removed by rotary evaporation. Enzymatic hydrolysis of raffinose derivatives is shown in Figure 2.

Product Purification and Isolation. The rotary evaporation residues were completely dissolved in a small amount of chloroform/methanol 1:1 (v/v). A gel column chromatograph (120 cm × 15 mm inner diameter, Yintebeier Experiment Instrument Plant, Guangzhou, China) filled with Sephadex LH-20 (GE Healthcare, Shanghai, China) was used with a solvent mixture of chloroform and methanol (1:1, v/v); thin-layer chromatography (TLC) was performed on silica gel GF_{254} 60A plates (purchased from Merck, Darmstadt, Germany) with a mixture of chloroform, methanol, and glacial acetic acid (75:25:4, v/v/v) as eluents, and spots were detected by dipping the plates into the 10% phosphomolybdic acid−ethanol solution (m/v), drying, and heating at 110 °C for 10 min. The same category collection of fluids was mixed, and the solvent was removed by evaporation. The isolated mono-*O*-lauroylsucrose crude product was extracted 20 times with hexane, and then the residues were dissolved in a small amount of chloroform, methanol, and glacial acetic acid (75:25:4, v/v/v); sole monoester was isolated by TLC and silica gel column chromatography (50 cm × 10 mm inner diameter, Yintebeier Experiment Instrument Plant).

Structural Identification of the Product. IR was recorded on an EQUINOX-55 spectrometer using KBr pellets, and absorption was reported in wavenumbers (cm^{-1}). ESI-MS analyses were performed at 4000Q Trap (AB SCIEX Co.). The chemical structures of the acylated derivatives were determined by ^{1}H NMR (500 MHz) and ^{13}C NMR

(126 MHz) in MeOD using a Bruker AC500 spectrometer (Bruker, Courtaboeuf, France). Chemical shift values and coupling constants are given in δ and hertz, respectively.

HPLC Analytical Process. Ultrapure water, purified using the Milli-Q system (Millipore, Bedford, MA), was used for analytical HPLC analysis. Analyses were conducted with an LC-20A system (Shimadzu, Japan), which was performed using a Diamonsil column (4.6 mm × 250 mm, Dikma Technologies, China) packed with 5 μm C18. The enzymatic samples were eluted using a mobile phase of methanol/water (80:20, v/v) at a flow rate of 1.0 mL/min. Twenty microliters of sample (20 mg/mL) was injected onto the column (40 °C), and enzymatic samples were detected with a refractive index detector (RID) (Shimadzu). The hydrolysis effect could be obtained quickly by using the following formula:

$$\text{hydrolysis effect} = \frac{\text{initial raffinose monoester} - \text{residual raffinose monoester}}{\text{initial raffinose monoester}} \times 100\%$$

Enzyme Reusability. At the end of each reaction batch, the immobilized α-galactosidase was recycled and washed with acetic acid−sodium acetate to remove any substrates or products. Then, the α-galactosidase was consecutively reused in each reaction cycle.

HLB Value. HLB is the balance of the size and strength of the hydrophilic and lipophilic moieties of a surfactant molecule.[23] HLB values could be obtained quickly by using the following formula:

$$\text{HLB} = 20 \times \frac{\text{hydrophilic group molecular wt}}{\text{total surfactant molecular wt}}$$

CMC and γ_{CMC} Evaluation. Surface tensions of the sugar esters have been measured according to the ring method using a JYW-200B automatic interfacial tension meter (Chengde, China). A platinum ring (platinum circle radius = 9.55 mm, platinum wire radius = 0.3 mm) is vertically hanged on the device of the tension meter. Homogenous aqueous surfactant solutions (50 mL) were contained in a large Petri dish. Before measuring, the ring and Petri dish were soaked for 12 h with 10% sodium carbonate solutions and then washed with ultrapure water to neutral. The inside temperature of the tension meter was controlled at 28 °C (±0.5 °C). Measurements were repeated three times, and the average values were considered. γ_{CMC} is the surface tension corresponding to the CMC.

Thermal Stability. Thermal stability assays were performed by weighing about 10 mg of mono-*O*-lauroylsucrose placed on the Thermo Gravimetric Analyzer (TGA, TG209F3-ASC, Germany). The temperature ranged from 30 to 400 °C, and the heating rate was 5 °C/min. The samples were protected by nitrogen (N_2) in the Al_2O_3 crucible.

■ RESULTS AND DISCUSSION

Parameters Choice of Enzymatic Hydrolysis. *Choice of α-Galactosidase and Buffer.* α-Galactosidase from plant, bacterial, and fungal sources had been used for hydrolysis of galacto-oligosaccharides of the raffinose family, respectively.[13] Studies[24,25] showed that the enzymes produced by fungal sources, such as *A. oryzae*, *Cl. cladosporioides*, *A. saitoi*, *M. vinacea*, and *A. awamori*, had different optimum hydrolysis conditions, which indicates that α-galactosidases from different organisms might have different activities. It can be seen from Figure 3 that α-galactosidase A had a better hydrolysis effect than α-galactosidase B, especially in acetic acid−sodium acetate buffer. The former's hydrolysis effect was approximately 20% higher than the latter's. As a result, α-galactosidase A was chosen as the required enzyme.

A suitable buffer is essential for the hydrolysis of raffinose lauric acid monoesters by α-galactosidase, and it should dissolve sufficient amounts of raffinose lauric acid monoester. In addition, the buffer should not adversely affect the stability and activity of α-galactosidase. Buffer selection is known to affect the enantioselectivity and specificity of the enzyme-catalyzed reactions.[26−29] The influence of different buffers on the immobilized α-galactosidase hydrolysis reaction was confirmed by the following: 95% ethanol, 1-butanol, acetic acid−sodium acetate (pH 4.4), and phosphate buffer solution (pH 4.4). The results of the hydrolysis effect of α-galactosidase indicated that all of the investigated buffers supported the enzyme hydrolysis (Figure 3). When acetic acid−sodium acetate was chosen as the hydrolysis reaction buffer, α-galactosidase A had a maximum hydrolysis effect of ca. 52.2%, which was almost 20% higher than that in phosphate buffer solution, nearly 30% higher than that in 1-butanol, and nearly 40% higher than that in 95% ethanol. As for α-galactosidase B, it also had the highest hydrolysis effect of ca. 30.3% in acetic acid−sodium acetate buffer, perhaps because acetic acid−sodium acetate has good dissolving ability to raffinose monoester and does not adversely affect the stability and activity of the enzyme. Therefore, acetic acid−sodium acetate was chosen as the required hydrolysis reaction buffer.

