Food Chemistry 112 (2009) 421–427

Contents lists available at ScienceDirect

Food Chemistry

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

Identification and Maillard reaction activities of dilauryl mannose isomers formed during lipase-catalyzed condensation

Wenbin Zhang, Chengsheng Jia, Yinjiao Wang, Jin Moon Kim, Pingping Jiang, Xiaoming Zhang *

State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu 214122, PR China

ARTICLE INFO

Article history:
Received 2 January 2008
Received in revised form 24 March 2008
Accepted 28 May 2008

Keywords:
Lauryl mannose
Lipase
Mannose
Lauric acid
Maillard reaction

ABSTRACT

Lauryl mannoses were synthesized by lipase-catalyzed condensation using D-mannose and lauric acid in acetone in the presence of molecular sieves at 50 °C. 6-*O*-Lauryl mannose, 1,6-di-*O*-lauryl mannose, 3,6-di-*O*-lauryl mannose, and 4,6-di-*O*-lauryl mannose were isolated and identified by FT-IR, MS, and NMR. Maillard reaction activities of mannose with L-cysteine and lauryl mannoses with L-cysteine were evaluated by headspace solid phase microextraction (HS-SPME) method combined with GC/MS. 1,6-Di-*O*-lauryl mannose exhibited the lowest Maillard reaction activity, compared with 6-*O*-lauryl mannose, 3,6-di-*O*-lauryl mannose and 4,6-di-*O*-lauryl mannose. Low activity of Maillard reaction by lauryl mannoses compared to that by mannose was due to the structures of mannose and lauryl mannoses determined by NMR analysis.

© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

Saccharide fatty acid esters (SFAEs) are biodegradable, non-toxic and amphiphilic derivatives, which have been widely used as surfactants and emulsifiers in the food, cosmetic, and pharmaceutical industries (Ferrer, Cruces, Plou, Bernabé, & Ballesteros, 2000; Scheckermann, Schlotterbeck, Schmidt, Wray, & Lang, 1995; Zhang et al., 2003).

Enzyme-catalyzed condensation or transesterification in non-aqueous or solvent-free systems offers an alternative to chemical synthesis due to the direct use of unmodified substrates, moderate reaction conditions, and high regioselectivity of the enzyme (Adachi, Nagae, & Matsuno, 1999; Arcos, Bernabé, & Otero, 1998a; Arcos, Bernabé, & Otero, 1998b; Zhang, Adachi, Watanabe, Kobayashi, & Matsuno, 2003). High regioselectivity is the most important reason for replacing chemical methods with enzyme-catalyzed syntheses of SFAE. Immobilized lipase, Novozyme 435, from *Candida antarctica*, is a common enzyme used for synthesis of sugar esters (Watanabe, Adachi, Nakanishi, & Matsuno, 2001). *C. antarctica* lipase prefers to catalyze the acylation of the primary hydroxyl group at C6 of hexose, as well as the secondary hydroxyl group (Arcos, Hill, & Otero, 2001; Arcos et al., 1998b; Ferrer, Cruces, Bernabé, Ballesteros, & Plou, 1999). Therefore, there is a possibility that di- and/or triacyl hexoses are produced in addition to the monoacyl hexose. Mannose is a stereoisomer of glucose, which has 5 hydroxyl groups. Theoretically, all of the hydroxyl groups can be acylated by electrophiles. In the previous reports, di- and higher esters that could have been formed during the enzymatic condensation of D-mannose and lauric acid in acetone in the presence of molecular sieves were not detected (Watanabe, Miyawaki, Adachi, Nakanishi, & Matsuno, 2000; Watanabe, Miyawaki, Adachi, Nakanishi, & Matsuno, 2001). However, in the previous work in our lab (Liu et al., 2007; Zhou et al., 2006), diesters formed in the enzymatic synthesis were detected by mass spectra (MS). However, the separation and purification of products have not been further investigated. Because sugar mono- and diesters have different surfactant properties, further work on regioselective acylation mechanism and kinetic course of esterification should be conducted to obtain the desirable esterified lauryl mannose for different food applications.

The Maillard reaction is a chemical reaction between a reducing sugar and an amino acid on heating or on storage, leading to browning and the formation of a variety of volatile substances (Jalbout, Shipar, & Navarro, 2007; Kim & Lee, 2007; Lertittikul, Benjakul, & Tanaka, 2007). SFAEs can be used as emulsifiers and preservatives in the food industry. If the SFAE keeps its reducing sugar moiety, it is possible that SFAE reacts with an amino acid to produce volatile substances and browning reaction. In some cases, it is not desirable to have the Maillard reaction during food processing.

In this study, enzymatic synthesis of sugar esters was conducted, and presence of di-/triesters that may be formed during the synthesis was investigated. Identification of diesters using FT-IR, MS, and NMR led to the proposal on the mechanism of the diester formation with their structures. Maillard reaction activities of the identified diesters were evaluated for the potential food application.

* Corresponding author. Tel.: +86 510 85919106; fax: +86 510 85884496.
E-mail address: xmzhang@jiangnan.edu.cn (X. Zhang).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.05.096

2. Experimental

2.1. Chemicals

Commercial immobilized lipase from *C. antarctica*, Novzyme 435, was purchased from Novozyme A/S (Bagsvaerd, Denmark). D-Mannose, lauric acid, L-cysteine, methanol, acetone, acetonitrile and *n*-hexane were purchased from Sinopharm Chemical Reagent Co., Ltd. China. The 3A 1/16 and 4 Å 1/16 molecular sieves were purchased from Shanghai UOP, Shanghai.

2.2. Lipase-catalyzed reactions

Lauric acid (1.00 mmol, 0.200 g), mannose (0.25 mmol, 0.045 g) and the immobilized lipase (75 mg) were weighed into a glass vial. Five milliliters of acetone as a solvent was added into the vial. The solvent had been predehydrated with molecular sieve 4 Å for at least 24 h. The vial was tightly screw-capped and immersed in a water-bath to conduct condensation. Condensation was carried out with vigorous shaking (150 rpm) at 50 °C for 3 days. If necessary, 3 Å molecular sieves (400 mg) was included.

The reaction mixture was analyzed by HPLC using a Sunfire-C18 column (4.6 × 250 mm, Waters, USA) eluted with methanol/water (90:10 v/v) at 1 ml/min, and detected by a Waters 2420 evaporative light scattering detector (ELSD). ELSD conditions were optimized at drift-tube temperature 45 °C; sprayer temperature 36 °C; carrier gas pressure 20 psi; and gain 1.

