Food Sci. Biotechnol. 22(3): 621-629 (2013)
DOI 10.1007/s10068-013-0123-8

RESEARCH ARTICLE

Optimization of Conditions for 2,2,6,6-Tetramethyl-1-piperidinyl Oxoammonium Ion/Sodium Hypochlorite-catalyzed Selective Oxidation of the Primary Alcohol in 1-Monolaurin

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Received: 29 August 2012 / Revised: 8 January 2013 / Accepted: 8 January 2013 / Published Online: 30 June 2013

Abstract Response surface methodology (RSM) was used to determine the optimum conditions for complete chemo-selective oxidization of the primary alcohol group in 1-monolaurin (1-ML) with dual catalysts, 2,2,6,6-tetramethyl-1-piperidine oxoammonium (TEMPO) and sodium hypochlorite (NaClO). Reaction conditions that required (i) the least amount of catalyst and (ii) the shortest reaction time were established. A statistical model of the degree of oxidation was proposed by response surface regression considering 5 factors: reactant pH, concentrations of the 2 catalysts, and reaction temperature, and time. Based on this proposed model, the relative effect of each factor could be predicted. The conditions that resulted in the lowest consumption of catalyst enabled oxidization of 2.744 g of 1-ML completely within 81 min with 18.4 mg TEMPO and 19.6 mL NaClO (pH 9.66, 34.5°C). The fastest reaction time (72 min) required 21.8 mg TEMPO and 19.9 mL NaClO (pH 10.98, 34.8°C). FT-IR and ^{13}C NMR analysis revealed that 1-ML was completely oxidized under 2 different optimal conditions and the chemo-selective oxidation of the primary alcohol occurred without oxidation of a secondary alcohol. After chemo-selective oxidation, 1-ML retained antibacterial activity against Gram-positive bacteria.

Keywords: 1-monolaurin, chemo-selective oxidation, 2,2,6,6-tetramethyl-1-piperidine oxoammonium (TEMPO), NaClO, response surface methodology

Introduction

1-Monolaurin (1-ML), a generally recognized as safe (GRAS) grade emulsifier and a derivative of monoacyl glycerol, is common food additive used in the food industry since 1977. Moreover, 1-ML receives much attention due to its antibacterial activity (1,2). However, the poor solubility and high melting temperature of 1-ML limit its application to the food industry (3).

Emulsifiers are generally classified on the basis of their hydrophilic-lipophilic balance (HLB) number, indicating the overall affinity of any emulsifier for aqueous or oil phases (4-6). An emulsifier with a low HLB number (4-6) is predominantly hydrophobic and readily solubilized into oil. This type of emulsifier is used for the formation of water-in-oil emulsions and reverse micelles in oil. An emulsifier having a high HLB number (8-18) is hydrophilic, dissolves in water, stabilizes oil-in-water emulsions, and forms micelles in water. An emulsifier with intermediate HLB number (about 7) has no particular preference for either solvent.

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The water solubility of low HLB (<6) monoacyl glycerols can be modified to increase their suitability for use in the food industry. Many organic acids, such as acetic, lactic, citric, and oxalic acids, have been used for the esterification of the hydroxyl group of monoacyl glycerol under high-temperature (about 250°C) and high-pressure (>2 atm) conditions using inorganic catalysts (7). This process is energy-intensive, and the end products are usually colored with an undersirable acidic taste.

Using a stable organic nitroxyl radical, the 2,2,6,6-tetramethyl-1-piperidinyl oxoammonium ion (TEMPO), sodium hypochlorite (NaClO), and sodium bromide (NaBr), deNooy *et al.* (8) developed a high yield method of selectively oxidizing the primary alcohol group, and successfully applied this to oxidization of, for example, methyl-α-D-glucopyranoside, methyl-β-D-glucopyranoside, α-trehalose, potato starch, and pullulan. Oxidation of the primary alcohol group of monoacyl glycerol using TEMPO can proceed under milder conditions and requires less energy. Also, using this TEMPO-mediated selective oxidation method, another research group has reported improved water solubilities of several polysaccharides, cyclomaltodextrins, and 1-monostrearyl glycerol (9-11). Ahn *et al.* (12) reported the chemo-selective oxidation of 1-ML using TEMPO/NaClO without NaBr, a compound toxic to the lungs and that is known to decrease fertility in rats.

As described above, the wide application of 1-ML to the food industry is limited by its poor water solubility. If there are the possible methods to increase the solubility of 1-ML, it could become very popular and acceptable emulsifier for the food production. Among the applicable methods, the oxidation of 1-ML could be considered. It is well known that several factors affect the oxidation of 1-ML but no study of the interaction of such factors and optimization of the process has been reported (12). Thus, response surface experimental design was used to determine the effects of 5 independent factors (pH, reaction time, reaction temperature, concentration of TEMPO, and amount of NaClO) on 1-ML oxidation in this study. Furthermore, we determined the antibacterial activity of oxidized 1-ML compared with native 1-ML.