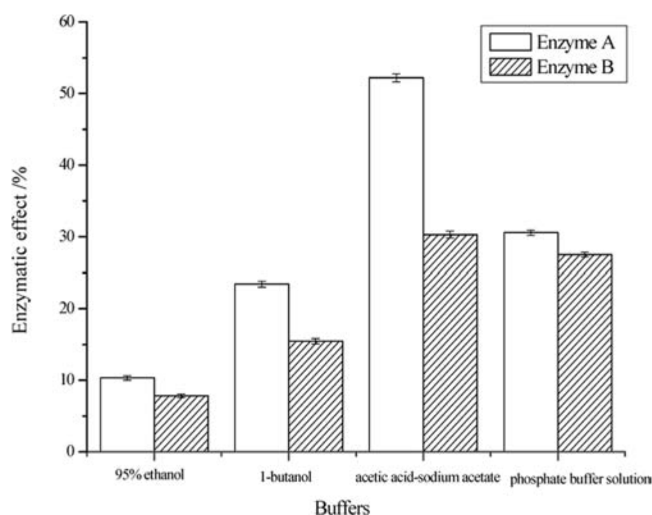

Figure 3. Effects of α-galactosidase and buffer on enzymatic hydrolysis of 1′-*O*-lauroylsucrose.

Effect of Buffer pH, Hydrolysis Time, and Temperature. α-Galactosidases have some common features such as acidic pH optima and long storage stability.[30−32] In recent years, many α-galactosidases have been of interest because of their potential applications in various degradation systems. Moreover, the enzyme is stable in a certain range of pH values. It may be lyophilized and stored for more than two years without a decrease of activity.[33]

The influence of buffer pH on immobilized α-galactosidase hydrolysis activity was determined at a pH range of 2.6−5.0 at 37 °C for 48 h using raffinose lauric acid monoester as substrate with respective controls (Figure 4a). It could be found that there was a significant change in the hydrolysis effect with different buffer pH values. The hydrolysis effect increased from ca. 28.4% to ca. 82.6% of 1′-*O*-lauroylraffinose and from ca. 27.9% to ca. 82.2% of 6′-*O*-lauroylraffinose with the rise in pH from 2.6 to 3.8. A pH exceeding 3.8 may destroy the enzyme's structure and therefore decrease hydrolysis effect to nearly 40% of the substrate. This might be due to the fact that every enzyme has its own optimal pH value; above or below that value, all affect the enzymatic effect.