2.3. Purification and identification of condensation products

The reaction mixture was filtered and the filtrate was then rotary-evaporated. The concentrated solution was applied to a semi-preparative HPLC with ELSD and a Sunfire-C18 column (5 μm, 19 × 150 mm, Waters, USA) to purify the products. The eluant was methanol/water (90:10, v/v), and the flow rate was 8.0 ml/min.

Finally, the purified products were identified: the infrared spectra were taken on a Nicolet Nexus FT-IR. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian INOVA (600 MHz and 150 MHz) and av300 (300 MHz and 75 MHz) spectrometer at 30 °C in CD_3OD. Chemical shifts were referred to the methanol multiplet, centered at 3.30 ppm for ^{1}H NMR and 49.0 ppm for ^{13}C NMR. The ^{1}H NMR spectra showed chemical shifts and coupling constants within ±0.005 and ±0.5 Hz, respectively. Mass spectra were obtained by mass spectrometry (Waters Platform ZMD 4000, Milford, MA, USA) with positive EI mode, and the ionisation conditions were as follows: capillary voltage 3.87 kV; cone voltage 30 V; and extractor voltage 7 V. The source block temperature was 120 °C, and the desolvation temperature was 300 °C. The electrospray probe flow was adjusted to 70 ml/min. Scanning of sample was recorded over the range of *m/z* 200–1000 with a scan time 1 s and an interscan delay of 0.1 s.

2.4. Maillard reaction

Mixtures of mannose/lauryl mannoses (0.01 mol) and L-cysteine (0.01 mol) were dissolved in 50 ml sodium phosphate buffer (0.2 mol/l, pH 5) to form the Maillard reaction solution. The Maillard reaction was immediately conducted in steam jacketed kettle pressure vessel (LS-B50 L, Shanghai, China) for 40 min or 3 h at 120 °C.

2.5. Headspace solid phase microextraction (HS-SPME)

SPME fused silica fibres (Supelco), coated with polydimethylsiloxane (CAR/PDMS, 75 μm) were used with a manual SPME holder. Samples (8 ml) solution saturated by NaCl were poured into open-top vials with PTFE/silicone septum (15 ml, clear glass) under agitation with Teflon-coated magnetic stirring bars at 40 °C to establish the equilibrium between the headspace and sample. After a set extraction time (40 min), the fibre was withdrawn into the needle and then introduced into the injection port of the GC for analysis.

2.6. Gas chromatography–mass spectroscopy (GC–MS) analysis

A gas chromatograph–mass spectrometer (Finnigan Trace GC/MS, Finnigan, USA) was used to determine the samples. A DB-WAX capillary column (30 m × 0.25 mm × 0.25 μm, J&W Scientific, Folsom, CA, USA) was used for the GC separation using pure nitrogen as carrier gas at 0.8 ml/min in a splitless mode. The column temperature program was as follows: initial temperature 40 °C for 4 min, increased to 50 °C at 10 °C/min, increased to 120 °C at 6 °C/min, increased to 220 °C at 10 °C/min, 220 °C was maintained for 7 min.

Mass spectroscopy condition was as follows: ionic source temperature, 200 °C; ionization mode, EI^+; electronic energy, 70 eV; ejection current, 200 μA; scanning quality scope, 33–450 amu.

Data were collected and processed by Xcalibur software, the identification of volatiles was achieved by comparing mass spectral data of samples with those of the NIST library and Wiley library, and quantitative analysis was performed according to integral calculus on peak area.

3. Results and discussion

3.1. Identification of condensation product

There were four peaks and the protonated molecular ions $(M+Na)^+$ of the products were at *m/z* 385.3, 567.5, 567.5, and

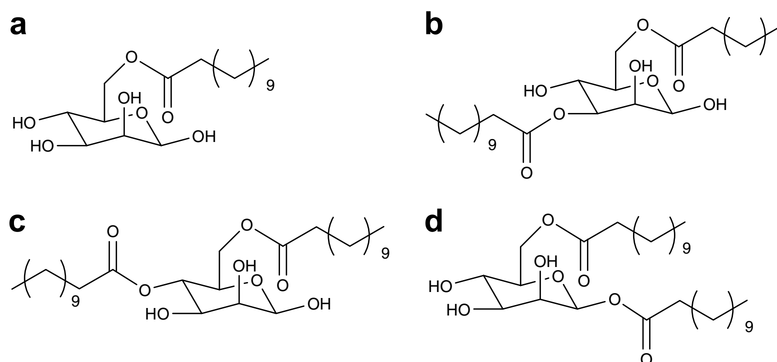

Fig. 1. Chemical structures of (a) 6-*O*-lauryl mannose (ME), (b) 3,6-di-*O*-lauryl mannose (D 3,6), (c) 4,6-di-*O*-lauryl mannose (D 4,6) and (d) 1,6-di-*O*-lauryl mannose (D 1,6).

567.5, respectively. The results indicated that the products included one monolauryl mannose and three positional isomers of dilauryl mannoses.

The purified products detected from this reaction were 6-*O*-lauryl mannose (ME), 3,6-di-*O*-lauryl mannose (D 3,6), 4,6-di-*O*-lauryl mannose (D 4,6), and 1,6-di-*O*-lauryl mannose (D 1,6). The yields of 6-*O*-lauryl mannose, 3,6-di-*O*-lauryl mannose, 4,6-di-*O*-lauryl mannose, and 1,6-di-*O*-lauryl mannose were 24%, 3%, 16% and 9%, respectively. The FT-IR and NMR analysis data were as follows:

(1) 6-*O*-Lauryl mannose: IR (ν, cm^{-1}) 3382 (br, O–H), 2956, 2922, 2853 (CH_2 and CH_3), 1722 (C=O), 1466 (CH_2 and CH_3), 1065 (C–O); ^1H NMR (300 MHz, δ, ppm): 4.96 (s, 1H, H-1), 4.29 (dd, 1H, *J* = 11.6, 1.7 Hz, H-6a), 4.12 (dd, 1H, *J* = 11.7, 6.1 Hz, H-6b), 3.86–3.81 (m, 1H, H-5), 3.72–3.65 (m, 2H, H-2 + H-3), 3.54 (d, 1H, *J* = 9.4 Hz, H-4), 2.26 (t, 2H, *J* = 7.4 Hz, CH_2CO), 1.53–1.51 (m, 2H, CH_2CH_2CO), 1.21 (m, 16H, $(CH_2)_8CH_3$), 0.82 (t, 3H, *J* = 6.8 Hz, CH_3); ^{13}C NMR (75 MHz, δ, ppm) 175.65 (C=O), 95.81 (C-1), 72.72 (C-5), 72.16 (C-2), 71.64 (C-3), 68.83 (C-4), 65.15 (C-6), 34.94 (CH_2CO), 33.01 ($CH_2CH_2CH_3$), 30.72 + 30.68 + 30.56 + 30.41 + 30.37 + 30.20 ($(CH_2)_6CH_2CH_2CH_3$), 25.93 (CH_2CH_2CO), 23.67 (CH_2CH_3), 14.43 (CH_3); ESIMS: *m/z* 385.3 $(M+Na)^+$; HPLC retention time (min): 2.96. Its structure is shown in Fig. 1a.