Materials and Methods

Materials 1-Monolaurin (1-ML) was obtained from Ilshin Wells (Seoul, Korea). 2,2,6,6-Tetramethyl-1-piperidinyl oxoammonium ion (TEMPO) and NaClO solution were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Yakuri Pure Chemicals (Kyoto, Japan), respectively. All other reagents were of the extra pure grade.

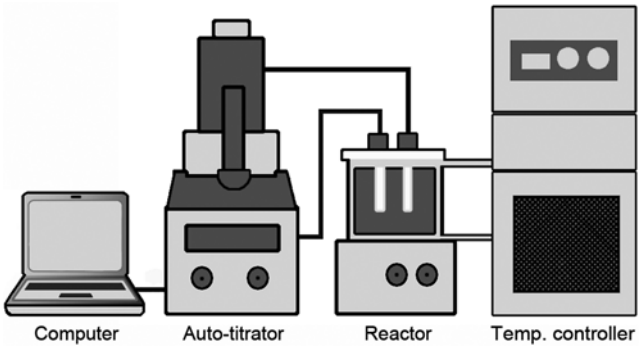

Fig. 1. Schematic representation of tools for the chemo-selective oxidation reaction.

Chemo-selective oxidation TEMPO and NaClO (12%, w/v) were dissolved in 100 mL distilled water to the appropriate concentrations. Reactant pH was monitored with a pH-Stat (Metrohm, Hensau, Switzerland). Solution pH was adjusted and maintained using 0.5 N HCl or 0.5 N NaOH as necessary. The oxidation reaction was initiated by mixing 1-ML (10 mmol, 2.744 g) with the appropriate catalysts and performed at pre-determined temperatures in a water bath. The volume of NaOH solution required to maintain initial pH was recorded at 10 min intervals using an automatic titrator (Fig. 1). The reaction was stopped when 20 mL of 0.5 N NaOH solution had been consumed.

Product recovery The oxidized 1-ML (OML) was collected through 2 different recovery methods. OML was precipitated by addition of 2 or 3 volumes of ethanol. After storage for 1 h, the supernatant was removed after centrifuging at 5,000×*g* for 20 min at 25°C and the precipitate was washed with acetone 3 or 4 times to remove TEMPO and NaClO. The precipitate was dried at 45°C *in vacuo* and followed by nitrogen gas flushing at 25°C. Another recovery method was based on the low water solubility of OML in cold temperature. OML solution was stored at 1-3°C so that in the precipitation of OML.

Degree of oxidation The degree of oxidation (DO) of the primary alcohol group of 1-ML was determined by measuring the amount of NaOH consumed to maintain initial pH according to the following equation:

$$\text{Degree of oxidation (DO, \%)} = \frac{\text{mol of NaOH consumed to maintain the initial pH}}{\text{initial mol of the primary alcohol in 1-ML}} \times 100$$

Experimental design A 5-factor-5-level central composite design was used to investigate the effect of each variable on DO of 1-ML. The independent variables were the pH (X_1), TEMPO concentration (X_2), NaClO amount (X_3), time

Table 1. Independent variables affecting the chemo-selective oxidation at the primary alcohol in 1-ML and their levels

Variables	Level					Interval
	-2	-1	0	1	2	
X_1 : pH	8.0	9.0	10.0	11.0	12.0	1.0
X_2 : TEMPO (mg)	10.0	15.0	20.0	25.0	30.0	5.0
X_3 : NaClO (mL)	5.0	10.0	15.0	20.0	25.0	5.0
X_4 : Reaction time (min)	40.0	60.0	80.0	100.0	120.0	20.0
X_5 : Temperature (°C)	20.0	25.0	30.0	35.0	40.0	5.0

(X_4), and temperature (X_5). According to the method of Cochran and Cox (13), the levels of independent variables were coded as −2, −1, 0, 1, and 2 (Table 1). The complete experimental design consisted of 32 combinations, including 6 replicates of the center point (Table 2). Experiments were performed in a random order.

Statistical analysis The results of each experiment were analyzed statistically using the SAS software (SAS Institute, Cary, NC, USA). Correlation coefficients (R^2) between subject variables were determined. The response surface regression (RSREG) procedure of SAS (JMP; SAS Institute) was used to fit the experimental data to the following second-order polynomial equation for DO (%, Y).

$$Y=\beta_0+\Sigma_{i=1}^{5}\beta_i X_i+\Sigma_{i=1}^{5}\beta_i X_i^2+\Sigma_{i=1}^{4}\Sigma_{j=i+1}^{5}\beta_{ij}X_i X_j \quad (1)$$

where, Y is the response variable (DO, %), β_0, β_i, β_{ii}, and β_{ij} are constant coefficients, and X_i, X_j represent independent variables defined previously. This second-order model was developed using multiple regression and analyses of variance (ANOVA). A $p<0.05$ were considered to indicate statistical significance.