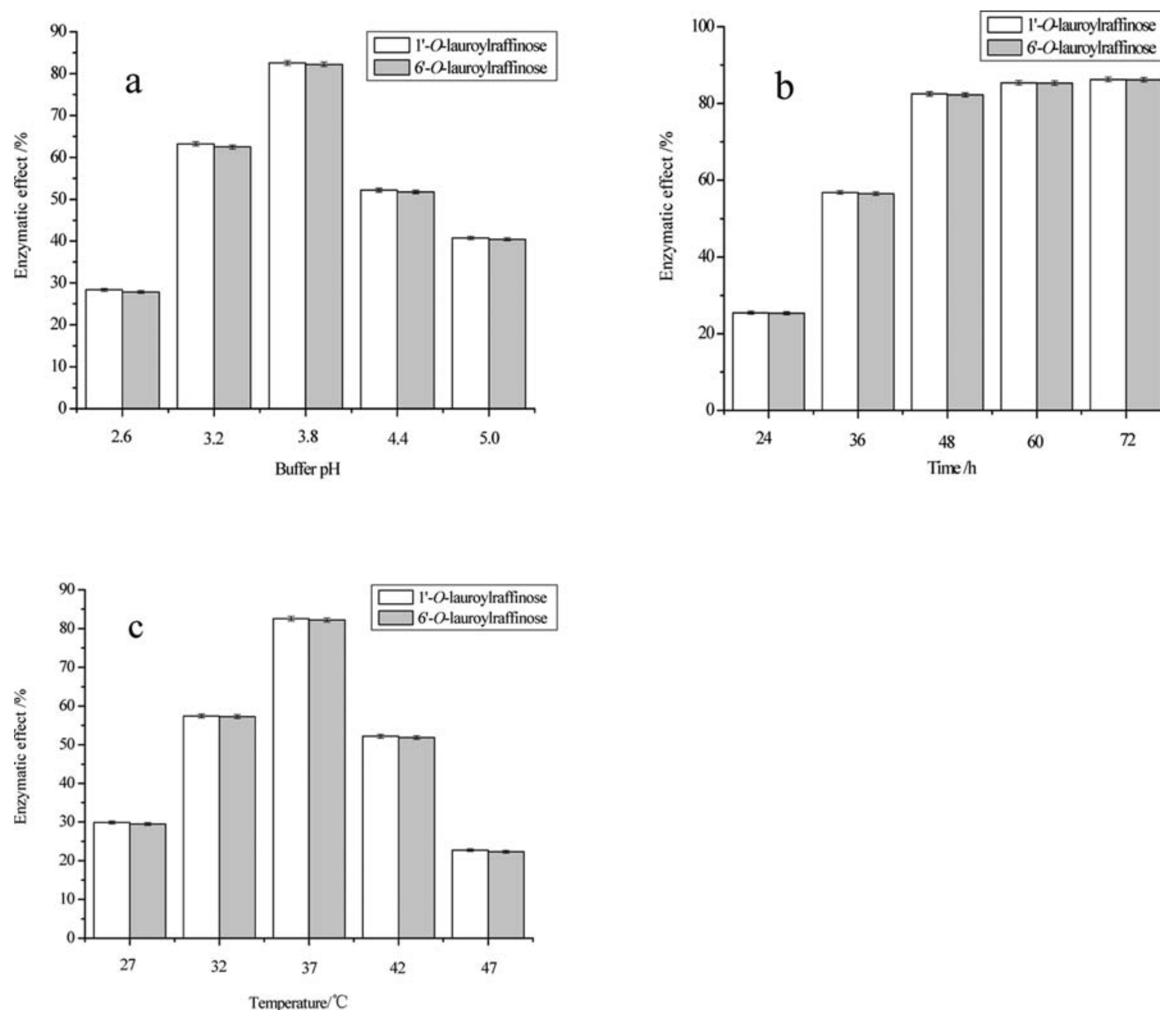

Figure 4. Effects of buffer pH, hydrolysis time, and temperature on enzymatic hydrolysis.

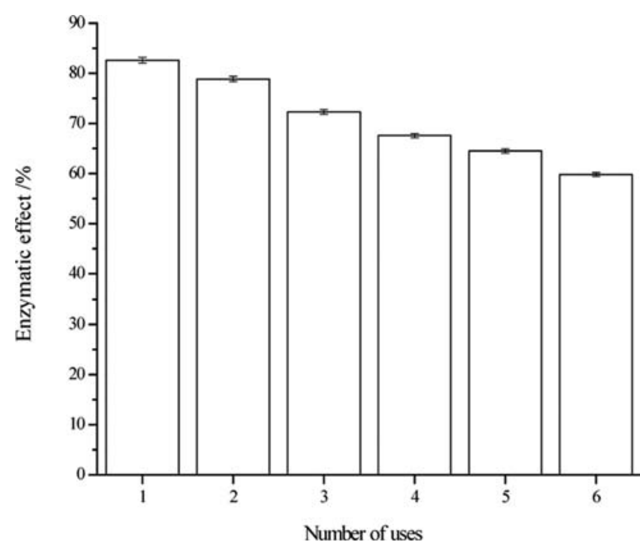

Figure 5. Effect of reusability of α-galactosidase on enzymatic hydrolysis.

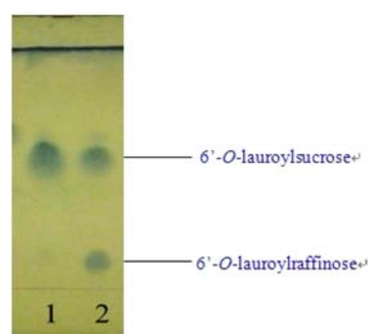

Figure 6. TLC of the hydrolysis of 6′-O-lauroylraffinose and isolated 6′-O-lauroylsucrose.

The influence of hydrolysis time on immobilized α-galactosidase hydrolysis effect was determined in 20 mL of pH 3.8 acetic acid–sodium acetate buffer at a time range of 24–72 h using raffinose lauric acid monoester as substrate with respective controls. The results are shown in Figure 4b. They indicated that as time was prolonged to 48 h, the hydrolysis effect increased accordingly, and the enzymatic effect could reach ca. 82.6% of 1′-O-lauroylraffinose and ca. 82.2% of 6′-O-lauroylraffinose. However, when the time exceeded 48 h, the hydrolysis effect of both remained almost unchanged at approximately 83%. This might be due to the fact that α-galactosidase reached maximum performance with little substrate.

The effect of temperature was studied by adding the immobilized α-galactosidase in 20 mL of pH 3.8 acetic acid–

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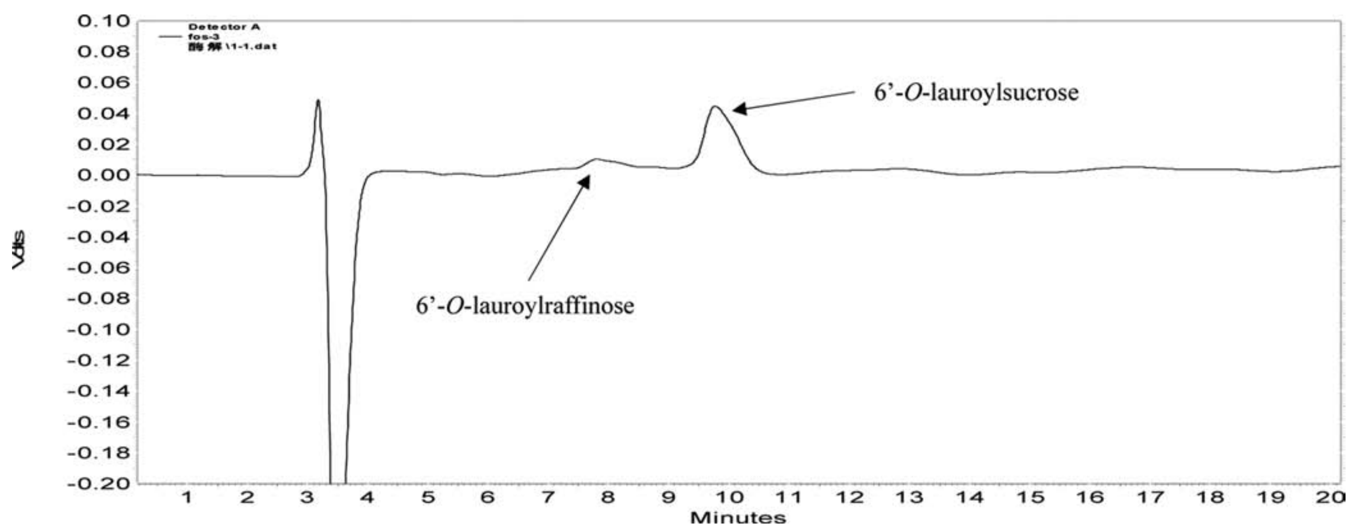

Figure 7. HPLC of the hydrolysis of 6′-*O*-lauroylraffinose.