(2) 3,6-Di-*O*-lauryl mannose: IR (ν, cm^{-1}) 3450 (br, O–H), 2955, 2920, 2852 (CH_2 and CH_3), 1744, 1728 (C=O), 1468, 1160 (C–O); ^1H NMR (600 MHz, δ, ppm): 5.05 (dd, 1H, *J* = 9.8, 3.4 Hz, H-5), 5.01 (s, 1H, H-1), 4.35 (dd, 1H, *J* = 11.7, 1.5 Hz, H-6a), 4.22 (dd, 1H, *J* = 11.7, 5.9 Hz, H-6b), 4.00–3.99 (m, 1H, H-2), 3.92–3.91 (m, 1H, H-3), 3.82 (t, 1H, *J* = 9.8 Hz, H-4), 2.38 (t, 2H, *J* = 7.8 Hz, CH_2CO), 2.32 (t, 2H, *J* = 7.8 Hz, CH_2CO), 1.63–1.56 (m, 4H, 2 × CH_2CH_2CO), 1.27 (m, 32H, 2 × $(CH_2)_8CH_3$), 0.88 (t, 6H, *J* = 6.8 Hz, 2 × CH_3); ^{13}C NMR (150 MHz, δ, ppm) 176.07 + 175.71 (2 × C=O), 96.34 (C-1), 75.80 (C-5), 72.29 (C-2), 71.11 (C-3), 66.71 **(C-4)**, 65.39 (C-6), 35.62 + 35.46 (2 × CH_2CO), 33.59 (2 × $CH_2CH_2CH_3$), 31.25 + 31.22 + 31.12 + 30.97 + 30.93 + 30.74 (2 × $(CH_2)_6CH_2CH_2$ CH_3), 26.48 + 26.45 (2 × CH_2CH_2CO), 24.23 (2 × CH_2CH_3), 14.95 (2 × CH_3); ESIMS: *m/z* 567.5 $(M+Na)^+$; HPLC retention time (min): 11.25. Its structure is shown in Fig. 1b.

(3) 4,6-Di-*O*-lauryl mannose: IR (ν, cm^{-1}) 3441 (br, O–H), 2955, 2920, 2851 (CH_2 and CH_3), 1736, 1720 (C=O), 1467, 1058 (C–O); ^1H NMR (600 MHz, δ, ppm): 5.01 (d, 1H, *J* = 5.8 Hz, H-5), 4.95 (s, 1H, H-1), 4.35 (d, 1H, *J* = 11.7 Hz, H-6a), 4.22 (dd, 1H, *J* = 11.7, 4.9 Hz, H-6b), 3.95–3.89 (m, 2H, H-2 + H-3), 3.65 (t, 1H, *J* = 9.8 Hz, H-4), 2.36 (t, 2H, *J* = 7.8 Hz, CH_2CO), 2.32 (t, 2H, *J* = 7.3 Hz, CH_2CO), 1.62–1.59 (m, 4H, 2 × CH_2CH_2CO), 1.27 (m, 32H, 2 × $(CH_2)_8CH_3$), 0.88 (t, 6H, *J* = 6.8 Hz, 2 × CH_3); ^{13}C NMR (150 MHz, δ, ppm) 175.89 + 175.45 (2 × C=O), 93.58 (C-1), 75.16 (C-5), 72.16 (C-3), 70.75 (C-2), 69.38 (C-4), 65.14 (C-6), 35.65 + 35.63 (2 × CH_2CO), 33.61 (2 × $CH_2CH_2CH_3$), 31.31 + 31.22 + 31.02 + 31.00 + 30.83 + 30.75 (2 × $(CH_2)_6CH_2CH_2CH_3$), 26.57 + 26.53 (2 × CH_2CH_2CO), 24.27 (2 × CH_2CH_3), 15.02 (2 × CH_3); ESIMS: *m/z* 567.5 $(M+Na)^+$; HPLC retention time (min): 16.01. Its structure is shown in Fig. 1c.

(4) 1,6-Di-*O*-lauryl mannose: IR (ν, cm^{-1}) 3396 (br, O–H), 2955, 2919, 2850 (CH_2 and CH_3), 1740 (C=O), 1467, 1059 (C–O); ^1H NMR (600 MHz, δ, ppm): 5.95 (d, 1H, *J* = 1.5 Hz, H-1), 4.35 (dd, 1H, *J* = 11.7, 1.5 Hz, H-6a), 4.17 (dd, 1H, *J* = 11.7, 6.8 Hz, H-6b), 3.79 (d, 1H, *J* = 2.0 Hz, H-2), 3.76–3.73 (m, 1H, H-5), 3.69–3.63 (m, 2H, H-3 + H-4), 2.35 (t, 2H, *J* = 7.3 Hz, CH_2CO), 2.30 (t, 2H, *J* = 7.3 Hz, CH_2CO), 1.63–1.57 (m, 4H, 2 × CH_2CH_2CO), 1.28 (m, 32H, 2 × $(CH_2)_8CH_3$), 0.88 (t, 6H, *J* = 6.8 Hz, 2 × CH_3); ^{13}C NMR (150 MHz, δ, ppm) 175.87 + 173.64 (2 × C=O), 95.54 (C-1), 74.92 (C-5), 72.74 (C-2), 71.43 (C-3), 68.73 (C-4), 65.33 (C-6), 35.55 + 35.53 (2 × CH_2CO), 33.58 (2 × $CH_2CH_2CH_3$), 31.27 + 31.14 + 30.99 + 30.96 + 30.74 + 30.64 (2 × $(CH_2)_6CH_2CH_2CH_3$), 26.53 + 26.51 (2 × CH_2CH_2CO), 24.24 (2 × CH_2CH_3), 14.97 (2 × CH_3); ESIMS: *m/z* 567.5 $(M+Na)^+$; HPLC retention time (min): 18.50. Its structure is shown in Fig. 1d.