Determination of water solubility Samples (1-ML and OML) were dispersed in 200 mL of distilled water. The sample solution was stirred at 25°C for 24 h and centrifuged at 5,000×*g* for 20 min. Ethanol (15 mL) was added to 5 mL of supernatant and centrifuged again at 5,000×*g* for 20 min. After the removal of supernatant, ethanol was evaporated at 45°C *in vacuo* for 12 h. The collected product was dried under nitrogen gas flushing and weighted to determine the amount of soluble material.

Structural analysis of oxidized 1-ML ^{13}C NMR spectra were obtained to determine the structure of the oxidation product of 1-ML using Bruker AMX-500 NMR instrument (Bruker Co., Bremen, Germany). An oxidized sample (2.5 mg/mL) was dissolved D_2O in a capillary tube, and denuterated dimethyl sulfoxide (DMSO) was used as an internal standard. IR analysis was performed on the oxidized 1-ML. Dry powder was applied with potassium bromide pellet as a control. Spectra were collected using a FTS-135 (Bio-Rad Co., Cambridge, MA, USA) to elucidate the change of 1-ML structure and confirm the formation of carboxyl group in OML.

Antibacterial effect of oxidized 1-ML Two strains each of *Escherichia coli* O157:H7 (ATCC 35150, ATCC 43889) and *Listeria monocytogenes* (ATCC 7644, ATCC 19115) were obtained from the bacterial culture collection of Seoul National University (Seoul, Korea). Each strain was cultured in tryptic soy broth (BD, Sparks, MD, USA) at 37°C for 24 h, harvested by centrifugation at 4,000×*g* for 20 min at 4°C, and washed 3 times with buffered peptone water (BPW) (BD). The final pellets were resuspended in BPW, corresponding to 10^6 to 10^7 CFU/mL. 1-ML or OML solution was prepared by pouring 95% ethanol containing the desired content of 1-ML or OML into 10 mL of the sterile distilled water. Then, 0.1 mL of suspension culture was applied to 10 mL of 1-ML or OML solution and the inoculated solutions were incubated for 30 min. To ensure the distribution of the bacterial culture, these solutions were well mixed immediately after inoculation and before enumeration. After 30 min incubation, 1 mL of the inoculated solution was transferred into 9 mL of the sterile buffered peptone water (BD) and was 10-fold serially diluted. The diluted solution (100 μL) was spread-plated onto selective medium including sorbitol MacConkey agar (SMAC) (BD), xylose lysine desoxycholate agar (XLD) (BD), Oxford agar base containing Oxford antimicrobic supplement (MOX) (BD), and Baird-Parker agar base (BPA) (BD), and enumerated. When low bacterial numbers were anticipated, 250 μL of the inoculated solution was plated onto 4 plates of each respective medium. The plates were incubated at 37°C for 24-48 h. Colonies were counted and calculated as log CFU/apple and log CFU/g in 1-ML or OML.

Results and Discussion

Product recovery The recovery of the OML was achieved by two methods. One is based on the precipitation of OML in organic solvent solution and another is the precipitation of OML at cold temperature. Compared to method using organic solvent, the precipitation at cold temperature could