Table 1. Solubility of Sucrose Monoesters[a]

solvent	H_2O	methanol	ether	chloroform	petroleum ether	*n*-butanol	benzene	ethyl acetate
6′-*O*-lauroylsucrose	±	+	−	±	±	+	−	+
1′-*O*-lauroylsucrose	±	+	−	±	±	+	−	+
6-*O*-lauroylsucrose	±	+	−	±	±	+	−	+

[a]±, partial solution; +, complete solution; −, insoluble.

sodium acetate buffer at various temperatures (ranging from 27 to 47 °C) with respective controls for 48 h using raffinose lauric acid monoester as substrate (Figure 4c). The hydrolysis effect, the solubility of the raffinose lauric acid monoester, and the stability as well as activity of the α-galactosidase are strongly related to the reaction temperature. As shown in Figure 4c, the hydrolysis effect increased from ca. 29.9% to 82.6% of 1′-*O*-lauroylraffinose and from ca. 29.5% to ca. 82.2% of 6′-*O*-lauroylraffinose with the rise in temperature from 27 to 37 °C. However, an obvious decrease in hydrolysis effect was observed when the temperature further increased above 37 °C because a temperature exceeding 37 °C would reduce the enzyme activity and therefore the hydrolysis effect of the substrate (about 22%). This is consistent with Katrolia's[34] and Cao's[35,36] studies. Besides, the hydrolysis effect obtained in this study was similar to those found by Kapnoor et al.,[18] who reported that under the optimum conditions, α-galactosidase, produced by *A. oryzae* on red gram plant waste−wheat bran, completely removed the raffinose oligosaccharides in soy milk.

Enzyme Reusability. Figure 5 shows the effect of repeatedly used α-galactosidase. It was observed that the immobilized α-galactosidase retained relatively high hydrolysis activity even after six uses. In more detail, in the first, second, third, fourth, fifth, and sixth cycles, the percent of hydrolysis was found to be ca. 82.6%, 78.8%, 72.3%, 67.5%, 64.5%, and 59.8%, respectively. This is consistent with Shankar's[17] study, who reported the repeated use of entrapped and cross-linked Con A−α-galactosidase entrapped in a calcium alginate complex. After eight cycles, the hydrolytic activity of both cross-linked Con A−α-galactosidase entrapped complex and soluble entrapped α-galactosidase declined, retaining 95% and 51%, respectively.

Enzymatic Hydrolysis Product Characterization by IR and ESI-MS Analyses. 6-*O*-Lauroylsucrose and 6′-*O*-lauroylsucrose synthesized by chemical method[22] and acylated raffinose derivatives' spectral data for octanoyl and lauroyl monoesters were presented previously.[5] IR spectrum and mass spectrometric data for the enzymatic hydrolysis preparation of mono-*O*-lauroylsucrose are shown below.

IR spectrum and mass spectrometric data for the monoesters of isomers (1′ and 6′):

Mono-O-lauroylsucrose: IR (cm^{-1}) 3347.46 (strong peak, O—H stretch of free hydroxyl in sucrose); 2921.75, 2855.30 (C—H stretch of methyl and methylene); 1717.88 (C═O stretch of ester); 1263.71 (C—O stretch of ester); 1051.40, 1112.67 (C—O stretch of C—O—C); 929.51 (glycosidic bond stretch of sucrose). ESI-MS (ES^-) *m/z* requires 559.5 $[M + Cl]^-$, found 559.5.

Mono-O-octanoylsucrose: IR (cm^{-1}) 3375.48 (strong peak, O—H stretch of free hydroxyl in sucrose); 2923.76, 2856.53 (C—H stretch of methyl and methylene); 1724.77 (C═O stretch of ester); 1261.89 (C—O stretch of ester); 1062.32, 1124.35 (C—O stretch of C—O—C); 934.65 (glycosidic bond stretch of sucrose). ESI-MS (ES^-) *m/z* requires 503.6 $[M + Cl]^-$, found 503.6.

Enzymatic Hydrolysis Product Characterization by ^1H and ^{13}C NMR Analyses. The carbon-bit number of sucrose and raffinose and the structure of sucrose monoester and raffinose monoester are given in Figures 1 and 2, respectively. Chemical shifts were referred to MeOD, centered at 3.33 ppm for ^1H NMR and at 47.61 ppm for ^{13}C NMR. Assignments of all chemical shifts are listed below.

1′-O-Octanoylsucrose: ^{13}C NMR (126 MHz, MeOD) δ 173.39 (C═O), 102.66 (C-2′), 92.71 (C-1), 82.41 (C-5′), 77.35 (C-3′), 73.54 (C-4′), 73.18 (C-5), 73.01 (C-3), 71.63 (C-2), 69.98 (C-4), 62.39 (C-1′), 61.83 (C-6′), 60.80 (C-6), 33.58 ($-CH_2-CO-$), 31.46, 30.23, 28.95, 28.59, 24.64, 22.27 ($-CH_2$-octanoyl backbone), 13.00 (CH_3-octanoyl).