In the reaction mixtures, a protonated molecular ion $(M+Na)^+$ at *m/z* 749.9 was also detected, which confirmed the formation of triester in this reaction system. However, it was not detected by ELSD since it was present in a very small amount.

As a result of these structure study, we propose in this paper the mechanism of enzymatic synthesis of lauryl mannoses as shown in Fig. 2. At first, the primary hydroxyl group at C6 of mannose is preferentially acylated by lauric acid to produce 6-*O*-lauryl mannose; and then the secondary hydroxyl groups at C1, C3, or C4 of 6-*O*-lauryl mannose is further acylated by lauric acid to produce dilauryl mannoses.

Among these products, 6-*O*-lauryl mannose has the same ^1H NMR spectra data as previously reported (Watanabe, Miyawaki,

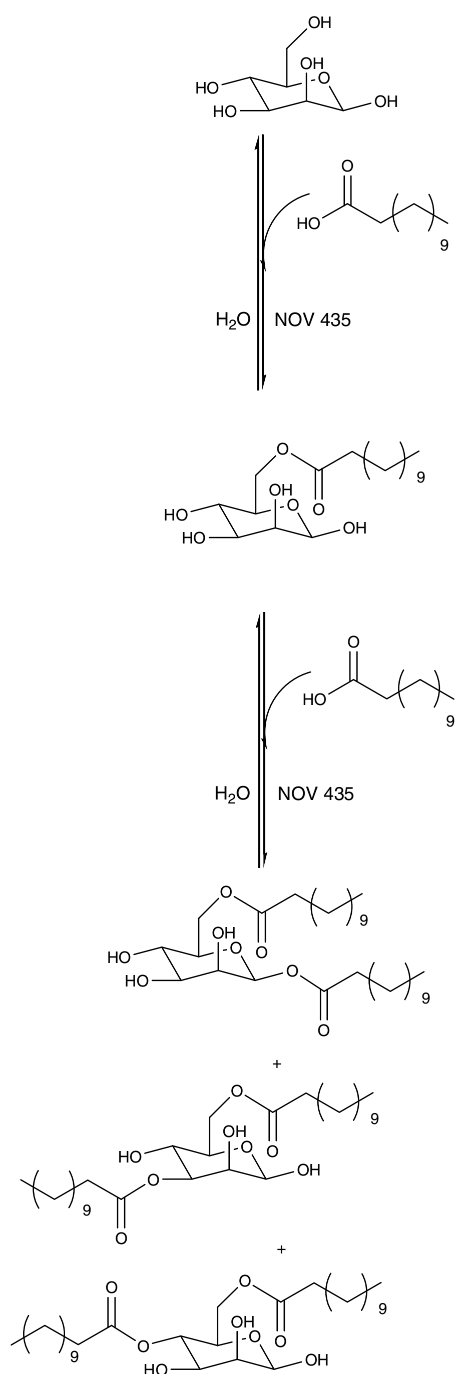

Fig. 2. A proposed mechanism of enzymatic synthesis of lauryl mannoses.