Table 2. Experimental conditions of central composite second-order design

Run no.	Variables[1)]					Response (%)	
	X_1	X_2	X_3	X_4	X_5	Observed	Predicted
1	-1 (9)	-1 (15)	-1 (10)	-1 (60)	-1 (25)	44.1±1.8	39.5
2	-1 (9)	-1 (15)	-1 (10)	1 (100)	1 (35)	55.0±3.1	59.3
3	-1 (9)	-1 (15)	1 (20)	-1 (60)	1 (35)	83.3±2.3	84.6
4	-1 (9)	-1 (15)	1 (20)	1 (100)	-1 (25)	71.9±1.3	74.0
5	-1 (9)	1 (25)	-1 (10)	-1 (60)	1 (35)	54.7±2.9	53.4
6	-1 (9)	1 (25)	-1 (10)	1 (100)	-1 (25)	53.6±2.8	53.1
7	-1 (9)	1 (25)	1 (20)	-1 (60)	-1 (25)	69.2±1.3	65.7
8	-1 (9)	1 (25)	1 (20)	1 (100)	1 (35)	106.0±1.7	105.5
9	1 (11)	-1 (15)	-1 (10)	-1 (60)	1 (35)	48.2±0.8	48.1
10	1 (11)	-1 (15)	-1 (10)	1 (100)	-1 (25)	51.4±2.6	52.0
11	1 (11)	-1 (15)	1 (20)	-1 (60)	-1 (25)	40.1±2.8	37.8
12	1 (11)	-1 (15)	1 (20)	1 (100)	1 (35)	108.0±2.6	106.6
13	1 (11)	1 (25)	-1 (10)	-1 (60)	-1 (25)	27.0±0.6	22.1
14	1 (11)	1 (25)	-1 (10)	1 (100)	1 (35)	86.5±1.8	90.5
15	1 (11)	1 (25)	1 (20)	-1 (60)	1 (35)	88.2±2.1	89.2
16	1 (11)	1 (25)	1 (20)	1 (100)	-1 (25)	84.3±4.1	86.1
17	-2 (8)	0 (20)	0 (15)	0 (80)	0 (30)	74.0±1.8	73.6
18	2 (12)	0 (20)	0 (15)	0 (80)	0 (30)	75.0±2.7	72.9
19	0 (10)	-2 (10)	0 (15)	0 (80)	0 (30)	79.9±2.9	77.2
20	0 (10)	2 (30)	0 (15)	0 (80)	0 (30)	93.0±1.2	93.2
21	0 (10)	0 (20)	-2 (5)	0 (80)	0 (30)	34.5±1.1	37.0
22	0 (10)	0 (20)	2 (25)	0 (80)	0 (30)	103.5±2.1	94.9
23	0 (10)	0 (20)	0 (15)	-2 (40)	0 (30)	33.9±1.7	42.3
24	0 (10)	0 (20)	0 (15)	2 (120)	0 (30)	108.5±2.1	89.0
25	0 (10)	0 (20)	0 (15)	0 (80)	-2 (20)	31.9±1.6	38.8
26	0 (10)	0 (20)	0 (15)	0 (80)	2 (40)	105.0±2.8	90.6
27	0 (10)	0 (20)	0 (15)	0 (80)	0 (30)	88.3±1.2	87.9
28	0 (10)	0 (20)	0 (15)	0 (80)	0 (30)	90.7±2.3	87.9
29	0 (10)	0 (20)	0 (15)	0 (80)	0 (30)	90.6±4.0	87.9
30	0 (10)	0 (20)	0 (15)	0 (80)	0 (30)	83.9±2.4	87.9
31	0 (10)	0 (20)	0 (15)	0 (80)	0 (30)	85.0±2.8	87.9
32	0 (10)	0 (20)	0 (15)	0 (80)	0 (30)	86.5±3.7	87.9

[1)]For the identification of X_1, X_2, X_3, X_4, and X_5, refer to Table 1.

be simple and less labor-intensive recovery method and it will be able to achieve solvent-free oxidized product. Recovery yield of OML by precipitation at cold temperature is expected to increase compared to that of previous solvent recovery because of the simplified and less repetition of recovery procedure. In addition, when considering commercialization of OML in the future, it is also crucial to minimize the residue of the 2 catalysts, NaClO and TEMPO involved in the selective oxidation. The 2 catalysts are both ionic compounds, easily dissolved even in ambient temperature water, which will make the catalysts to be removed by several times of washing with water.

Statistical analysis A central composite experimental design was used to identify the optimum conditions for complete chemo-selective oxidization of the primary alcohol group of 1-ML (Table 2). The data were fitted to a second-order polynomial model. Regression coefficients were calculated and the equation for predicting the degree of oxidation was:

$$DO\ (\%) = -234.725 + 23.634X_1 - 4.230X_2 + 7.524X_3 - 0.045X_4 + 6.743X_5 - 3.669X_1^2 - 0.027X_2^2 - 0.219X_3^2 - 0.014X_4^2 - 0.232X_5^2 + 0.289X_1X_2 - 0.219X_1X_3 + 0.280X_1X_4 + 0.824X_1X_5 + 0.058X_2X_3 + 0.014X_2X_4 + 0.041X_2X_5 + 0.002X_3X_4 + 0.094X_3X_5 - 0.009X_4X_5 \quad (2)$$

where, Y represents the response variable (dependent variable), DO (%); and X_1, X_2, X_3, X_4, and X_5 independent variables) represent pH, TEMPO, NaClO, reaction time, and temperature, respectively. The adequacy and fit quality of the second-order model equation was confirmed by ANOVA (Table 3). The fit of the model was also assessed

Table 3. Analysis of variance for the second-order model obtained from central composite experimental design

Source of variation		DF	Sum of square	*F* value	Prob>*F*
Regression	Linear	5	12,700.000	46.68	<0.0001
	Qudratic	5	2,575.856	9.47	0.0011
	Cross product	10	1,008.386	1.85	0.1630
	Total model	20	16,284.000	14.96	<0.0001
Residual	Lack of fit	6	557.804	11.42	0.0086
	Pure error	5	40.700		
	Total error	11	11,598.504		

R^2=0.9645

by the coefficient of multiple determination of the polynomial model, R^2, which was found to be 0.9645. This indicates that 96% of the variability of the response can be explained by the second-order polynomial model (Table 3).