1′-O-Lauroylsucrose: ^{13}C NMR (126 MHz, MeOD) δ 173.38 (C═O), 102.65 (C-2′), 92.71 (C-1), 82.41 (C-5′), 77.35 (C-3′), 73.52 (C-4′), 73.18 (C-5), 73.02 (C-3), 71.63 (C-

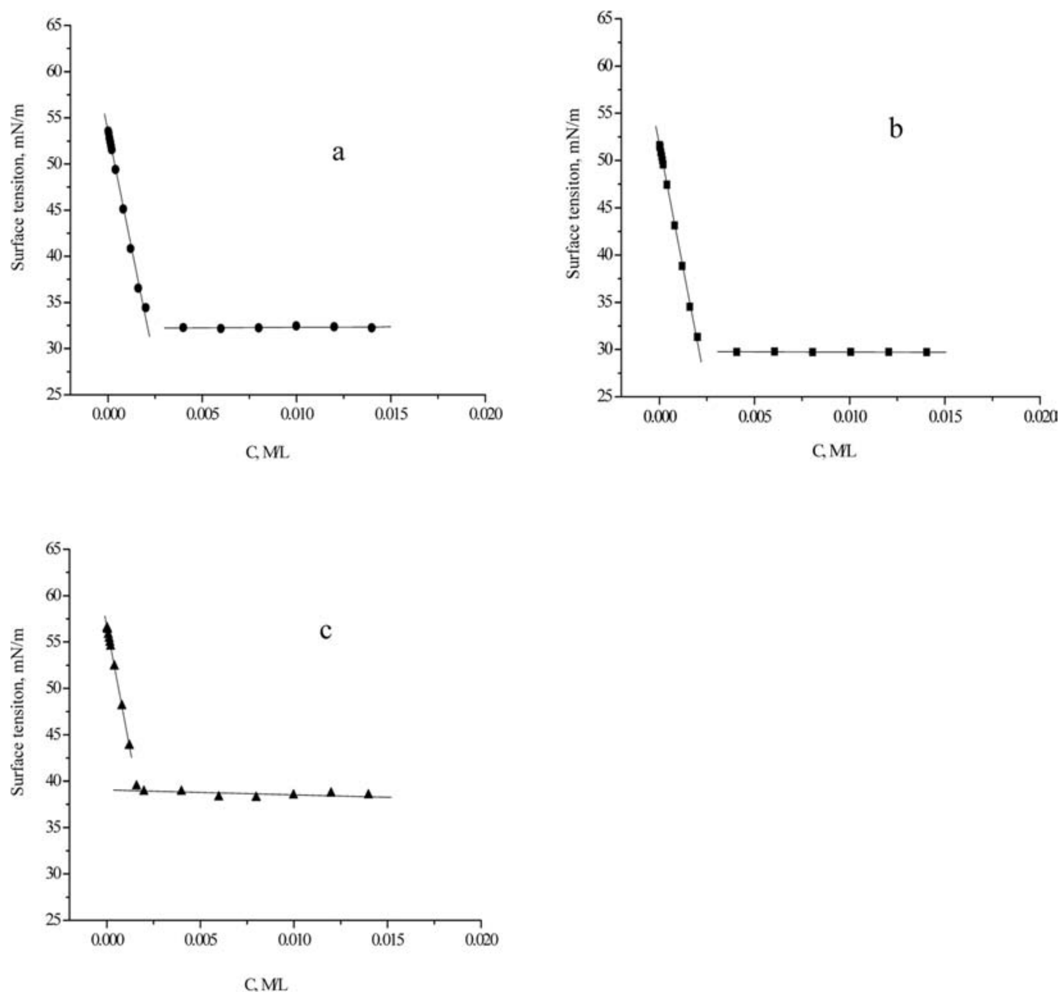

Figure 8. CMC of sucrose monoesters: (a) 6′-*O*-lauroylsucrose; (b) 1′-*O*-lauroylsucrose; (c) 6-*O*-lauroylsucrose.

Table 2. Calculated HLB, R_f Value, CMC, and γ_{CMC} of Monoesters

compound	chain length	HLB	R_f value	CMC(M/L)	γ_{CMC} (mN/m)
6′-*O*-lauroylsucrose	C12	13.02	0.52	2.0×10^{-3}	32.26
1′-*O*-lauroylsucrose	C12	13.02	0.58	2.5×10^{-3}	29.86
6-*O*-lauroylsucrose	C12	13.02	0.67	1.7×10^{-3}	38.45

2), 69.98 (C-4), 62.40 (C-1′), 61.81 (C-6′), 60.80 (C-6), 33.58 ($-CH_2-CO-$), 31.67, 29.35, 28.55, 24.64, 22.33 ($-CH_2$-lauroyl backbone), 13.03 (CH_3-lauroyl).

6′-O-Lauroylsucrose: ^{1}H NMR (500 MHz, MeOD) δ 5.37 (d, J = 3.8 Hz, H-1), 4.40 (dt, J = 13.7, 6.9 Hz, H-3′), 4.11 (d, J = 8.2 Hz, H-6′), 4.03 (t, J = 8.1 Hz, H-4′), 3.85 (dt, J = 7.6, 2.2 Hz, H-5′), 3.76−3.69 (m, H-3, H-5, H-6), 3.64 (d, J = 3.7 Hz, H-1′), 3.43 (dd, J = 9.8, 3.8 Hz, H-2), 3.36 (dd, J = 10.8, 2.8 Hz, H-4), 2.37 (t, J = 7.5 Hz, 2H), 1.63 (dd, J = 14.4, 7.2 Hz, 2H), 1.32 (s, J = 8.4 Hz, 16H), 0.92 (t, J = 7.0 Hz, 3H).

^{13}C NMR (126 MHz, MeOD) δ 174.12 (C═O), 104.13 (C-2′), 92.08 (C-1), 79.29 (C-5′), 77.58 (C-3′), 75.50 (C-4′), 73.34 (C-5), 72.84 (C-3), 71.91 (C-2), 70.14 (C-4), 65.50 (C-6′), 62.41 (C-1′), 61.12 (C-6), 33.54 ($-CH_2-CO-$), 31.67, 29.28, 28.80, 24.58, 22.33 ($-CH_2$-lauroyl backbone), 13.04 (CH_3-lauroyl).

Identification of the mono-*O*-lauroylsucrose was based on the identification of the mono-*O*-lauroylraffinose.[5] Besides, the structure of mono-*O*-lauroylsucrose was also confirmed by the carbon atoms' chemical shifts. For example, ^{13}C NMR of the monoester showed the typical acylation featured with an upfield chemical shift for C-2′ of 1′-*O*-lauroylsucose compared to C-2′ of sucrose: 104.46 versus 102.65 ppm. The chemical shift of 1′-*O*-octanoylsucose is similar to that of 1′-*O*-lauroylsucose. As for 6′-*O*-lauroylsucrose, the typical acylation featured with an upfield chemical shift for C-5′ of 6′-*O*-lauroylsucose compared to C-5′ of sucrose: 82.41 versus 79.29 ppm and downfield chemical shifts for C-6′ of the same compounds: 61.81 versus 65.50 ppm.