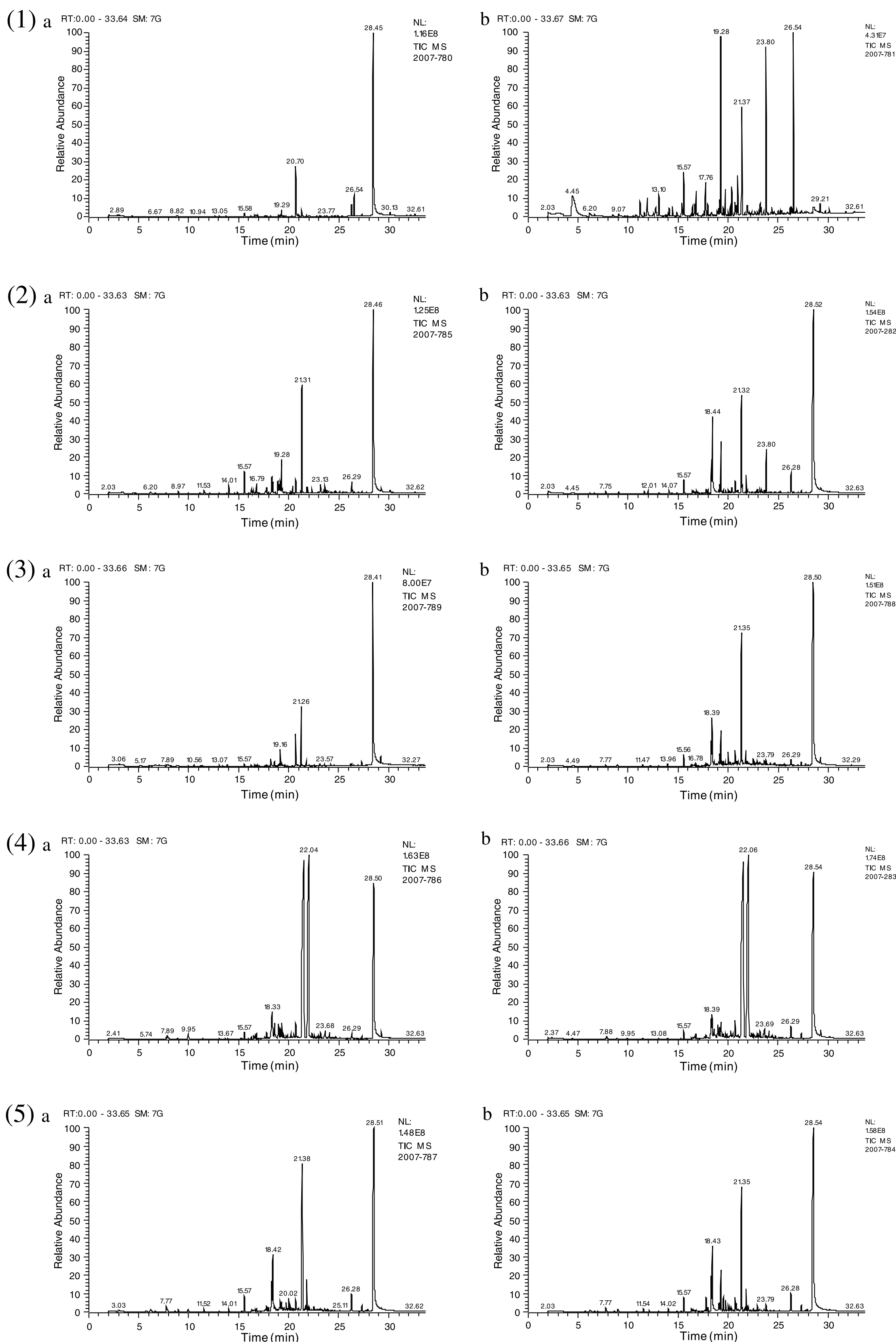

Fig. 3. GC spectra of the Maillard reaction of mannose/lauryl mannoses-L-cysteine systems for 40 min and 3 h, respectively. (1) (a) mannose (M) heated for 40 min; (b) mannose heated for 3 h; (2) (a) 6-*O*-lauryl mannose (ME) heated for 40 min; (b) 6-*O*-lauryl mannose (ME) heated for 3 h; (3) (a) 3,6-di-*O*-lauryl mannose (D 3,6) heated for 40 min; (b) 3,6-di-*O*-lauryl mannose (D 3,6) heated for 3 h; (4) (a) 4,6-di-*O*-lauryl mannose (D 4,6) heated for 40 min; (b) 4,6-di-*O*-lauryl mannose (D 4,6) heated for 3 h; and (5) (a) 1,6-di-*O*-lauryl mannose (D 1,6) heated for 40 min; (b) 1,6-di-*O*-lauryl mannose (D 1,6) heated for 3 h.

& Adachi, 2001). In this study, three other dilauryl mannoses were identified for the first time.

In Fig. 1, owing to the different degree and position of substitution, lauryl mannoses may exhibit different properties. The Maillard reaction was induced to compare the structure–reaction activities of lauryl mannoses.

3.2. Maillard reaction activity of mannose and various lauryl mannoses

The Maillard reaction of mannose/lauryl mannoses-L-cysteine systems was conducted for 40 min and for 3 h, and analyzed by HS-SPME method combined with GC/MS (Fig. 3).

The volatile compounds identified by MS corresponding to GC spectra from 14th to 25th minute were quite different for different reaction systems. The results are shown in Table 1 (reaction for 40 min) and Table 2 (reaction for 3 h). Volatile compounds formed by the system of (ME)-, (D 1,6)-, (D 3,6)-, and (D 4,6)-L-cysteine, with the retention times corresponding to those of the 8 volatile compounds formed by the mannose (M)-L-cysteine system are listed together from Nos. 1 to 8 (mannose moiety in lauryl mannose) in Table 1. While 8 volatile compounds, such as furfural, 2-furanmethanol, were produced by (M)-L-cysteine Maillard reaction system, only 1, 4, 5, and 6 volatile compounds, with the retention times corresponding to those of the 8 volatile compounds formed by (M)-L-cysteine system, were produced by (D 1,6)-, (D 3,6)-, (D 4,6)-, and (ME)-L-cysteine reaction systems, respectively. The result indicated that (D 1,6) significantly retarded the Maillard reaction compared to three other lauryl mannoses. Significant retardation of the Maillard reaction by (D 1,6) is attributed to its structure as shown in Fig. 1: hemiacetal group of mannose, essential for the early stage of the Maillard reaction is esterified to form

Table 1
The volatile compounds identified by MS corresponding to GC spectra from 14th to 25th minute during 40 min of Maillard reaction of mannose/lauryl mannoses with L-cysteine

No.	Retention time	Volatile compounds identified	Formula	Area of GC ($\times 10^7$)				
				M	ME	D 1,6	D 3,6	D 4,6
1	15.57	Furfural	$C_5H_4O_2$	1.22	6.23	0.39	2.10	5.24
2	16.75	Benzaldehyde	C_7H_6O	0.41	2.25	–[a]	1.46	0.76
3	17.79	2-Furancarboxaldehyde,5-methyl-	$C_{11}H_{22}O$	0.07	1.70	–	2.12	1.64
4	18.99	Cyclohexanol,1-methyl-4-(1-methylethyl)-	$C_{10}H_{20}O$	0.29	1.91	–	–	–
5	19.28	2-Furanmethanol	$C_5H_6O_2$	1.21	5.92	–	–	1.90
6	20.70	Oxime-,methoxy-phenyl-	$C_8H_9NO_2$	8.80	2.65	–	3.99	2.69
7	20.95	3-Methyl-2-thiophenecarboxaldehyde	C_6H_6OS	1.15	–	–	–	–
8	23.21	1-Dodecanol	$C_{12}H_{26}O$	1.33	–	–	–	–
9	14.85	Benzene,1,2,4,5-tetramethyl-	$C_{10}H_{14}$	–	0.59	–	–	–
10	16.25	1-Hexanol,2-ethyl-	$C_8H_{18}O$	–	1.28	0.24	–	–
11	16.28	Decanal	$C_{10}H_{20}O$	–	–	–	0.83	0.77
12	16.46	Ethanone,1-(2-furanyl)-	$C_6H_6O_2$	–	0.99	–	–	–
13	16.50	Ethyl sorbate	$C_8H_{12}O_2$	–	–	–	1.18	–
14	16.55	Pentadecane	$C_{15}H_{32}$	–	–	–	–	1.03
15	17.62	1-Octanol	$C_8H_{18}O$	–	–	0.17	–	–
16	17.69	Longiborneol	$C_{15}H_{26}O$	–	–	–	–	0.87
17	17.98	2-Cyclopentene-1,4-dione	$C_5H_4O_2$	–	0.31	–	–	1.19
18	18.14	Decanoic acid,methyl ester	$C_{11}H_{22}O_2$	–	–	0.07	–	–
19	18.22	2-Undecanone	$C_{11}H_{22}O$	–	3.06	0.92	5.84	9.51
20	18.40	Undecanal	$C_{11}H_{22}O$	–	2.69	0.49	12.60	17.30
21	18.59	Benzaldehyde 4-methyl-	C_8H_8O	–	–	0.78	–	1.26
22	18.60	Benzoic acid,methyl ester	$C_8H_8O_2$	–	–	–	5.72	–
23	18.63	Ethanedione (4-methylphenyl) phenyl-	$C_{15}H_{12}O_2$	–	0.49	–	–	–
24	18.92	Benzeneacetaldehyde	C_8H_8O	–	3.29	–	–	–
25	18.95	Decanoic acid,ethyl ester	$C_{12}H_{24}O_2$	–	–	–	5.82	–
26	19.02	Cyclohexanol, 5-methyl-2-(1-methylethyl)-	$C_{10}H_{20}O$	–	–	0.21	–	–
27	19.10	Benzene 1,3,5-trmethyl-	C_9H_{12}	–	0.54	–	–	–
28	19.16	Benzene (1-bromoethyl)-	C_8H_9Br	–	2.90	–	–	–
29	19.31	Benzoic acid,ethyl ester	$C_9H_{10}O_2$	–	0.78	–	4.92	–
30	19.36	Hexadecane,2-methyl-	$C_{17}H_{36}$	–	–	–	–	0.60
31	19.54	Hexadecane,3-methyl-	$C_{17}H_{36}$	–	–	–	–	1.02
32	19.73	2-Thiophenecarboxaldehyde	C_5H_4OS	–	–	–	0.97	1.59
33	19.99	Heptadecane	$C_{17}H_{36}$	–	–	–	–	2.55
34	20.18	Thiophene 2,3-dimethyl-	C_6H_8S	–	–	–	–	0.85
35	20.40	Naphthalene	$C_{10}H_8$	–	1.41	–	–	–
36	21.26	Dodecanoic acid,methyl ester	$C_{13}H_{26}O_2$	–	23.25	6.15	209.09	74.01
37	21.69	Dodecanoic acid,1-methylethyl ester	$C_{15}H_{30}O_2$	–	–	–	–	1.02
38	21.79	Dodecanoic acid ethyl ester	$C_{14}H_{28}O_2$	–	0.92	0.53	170.46	5.45
39	22.04	1-Undecanol	$C_{11}H_{24}O$	–	–	0.17	–	–
40	22.31	Naphthalene,2-methyl-	$C_{11}H_{10}$	–	0.83	–	–	–
41	22.92	2-Thiophenemethanol	C_5H_6OS	–	0.24	–	0.36	0.35
42	23.13	Benzothiazole	C_7H_5NS	–	1.36	–	1.23	0.64
43	23.19	Naphthalene,2,6-dimethyl-	$C_{12}H_{12}$	–	0.77	–	–	–
44	23.22	Naphthalene,1,7-dimethyl-	$C_{12}H_{12}$	–	0.45	–	–	–
45	23.80	3-(Vinythio)thiophene	$C_6H_6S_2$	–	0.49	–	–	–
46	24.22	Caryophyllenyl alcohol	$C_{15}H_{26}O$	–	–	0.21	–	–
47	24.70	Naphthalene,1,4,5-trimethyl-	$C_{13}H_{14}$	–	0.46	–	–	–