Analysis of the response surface The relationship between variables and the response was investigated by a 3-dimensional representation of the response surface curve. The response surface curves consist of the DO (%) against 2 of the variables while the remaining 3 variables are held constant at their mean values (Fig. 2). Chemo-selective oxidation was enhanced by increasing the amount of TEMPO (Fig. 2A). A high DO can be obtained using large amounts of TEMPO (over 25 mg) and at pH about 10. In contrast, use of large amounts of NaClO increased chemo-selective oxidation throughout the pH range (Fig. 2B). NaClO converted TEMP (the reduced form of TEMPO) to TEMPO, which then facilitated the oxidation reaction (8). Thus, the increase in DO as NaClO amount increased can be explained by the fast regeneration of TEMPO. The effect of varying reactant pH and reaction time on chemo-selective oxidation as shown in Fig. 2C while the remaining 3 variables were maintained at their mean levels (20 mg TEMPO, 15 mL NaClO, 30°C). Chemo-selective oxidation decreased at low pH and with shorter reaction time. Lower temperature dramatically decreased DO at high pH values (Fig. 2D). As the reaction temperature became closer to the melting temperature of 1-ML (approximately 64°C), 1-ML underwent a phase transition from solid to liquid and the collision frequency between 1-ML and TEMPO increased. As the concentration of both TEMPO and NaClO was raised, the rate of chemo-selective oxidation also increased (Fig. 2E). However, NaClO had a greater effect than did TEMPO. This suggested that the concentration of NaClO, but not TEMPO, represented the rate-limiting factor during TEMPO-mediated selective oxidation in the absence of NaBr. Because NaClO regenerates TEMPO from its reduced form, NaClO acts as the oxygen provider during chemo-selective oxidation. It was possible to scale-up the reaction system by increasing the supply of not only substrate, but also NaClO. These data suggest that a semi-continuous TEMPO-mediated selective oxidation system is feasible even without NaBr; indeed, this was successfully applied to chemo-selective oxidation of the primary alcohol groups of many substrates (14,15). DO increased with reaction time regardless of TEMPO concentration (Fig. 2F). Higher reaction temperature accelerated chemo-selective oxidation, but only up to 35°C (Fig. 2G, 2I). Other authors have reported depolymerization of starch or glucopyranose during TEMPO-mediated oxidation under alkaline conditions (pH>9.0) and high temperatures (>25°C) (15,16). The efficiency of chemo-selective oxidation under such conditions was influenced by the occurrence of side reactions, such as thermal oxidation of secondary alcohols. The greatest DO resulted from use of a high amount of NaClO and a long reaction time at high temperature (Fig. 2H, 2J).

Optimization of chemo-selective oxidation and verification Conditions that resulted in 100% oxidation of the primary alcohol group of 1-ML were established. In most processes, 'optimum conditions' generally refers to the combination of the values of each variable those results in the desired effect. In this study, however, many such combinations resulted in 100% oxidation, because reaction time was included as a variable. Process conditions that resulted in the lowest (i) reaction time and (ii) consumption of catalyst were determined. At pH 9.2, the lowest that enabled complete oxidation, the reaction time at 33°C was 80 min (Fig. 2C, 2D). However, the fastest reaction required 72 min at pH 10.9 (Fig. 2C). Although the lowest temperature at which complete oxidation was possible was 32°C, temperature optimization was problematic because of loss of selectivity above 35°C. The amount of NaClO required for complete oxidation varied depending on the pH (9.5-11.8, Fig. 2B). Although at least 20 mg of TEMPO was required to achieve 100% of DO, reduced amounts of TEMPO could be compensated for by increasing the NaClO concentration (Fig. 2E).

According to our model, the conditions under which complete oxidation occurred in the least time (72 min) were: 34.8°C, pH 10.9, 21.8 mg TEMPO, and 19.9 mL