TLC and HPLC Analysis. The hydrolysis of raffinose derivatives by α-galactosidase was indicated by TLC and HPLC (Figures 6 and 7). TLC of the isolated 6′-*O*-lauroylsucrose (lane 1) and hydrolysis of 6′-*O*-lauroylraffinose (lane 2) is shown in Figure 6. HPLC of the hydrolysis of 6′-*O*-lauroylraffinose is shown in Figure 7, and according to HPLC analysis, the percent of hydrolysis could exceed 80%.

Comparation of Three Mono-*O*-lauroylsucrose Isomers. *Solubility in Solvent.* Solubility of sucrose monoesters is shown in Table 1. All three monoesters have similar solubilities and are easily dissolved in a polar solvent, followed by moderately polar solvent, and poorly dissolved in nonpolar solvents. This may be because the three monoesters have the

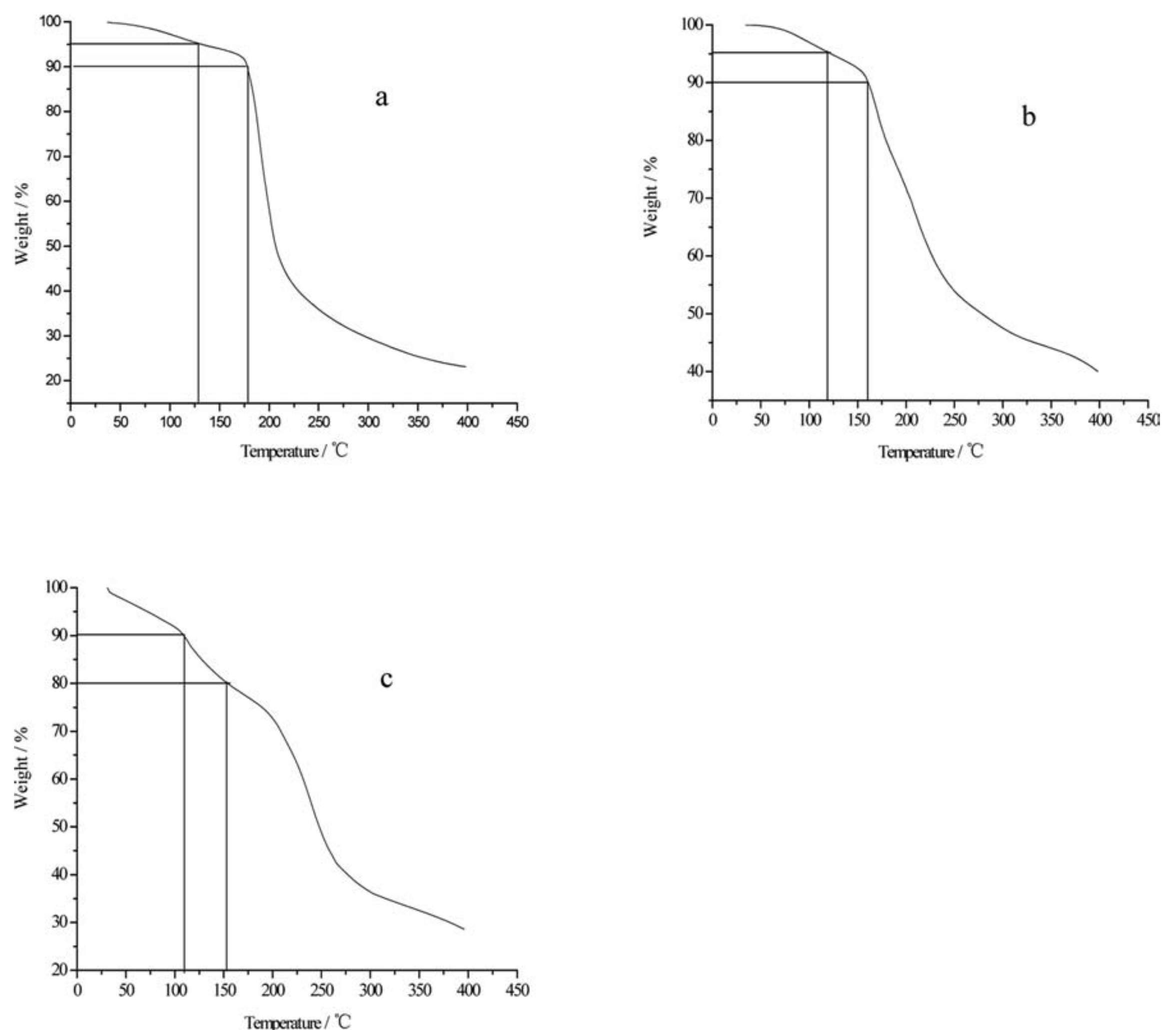

Figure 9. TG of sucrose monoesters: (a) 6-*O*-lauroylsucrose; (b) 6′-*O*-lauroylsucrose; (c) 1′-*O*-lauroylsucrose.

same molecular weight, group compositions, and similar spatial structures. These results coincided with Huang's.[37]

HLB Value, Retardation Factor (R_f) Value, CMC, and γ_{CMC} of Monoesters. CMC values of each mono-*O*-lauroylsucrose in aqueous solutions were calculated and are shown in Figure 8. All three monoesters have similar CMC and γ_{CMC} values (Table 2). 6-*O*-Lauroylsucrose's surface tension is the highest with 38.45 mN/m followed by 6′-*O*-lauroylsucrose and 1′-*O*-lauroylsucrose, with 32.26 and 29.86 mN/m, respectively. These results coincided with Bazin's[38] and Samia's.[39]