M: Mannose; ME: 6-*O*-lauryl mannose; D 1,6: 1,6-di-*O*-lauryl mannose; D 3,6: 3,6-di-*O*-lauryl mannose; D 4,6: 4,6-di-*O*-lauryl mannose.
Volatile compounds formed by the system of (ME)-, (D 1,6)-, (D 3,6)-, and (D 4,6)-L-cysteine, with the retention times corresponding to those of the 8 volatile compounds formed by the system of (M)-L-cysteine, were listed together from Nos. 1 to 8 (mannose moiety in lauryl mannose); volatile compounds, with the retention times different from those of 8 compounds formed by the system of (M)-L-cysteine, were listed from Nos. 9 to 47 (lauric acid moiety in lauryl mannose).
[a] Undetected.

Table 2
The volatile compounds identified by MS corresponding to GC spectra from 14th to 25th minute during 3 h of Maillard reaction of mannose/lauryl mannoses with L-cysteine

No.	Retention time	Volatile compounds identified	Formula	Area of GC ($\times 10^7$)				
				M	ME	D 1,6	D 3,6	D 4,6
1	15.57	Furfural	$C_5H_4O_2$	4.49	5.32	3.61	2.89	5.56
2	16.75	Benzaldehyde	C_7H_6O	2.16	1.14	0.84	1.51	–[a]
3	19.28	2-Furanmethanol	$C_5H_6O_2$	12.2	12.2	7.44	3.90	9.74
4	20.70	Oxime-,methoxy-phenyl-	$C_8H_9NO_2$	0.87	2.96	–	5.00	3.07
5	23.21	1-Dodecanol	$C_{12}H_{26}O$	0.64	–	–	–	–
6	14.00	Pyrazine,2-ethyl-6-methyl-	$C_7H_{10}N_2$	0.21	–	–	–	–
7	14.13	4-Pyridinamine,*N*,*N*-dimethyl-	$C_7H_{10}N_2$	0.93	–	–	–	–
8	14.44	Pyrazine,trimethyl-	$C_7H_{10}N_2$	0.77	–	–	–	–
9	14.89	2(3*H*)-Furanone,5-methyl-	$C_5H_6O_2$	0.38	–	–	–	–
10	15.37	Acetic acid	$C_2H_4O_2$	1.49	–	–	–	–
11	16.46	Ethanone,1-(2-furanyl)-	$C_6H_6O_2$	1.09	–	–	–	–
12	16.67	1*H*-Pyrrole	C_4H_5N	1.14	–	–	–	–
13	17.62	1-Octanol	$C_8H_{18}O$	0.33	–	–	–	–
14	17.76	1*H*-Pyrrole,3-methyl-	C_5H_7N	2.80	–	–	–	–
15	17.98	2-Cyclopentene-1,4-dione	$C_5H_4O_2$	0.94	1.09	–	0.36	–
16	18.35	Ethanone,1-(2-pyridinyl)-	C_7H_7NO	0.21	–	–	–	–
17	19.04	2-Acetylthiazole	C_5H_5NOS	0.53	–	–	–	1.97
18	19.52	3-Thiophenecarboxaldehyde	C_5H_4OS	0.55	–	–	–	3.42
19	19.73	2-Thiophenecarboxaldehyde	C_5H_4OS	1.59	0.63	–	–	2.35
20	20.02	5-Methyl-2-thiophenecarboxaldehyde	C_6H_6OS	0.26	–	–	–	–
21	20.43	2-Furancarboxaldehyde,5-(hydroxymethyl)-	$C_6H_6O_3$	1.37	–	–	–	–
22	20.82	Ethanone,1-(3-thienyl)-	C_6H_6OS	0.85	–	–	–	2.00
23	20.90	2-Ethyl-5-methyl thiophene	$C_7H_{10}S$	0.38	–	–	–	–
24	20.97	5-Methyl-2-thiophenecarboxaldehyde	C_6H_6OS	9.52	–	–	–	–
25	22.92	2-Thiophenemethanol	C_5H_6OS	0.23	0.80	–	1.13	1.40
26	23.13	Benzothiazole	C_7H_5NS	0.23	0.98	–	1.47	0.58
27	23.24	Benzenethiol,2-amino-	C_6H_7NS	0.66	–	–	–	–
28	23.32	Thieno[3,2-*b*]thiophene	$C_6H_4S_2$	0.47	–	–	–	–
29	23.80	3-(Vinythio)thiophene	$C_6H_6S_2$	10.72	10.10	–	–	–
30	24.39	1,5,7-Thiazabicyclo[4.4.0]dec-5-ene	$C_7H_{13}N_3$	0.49	–	–	–	–
31	17.79	2-Furancarboxaldehyde,5-methyl-	$C_{11}H_{22}O$	–	1.32	0.91	1.49	4.29
32	16.28	Decanal	$C_{10}H_{20}O$	–	0.91	0.58	–	–
33	18.22	2-Undecanone	$C_{11}H_{22}O$	–	10.28	7.65	8.20	14.62
34	18.40	Undecanal	$C_{11}H_{22}O$	–	26.68	16.10	9.66	19.52
35	18.60	Benzoic acid,methyl ester	$C_8H_8O_2$	–	–	–	3.71	–
36	18.95	Decanoic acid,ethyl ester	$C_{12}H_{24}O_2$	–	–	–	5.56	–
37	19.16	Benzene (1-bromoethyl)-	C_8H_9Br	–	–	–	3.21	–
38	19.31	Benzoic acid,ethyl ester	$C_9H_{10}O_2$	–	–	–	2.53	–
39	19.99	Heptadecane	$C_{17}H_{36}$	–	0.74	2.91	2.28	1.70
40	20.50	Undecanoic acid,ethyl ester	$C_{13}H_{26}O_2$	–	–	–	1.75	–
41	21.26	Dodecanoic acid,methyl ester	$C_{13}H_{26}O_2$	–	30.28	55.12	232.01	51.66
42	21.79	Dodecanoic acid ethyl ester	$C_{14}H_{28}O_2$	–	3.31	2.88	193.92	4.15
43	21.93	1-Butanone,1-(2-thienyl)-	$C_8H_{10}OS$	–	0.70	–	–	1.26
44	22.48	Nonadecane	$C_{19}H_{40}$	–	–	1.24	–	–
45	22.58	Tridecanoic acid,methyl ester	$C_{14}H_{28}O_2$	–	–	–	0.72	–
46	23.22	Naphthalene,1,7-dimethyl-	$C_{12}H_{12}$	–	–	–	2.64	–
47	23.58	Eicosane	$C_{20}H_{42}$	–	–	1.03	–	–
48	23.69	Tetradecanoic acid,methyl ester	$C_{15}H_{30}O_2$	–	–	–	1.72	–
49	24.09	Tetradecanoic acid,ethyl ester	$C_{16}H_{32}O_2$	–	–	–	1.58	–
50	24.66	Heneicosane	$C_{21}H_{44}$	–	–	0.57	–	–