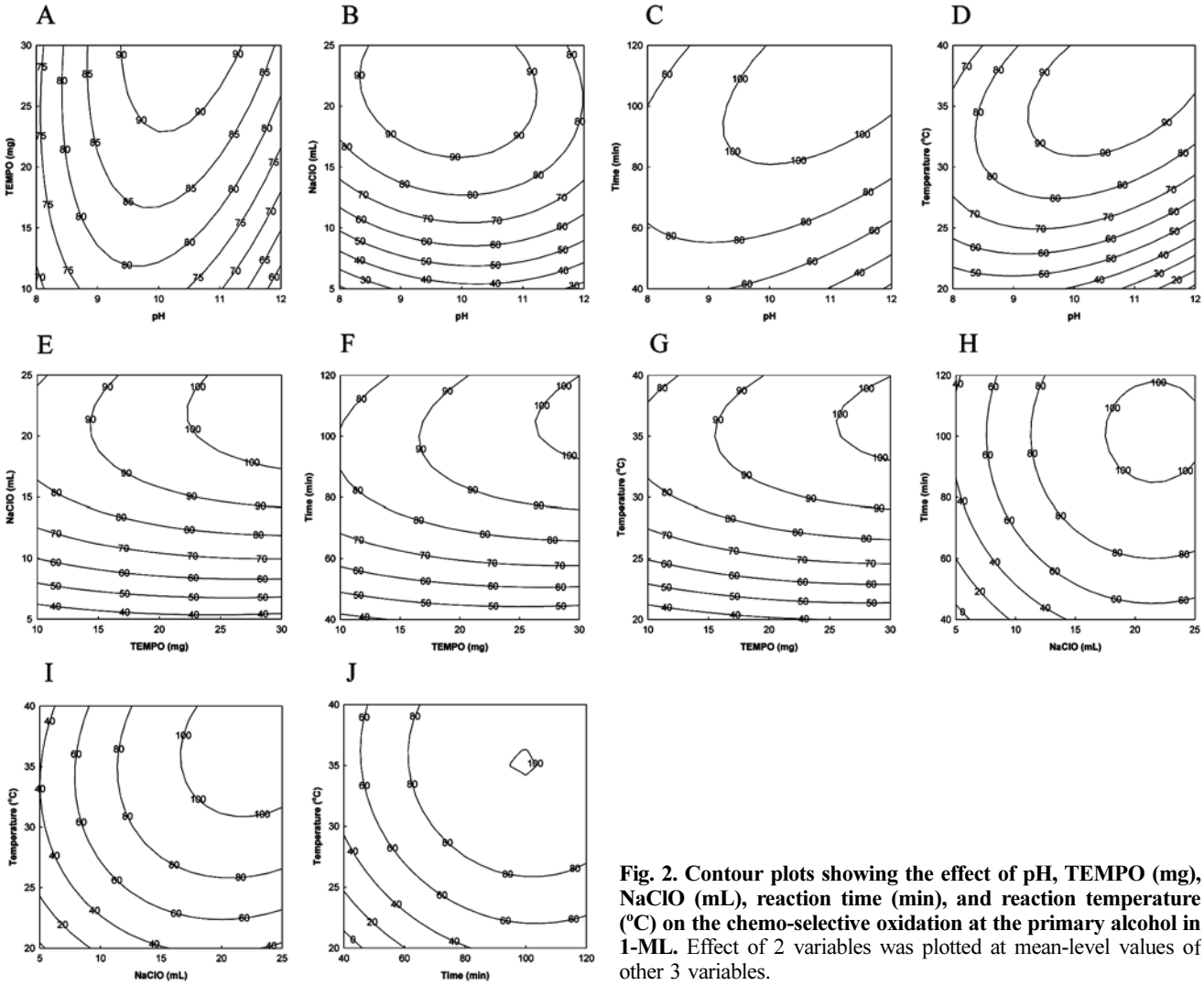

Fig. 2. Contour plots showing the effect of pH, TEMPO (mg), NaClO (mL), reaction time (min), and reaction temperature (°C) on the chemo-selective oxidation at the primary alcohol in 1-ML. Effect of 2 variables was plotted at mean-level values of other 3 variables.

NaClO. Minimal consumption of both TEMPO and NaClO required 18.4 mg TEMPO, 19.6 mL NaClO, pH 9.66, 34.5°C, and an 81 min reaction time. Complete oxidation was attained experimentally within 75 and 83 min (the fastest process and the lowest catalyst consumption, respectively). These values were similar to those predicted by our model, indicating the model's value. In comparison with the study of chemo-selective oxidation of 1-ML by Chang *et al*. (10), the reaction time we report here was markedly shorter, thereby allowing improvement of the efficiency of chemo-selective oxidation without the need for hazardous NaBr.

Water solubility of oxidized 1-ML At 100% of degree of oxidation, water solubility drastically increased from 0.13 to 4.32 mg/mL. This dramatic increase in water solubility after chemo-selective oxidation could be due to the relatively strong hydrophilicity of the carboxyl group that was newly introduced. Our result has good agreement with the elevation of water solubility of 1-ML after TEMPO-mediated oxidation was increased up to 7.3 mg/mL (10).

Structural analysis of the oxidized 1-ML The structural changes of 1-ML were determined before and after chemo-selective oxidation. 1-ML has 2 alcohol groups in it; the primary (-CH_2OH) and secondary (-CHOH-) alcohols. During the oxidation reaction, primary alcohol could be oxidized to carboxylic acid via aldehyde and secondary alcohol is oxidized to ketone. Comparing the structural properties of OML to the native 1-ML by determining the presence of carboxylic or ketone group, it could be confirmed which alcohol group is oxidized. IR spectra of 1-ML and OML are shown in Fig. 3. IR spectra of OML showed a sharp peak at 1,629/cm, regardless of the oxidation conditions. The sharp peak at 1,629/cm is rigid clue to the presence of a C=O bond in OML. The broad peak in the range of 3,200-3,600/cm was observed in 1-ML and OML, responding the presence of hydroxyl group. This observation suggests that OML still has one and more alcohol groups in it. If the secondary alcohol in 1-ML was