Thermal Stability. TG of sucrose monoesters is shown in Figure 9. As can be seen from Figure 9, when 6-*O*-lauroylsucrose (Figure 9a) lost 5% and 10% weight, the temperature reached 132 and 178 °C, respectively; when 6′-*O*-lauroylsucrose (Figure 9b) lost 5% and 10% weight, the temperature reached 122 and 161 °C, respectively, which indicates that the two monoesters have higher thermal stability. These results coincided with Huang's.[37] However, when 1′-*O*-lauroylsucrose (Figure 9c) lost 10% and 20% weight, the temperature reached only 110 and 155 °C, respectively. This might be caused by the steric effect, which could affect the thermal stability of compounds.[40]

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Notes

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ABBREVIATIONS USED

HLB, hydrophile−lipophile balance; CMC, critical micelle concentration; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; IR, infrared spectroscopy; ESI-MS, electrospray ionization mass spectrometry; NMR, nuclear magnetic resonance; HPLC, high-performance liquid chromatography;

TLC, thin-layer chromatography; RID, refractive index detector; TG, thermogravimetric; N_2, nitrogen; R_f, retardation factor

■ REFERENCES

(1) Carnero-Ruiz, C., Ed. *Sugar-Based Surfactants: Fundamentals and Applications*; CRC Press: Boca Raton, FL, 2010.
(2) Maire, M. l.; Champeil, P.; Moller, J. V. Interaction of membrane proteins and lipids with solubilizing detergents. *Biochim. Biophys. Acta—Biomembranes* **2000**, *1508*, 86−111.
(3) Prive, G. G. Detergents for the stabilization and crystallization of membrane proteins. *Methods* **2007**, *41* (4), 388−397.
(4) Perez-Victoria, I.; Perez-Victoria, F. J.; Roldan-Vargas, S.; Garcia-Hernandez, R.; Carvalho, L.; Castanys, S.; Gamarro, F.; Morales, J. C.; Perez-Victoria, J. M. Non-reducing trisaccharide fatty acid monoesters: novel detergents in membrane biochemistry. *Biochim. Biophys. Acta—Biomembranes* **2011**, *1808* (3), 717−726.
(5) Lu, Y.; Yan, R.; Ma, X.; Wang, Y. Synthesis and characterization of raffinose fatty acid monoesters under ultrasonic irradiation. *Eur. Food Res. Technol.* **2013**, DOI: 10.1007/s00217-013-1985-y.
(6) Wang, K.; Jiang, J.; Gao, Y.; Li, X. Research on sucrose esters-natural glycosyl surfactantsa review. *Chem. Ind. For. Prod.* **2011**, *31* (6), 100−106.
(7) Riva, S.; Chopineau, J.; Kieboom, A. P. G.; Klibanov, A. M. Protease-catalyzed regioselective esterification of sugars and related compounds in anhydrous dimethylformamide. *J. Am. Chem. Soc.* **1988**, *110*, 584−589.
(8) Riva, S.; Nonini, M.; Ottolina, G.; Danieli, B. Subtilisin-catalyzed esterification of di- and oligosaccharides containing a D-fructose moiety. *Carbohydr. Res.* **1998**, *314*, 259−266.
(9) Ferrer, M.; Soliveri, J.; Plou, F. J.; López-Cortés, N.; Reyes-Duarte, D.; Christensen, M.; Copa-Patiño, J. L.; Ballesteros, A. Synthesis of sugar esters in solvent mixtures by lipases from *Thermomyces lanuginosus* and *Candida antarctica* B, and their antimicrobial properties. *Enzyme Microb. Technol.* **2005**, *36* (4), 391−398.
(10) Habulin, M.; Šabeder, S.; Knez, Ž. Enzymatic synthesis of sugar fatty acid esters in organic solvent and in supercritical carbon dioxide and their antimicrobial activity. *J. Supercrit. Fluid* **2008**, *45* (3), 338−345.
(11) Chauvin, C.; Plusquellec, D. A new chemoenzymatic synthesis of 6′-*O*-acylsucroses. *Tetrahedron Lett.* **1991**, *32* (29), 3495−3498.
(12) Shi, Y. G.; Li, J. R.; Chu, Y. H. Enzyme-catalyzed regioselective synthesis of sucrose-based esters. *J. Chem. Technol. Biotechnol.* **2011**, *86*, 1457−1468.
(13) Praveen Kumar, S. K.; Mulimani, V. H. Continuous hydrolysis of raffinose family oligosaccharides in soymilk by fluidized bed reactor. *LWT—Food Sci. Technol.* **2010**, *43* (2), 220−225.
(14) Anisha, G. S.; John, R. P.; Prema, P. Substrate specificities and mechanism of action of multiple α-galactosidases from *Streptomyces griseoloalbus*. *Food Chem.* **2011**, *124* (1), 349−353.
(15) Ismail, S. A.-A.; Hashem, A. M. Nutrition requirement for the production of *Penicillium chrysogenum* α-galactosidase and its potential for hydrolysis of raffinose family oligosaccharides. *J. Appl. Sci. Res.* **2012**, *8* (2), 945−952.
(16) Kurakake, M.; Moriyama, Y.; Sunouchi, R.; Nakatani, S. Enzymatic properties and transglycosylation of α-galactosidase from *Penicillium oxalicum* SO. *Food Chem.* **2011**, *126* (1), 177−182.
(17) Shankar, S. K.; Kumar, S. K.; Mulimani, V. H. Calcium alginate entrapped preparation of α-galactosidase: its stability and application in hydrolysis of soymilk galactooligosaccharides. *J. Ind. Microbiol. Biotechnol.* **2011**, *38* (9), 1399−1405.
(18) Kapnoor, S.; Mulimani, V. H. Production of α-galactosidase by *Aspergillus oryzae* through solid-state fermentation and its application in soymilk galactooligosaccharide hydrolysis. *Braz. Arch. Biol. Technol.* **2010**, *53* (1), 211−218.