M: Mannose; ME: 6-*O*-lauryl mannose; D 1,6: 1,6-di-*O*-lauryl mannose; D 3,6: 3,6-di-*O*-lauryl mannose; D 4,6: 4,6-di-*O*-lauryl mannose.
Volatile compounds formed by the system of (ME)-, (D 1,6)-, (D 3,6)-, and (D 4,6)-L-cysteine, with the retention times corresponding to those of the 30 volatile compounds formed by the system of (M)-L-cysteine, were listed together from Nos. 1 to 30 (mannose moiety in lauryl mannose); volatile compounds with the retention times different from those of 30 compounds formed by the system of (M)-L-cysteine were listed from Nos. 31 to 50 (lauric acid moiety in lauryl mannose).
[a] Undetected.

(D 1,6). As reaction time was lengthened to 3 h (Table 2), more volatile compounds were produced in all Maillard reaction systems, (M)-L-cysteine and lauryl mannose [(ME), (D 1,6)-, (D 3,6)-, and (D 4,6)]-L-cysteine Maillard reaction systems. Volatile compounds, such as thiophene, pyrrole, pyrazine, and thiazole are the products at the final stage of the Maillard reaction. Thirty volatile compounds were formed by the (M)-L-cysteine Maillard reaction system after 3 h reaction, while 3, 7, 9 and 9 volatile compounds, with the retention times corresponding to those of 30 volatile compounds formed by the (M)-L-cysteine Maillard reaction system, were formed by (D 1,6)-, (D 3,6)-, (D 4,6)-, and (ME)-L-cysteine reaction systems, respectively. This confirms that (D 1,6) significantly retards the Maillard reaction. If the volatile compounds detected in (M)-L-cysteine reaction system were regarded as Maillard reaction products (MRPs) of mannose moiety in lauryl mannose, MRPs produced by mannose reaction system were greater than those produced by lauryl mannose reaction system in numbers in the case of mannose moiety in lauryl mannose (Table 1). This phenomenon became more pronounced when the Maillard reaction was extended to 3 h (Table 2). However, concentrations of most MRPs formed by lauryl mannose systems in mannose moiety in lauryl mannose were greater than those by mannose system according to the peak area in GC spectra except for (D 1,6). It seems that the acylation of sugar would change the Maillard reaction pattern in the second phase of the Maillard reaction, especially affecting the formation of carbohydrate fragments. This may result the

production of fewer numbers but greater concentration of flavour substances (Tables 1 and 2).

Meanwhile, many other volatile products were formed in lauryl mannose Maillard reaction systems as seen from Nos. 9 to 47 of Table 1 and Nos. 31 to 50 in Table 2. Those products, such as decanal, 1-octanol, decanoic acid methyl ester, undecanone, undecanal and undecanol are typical volatiles produced as a result of interaction of Maillard reaction and fat degradation products coming from the heat-induced degradation of lauric acid moiety of lauryl mannoses. When reaction time was extended to 3 h, amounts of volatile compounds from lauryl mannose reaction systems in lauric acid moiety in lauryl mannose was substantially high compared to those from mannose moiety in lauryl mannose. This is because more lauric acid moieties in lauryl mannose were decomposed to volatile products under high temperature during the extended reaction time. In contrast, mannose just produces more primary carbohydrate fragments and converts them to secondary products at different Maillard reaction stages during the prolonged reaction time. The concentrations of volatile compounds produced by (D 1,6) was significantly lower than those by of mannose and other lauryl mannoses, indicating that (D 1,6) has quite poor Maillard reaction activity producing low numbers and amounts of volatile compounds compared to mannose or other lauryl mannoses.

All lauryl mannoses were composed of lauric acid and mannose moieties, the only difference is the acylated position of hydroxyl group (Fig. 1). The early stage of the Maillard reaction involves the Schiff base formation between the amino group of amino acid and the aldehyde group of the reducing sugar (Nobuyasu et al., 2002). The hemiacetal group, which can be converted to aldehyde group in some conditions, is necessary in the Maillard reaction, but the hemiacetal group of mannose is esterified to (D 1,6) by lauric acid. This might be the reason for poor Maillard reaction activity of (D 1,6).