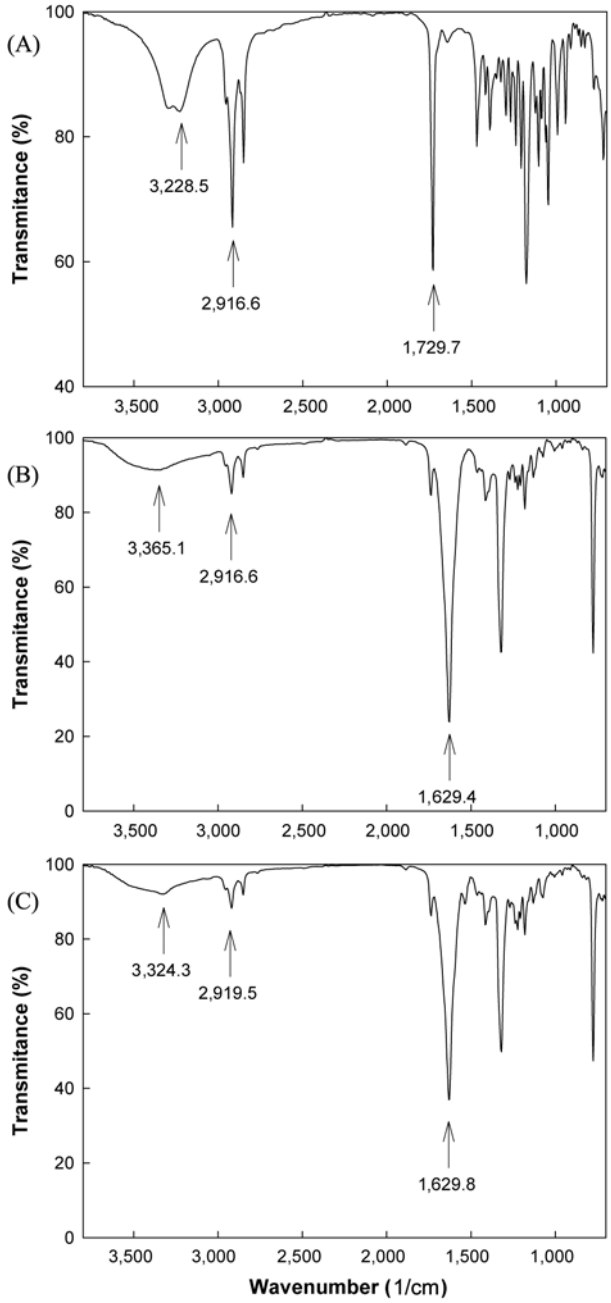

Fig. 3. FT-IR spectra of 1-ML and oxidized 1-ML. (A) 1-ML, (B) oxidized 1-ML under the condition for the fastest process, (C) oxidized 1-ML under the condition for the minimum catalyst consumption

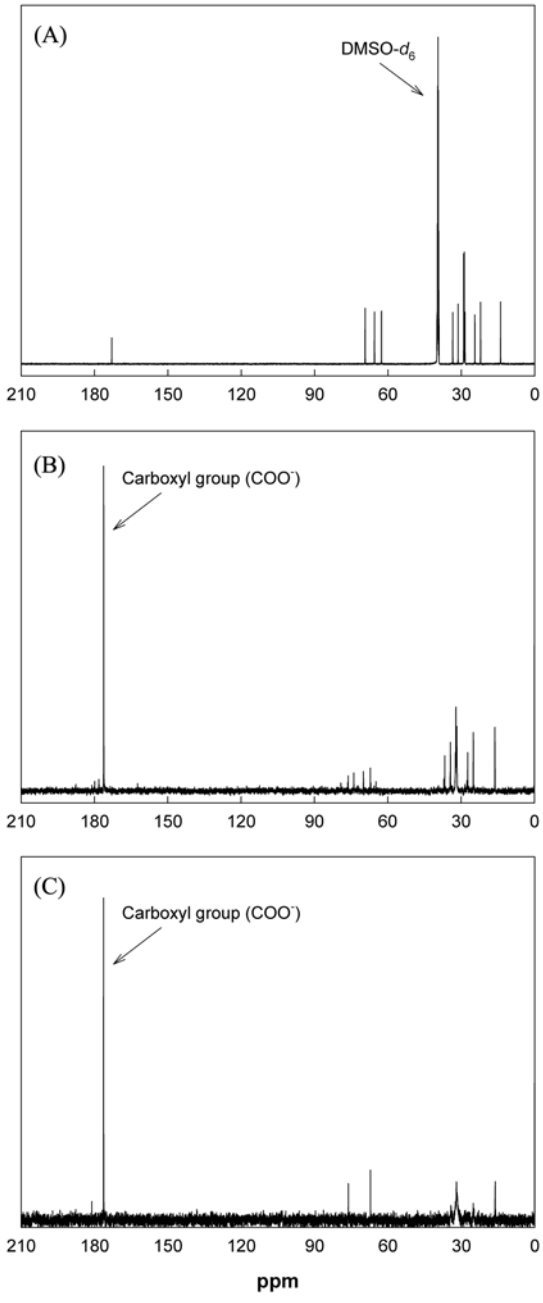

Fig. 4. ^{13}C NMR spectra of 1-ML and oxidized 1-ML. (A) 1-ML, (B) oxidized 1-ML under the condition for the fastest process, (C) oxidized 1-ML under the condition for the minimum catalyst consumption