(19) Clarke, J. H.; Davidson, K.; Rixon, J. E.; Halstead, J. R.; Fransen, M. P.; Gilbert, H. J.; Wood, G. P. H. A comparison of enzyme-aided bleaching of softwood paper pulp using combinations of xylanase, mannanase and α-galactosidase. *Appl. Microbiol. Biotechnol.* **2000**, *53*, 661−667.
(20) Thippeswamy, S.; Mulimani, V. H. Enzymic degradation of raffinose family oligosaccharides in soymilk by immobilized α-galactosidase from *Gibberella fujikuroi*. *Process Biochem.* **2002**, *38*, 635−640.
(21) Anisha, G. S.; Prema, P. Reduction of non-digestible oligosaccharides in horse gram and green gram flours using crude α-galactosidase from *Streptomyces griseoloalbus*. *Food Chem.* **2008**, *106* (3), 1175−1179.
(22) Lu, Y.; Yan, R.; Ma, X. Synthesis, purification and identification of the 6-*O*-lauroylsucrose, 6′-*O*-lauroylsucrose under ultrasonic condition. *Sci. Technol. Food Ind.* **2013**, *34* (04), 129−133.
(23) Soultani, S.; Ognier, S.; Engasser, J. M.; Ghoul, M. Comparative study of some surface active properties of fructose esters and commercial sucrose esters. *Colloid Surf. A* **2003**, *227* (1−3), 35−44.
(24) Cruz, R.; Park, Y. K. Production of fungal α-galactosidase and its application to the hydrolysis of galacto-oligosaccharides in soybean milk. *J. Food Sci.* **1982**, *47*, 1973−1975.
(25) Cruz, R.; Batistela, J. C.; Wosiacki, G. Microbial α-galactosidase for soymilk processing. *J. Food Sci.* **1981**, *46*, 1196−1200.
(26) Klibanov, A. M. Asymmetric transformations catalyzed by enzymes in organic solvents. *Acc. Chem. Res.* **1990**, *23*, 114−120.
(27) Takayasu, S.; Alexey, L. M.; Alan, J. R.; Alexander, M. K. Control of enzyme enantioselectivity by the reaction medium. *J. Am. Chem. Soc.* **1988**, *110*, 7236−7237.
(28) Wescott, C.; Klibanov, A. The solvent dependence of enzyme specificity. *Biochim. Biophys. Acta—Proteins Proteom.* **1994**, *1206*, 1−9.
(29) Liu, Y.; Wang, F.; Tan, T. Effects of alcohol and solvent on the performance of lipase from *Candida* sp. in enantioselective esterification of racemic ibuprofen. *J. Mol. Catal. B—Enzym.* **2009**, *56* (3), 126−130.
(30) Du, F.; Zhu, M.; Wang, H.; Ng, T. Purification and characterization of an α-galactosidase from *Phaseolus coccineus* seeds showing degrading capability on raffinose family oligosaccharides. *Plant Physiol. Biochem..* **2013**, DOI: 10.1016/ j.plaphy.2013.04.017.
(31) Singh, N.; Kayastha, A. M. Purification and characterization of α-galactosidase from white chickpea (*Cicer arietinum*). *J. Agric. Food Chem.* **2012**, *60* (12), 3253−3259.
(32) Praveen Kumar, S. K.; Mulimani, V. H. Immobilization of *Aspergillus oryzae* with κ-carrageenan for soybean oligosaccharide hydrolysis. *Food Sci. Biotechnol.* **2011**, *20* (6), 1691−1697.
(33) Shabalina, K. A.; Kulminskayaa, A. A.; Savel'evb, A. N.; Shishlyannikova, S. M.; Neustroeva, K. N. Enzymatic properties of α-galactosidase from *Trichoderma reesei* in the hydrolysis of galactooligosaccharides. *Enzyme Microb. Technol.* **2002**, *30*, 231−239.
(34) Katrolia, P.; Jia, H.; Yan, Q.; Song, S.; Jiang, Z.; Xu, H. Characterization of a protease-resistant α-galactosidase from the thermophilic fungus *Rhizomucor miehei* and its application in removal of raffinose family oligosaccharides. *Bioresour. Technol.* **2012**, *110*, 578−586.
(35) Cao, Y.; Wang, Y.; Luo, H.; Shi, P.; Meng, K.; Zhou, Z.; Zhang, Z.; Yao, B. Molecular cloning and expression of a novel protease-resistant GH-36 agalactosidase from *Rhizopus* sp. F78 ACCC 30795. *J. Microbiol. Biotechnol.* **2009**, *19* (11), 1295−1300.
(36) Cao, Y.; Wang, Y.; Meng, K.; Bai, Y.; Shi, P.; Luo, H.; Yang, P.; Zhou, Z.; Zhang, Z.; Yao, B. A novel protease-resistant α-galactosidase with high hydrolytic activity from *Gibberella* sp. F75: gene cloning, expression, and enzymatic characterization. *Appl. Microbiol. Biotechnol.* **2009**, *83* (5), 875−884.
(37) Huang, D.; Zhong, K.; Zhang, W.; Gao, W. Ultrasonic synthesis, characteristic and application of sucrose octanoate. *Chinese J. Org. Chem.* **2009**, *29* (12), 1951−1955.
(38) Bazin, H. G.; Polat, T.; Linhardt, R. J. Synthesis of sucrose-based surfactants through regioselective sulfonation of acylsucrose and the nucleophilic opening of a sucrose cyclic sulfate. *Carbohydr. Res.* **1998**, *309*, 189−205.

(39) Samia, S.; Stéphanie, O.; Jean-Marc, E.; Mohamed, G. Comparative study of some surface active properties of fructose esters and commercial sucrose esters. *Colloid Surf. A* **2003**, *227*, 35−44.
(40) Qin, C. Y.; Liu, F.; Peng, B.; Huang, W.; Wei, W. Synthesis, characterization and thermal stability of hyperbranched polyfluorenes containing sterically hindered groups. *Acta Polym. Sin.* **2008**, *6*, 537−543.