In many cases, the Maillard reaction is undesirable during food processing and storage; colour changes will have the negative effect on the food quality, especially for fruit juices and beverages. Sugar esters are commonly used as emulsifiers and preservatives in beverage productions. As (D 1,6) retards the Maillard reaction, it may be useful in some food processing where browning reaction is not desirable.

4. Conclusion

This study demonstrated the presence of di-/triesters formed during the enzyme-catalyzed synthesis of SFAE, and identified three dilauryl mannose isomers. The results indicated that the enzymatic synthesis of sugar ester was not so effectively regioselective as has been expected.

Not only the degree of substitution and fatty acid chain length, but also the position of substitution is vital to the physicochemical properties of sugar ester. 1,6-Di-*O*-lauryl mannose retards the Maillard reaction, so it may be useful in some food processing operations, where browning reactions are undesirable. Selective synthesis of SFAEs with desired properties is essential for the specific food application. And there should be further studies, such as regioselective acylation mechanism and kinetic pattern of esterification, in order to obtain the desirable esterified lauryl mannose for the particular food applications.

Acknowledgements

We are grateful for financial support of this work by the National High-Tech Research and Development Program of China (863 Program 2006AA10Z312), the HI-TECH Sustentation Fund of Jiangsu (BG2005015) and PCSIRT under Grant IRT0532.

References

Adachi, S., Nagae, K., & Matsuno, R. (1999). Lipase-catalyzed condensation of erythritol and medium-chain fatty acids in acetonitrile with low water content. *Journal of Molecular Catalysis B: Enzymatic, 6*, 21–27.

Arcos, J. A., Bernabé, M., & Otero, C. (1998a). Quantitative enzymatic production of 6-*O*-acylglucose esters. *Biotechnology and Bioengineering, 57*, 505–509.

Arcos, J. A., Bernabé, M., & Otero, C. (1998b). Quantitative enzymatic production of 1,6-diacyl fructofuranoses. *Enzyme and Microbial Technology, 22*, 27–35.

Arcos, J. A., Hill, C. G., Jr., & Otero, C. (2001). Kinetics of the lipase-catalyzed synthesis of glucose esters in acetone. *Biotechnology and Bioengineering, 73*, 104–110.

Ferrer, M., Cruces, M. A., Bernabé, M., Ballesteros, A., & Plou, F. J. (1999) Lipase-catalyzed regioselective acylation of sucrose in two-solvent mixtures. *Biotechnology and Bioengineering, 65*, 10–16.

Ferrer, M., Cruces, M. A., Plou, F. J., Bernabé, M., & Ballesteros, A. (2000). A simple procedure for the regioselective synthesis of fatty acid esters of maltose, leucrose, maltotriose and *n*-dodecyl maltosides. *Tetrahedron, 56*, 4053–4061.

Jalbout, A. F., Shipar, M. A., & Navarro, J. L. (2007). Density functional computational studies on ribose and glycine Maillard reaction: Formation of the Amadori rearrangement products in aqueous solution. *Food Chemistry, 103*, 919–926.

Kim, J. S., Lee, Y. S. (2007). Effect of reaction pH on enolization and racemization reactions of glucose and fructose on heating with amino acid enantiomers and formation of melanoidins as result of the Maillard reaction. *Food Chemistry*, doi:10.1016/j.foodchem.2007.11.014.

Lertittikul, W., Benjakul, S., & Tanaka, M. (2007). Characteristics and antioxidative activity of Maillard reaction products from a porcine plasma protein–glucose model system as influenced by pH. *Food Chemistry, 100*, 669–677.

Liu, Q., Jia, C., Jiang, P., Zhang, X., Feng, B., Xu, S. (2007). Lipase-catalyzed selective synthesis of monolauroyl maltose using continuous stirred tank reactor. *Biotechnology Letters*, doi:10.1007/s10529-007-9560-x.

Nobuyasu, M., Tadashi, A., Chihiro, S., Hiroyuki, K., Mitsuharu, O., Junichi, H., et al. (2002). Screening system for the Maillard reaction inhibitor from natural product extracts. *Journal of Health Science, 48*, 520–526.

Scheckermann, C., Schlotterbeck, A., Schmidt, M., Wray, V., & Lang, S. (1995). Enzymatic monoacylation of fructose by two procedures. *Enzyme and Microbial Technology, 17*, 157–162.

Watanabe, Y., Adachi, S., Nakanishi, K., & Matsuno, R. (2001). Lipase-catalyzed synthesis of unsaturated acyl l-ascorbates and their ability to suppress the autoxidation of polyunsaturated fatty acids. *JAOCS, 78*, 823–826.

Watanabe, Y., Miyawaki, Y., Adachi, S., Nakanishi, K., & Matsuno, R. (2000). Synthesis of lauroyl saccharides through lipase-catalyzed condensation in microaqueous water-miscible solvents. *Journal of Molecular Catalysis B: Enzymatic, 10*, 241–247.

Watanabe, Y., Miyawaki, Y., & Adachi, S. (2001). Continuous production of acyl mannoses by immobilized lipase using a packed-bed reactor and their surfactant properties. *Biochemical Engineering Journal, 8*, 213–216.

Watanabe, Y., Miyawaki, Y., & Adachi, S. Nakanishi, K., & Matsuno, R. (2001). Equilibrium constant for lipase-catalyzed condensation of mannose and lauric acid in water-miscible organic solvents. *Enzyme and Microbial Technology, 29*, 494–498.

Zhang, X., Adachi, S. , Watanabe, Y., Kobayashi, T., & Matsuno, R. (2003). Prediction of the equilibrium conversion for the synthesis of acyl hexose through lipase-catalyzed condensation in water-miscible solvent in the presence of molecular sieve. *Biotechnology Progress, 19*, 293–297.

Zhang, X., Kobayashi, T. , Watanabe, Y., Fujii, T., Adachi, S., & Matsuno, R. (2003). Lipase-catalyzed synthesis of monolauroyl maltose through condensation of maltose and lauric acid. *Food Science and Technology Research, 9*, 110–113.

Zhou, J., Tao, G., Liu, Q., Li, H., Zhang, X., & Adach, S. (2006). Equilibrium yields of mono- and di-lauroyl mannoses through lipase-catalyzed condensation in acetone in the presence of molecular sieve. *Biotechnology Letters, 28*, 395–400.