predominately oxidized during the reaction, the peak in the range of 3,200-3,600/cm was expected to disappear and the peak representing ketone group could be appeared. The peak for carboxylic group and no peak for ketone group in the spectra of OML revealed the presence of a secondary alcohol in OML, indicating there was no oxidation of secondary alcohol. The result obtained from ^{13}C NMR analysis has a good agreement with that from IR analysis (Fig. 4). The new resonance in 176 ppm in OML, independent on the oxidation condition, could be resulted from the formation of carboxyl group. No resonance in the region of 198-205 ppm in the OML suggested that the ketone group, the oxidative product of secondary alcohols, was not formed during the oxidation reaction.

FT-IR and ^{13}C NMR analysis confirmed that chemo-selective oxidation was performed at only primary alcohol in 1-ML without the oxidation of secondary alcohol and that there was no structural difference between OML under

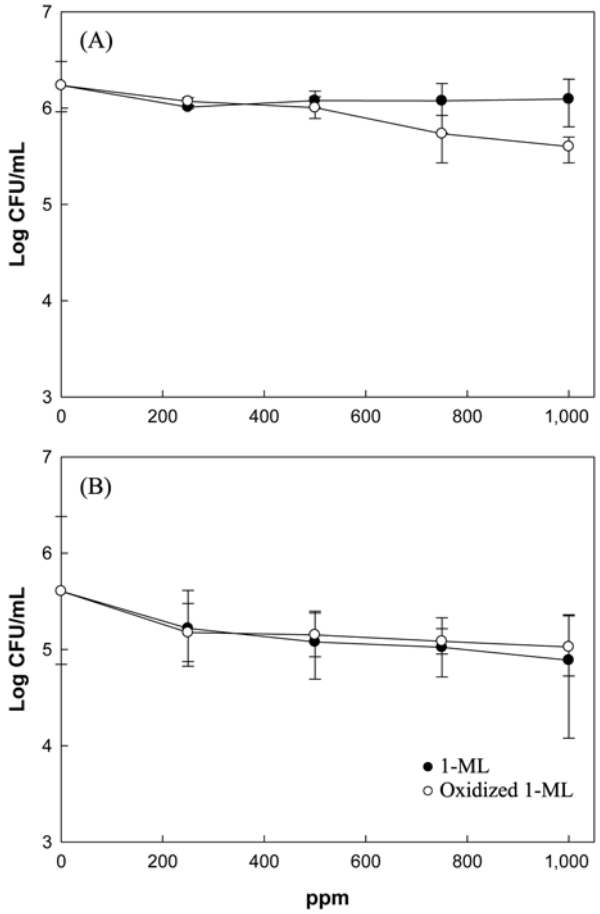

Fig. 5. Antibacterial activity of 1-ML and oxidized 1-ML on *E. coli* (A) and *L. monocytogenes* (B).

the distinguishable conditions evaluated by response surface method.

Antibacterial activity of oxidized 1-ML It is well known that monolaurin has antibacterial activity, not much high like as the commercially available antibacterial agents. Generally, monolaurin shows more antibacterial effect on Gram-positive bacteria such as *Staphylococcus* and *Streptococcus* and *L. monocytogenes* (17,18). Although monolaurin shows very weak or no antibacterial activity against Gram-negative bacteria such as *E. coli* and *Klebsiella pneumonia*, monolaurin sometimes has antibacterial activity against *Helicobacter pylori* and *Hemophilus influenza* (19). Comparing the commercial antibacterial agents, 1-ML and OML showed the very low antibacterial activity against *E. coli* and *L. monocytogenes* at any concentration tested (Fig. 5). OML showed very low antibacterial activity (0.74 log) against *E. coli* at a high dosage (1,000 ppm) when 1-ML did not. In case of *L. monocytogenes* (Fig. 5B), both of 1-ML and OML showed the count reduction to the negligible extent. The incubation at 1,000 ppm 1-ML or OML reduced counts by approximately 0.65 log. This observation suggests that 1-ML does not lose its antibacterial activity after oxidation because there is no significant difference in antibacterial activity before and after oxidation. Our observation had a good agreement to the previous studies (17-19).

Acknowledgments This work was financially supported in part by a grant (10162KFDA995) from Korea Food & Drug Administration in 2012 and by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2011-0010786).

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