Physicochemical Properties of Selectively Oxidized 1-Monolaurin from 2,2,6,6-Tetramethyl-1-Piperidinyl Oxoammonium Ion/Sodium Hypochlorite-Mediated Reaction

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The primary alcohol group of 1-monolaurin (1-ML) was selectively oxidized using 2,2,6,6-tetramethyl-1-piperidine oxoammonium ion/sodium hypochlorite (NaOCl) without NaBr at two different conditions. The selective oxidation occurred more efficiently at 35 °C and 32.2 mmol of NaOCl than at 25 °C and 18.7 mmol of NaOCl. Regioselective oxidation of the primary alcohol without oxidation of a secondary alcohol was confirmed by a chemical shift at 175 ppm and no resonance between 198 and 205 ppm in ^{13}C NMR and the presence of a peak at 1560–1570 cm^{-1} in IR spectra. The water solubility of oxidized 1-monolaurin (OML) was remarkably increased by 33.2 times as compared to that of 1-ML. Creaming velocities resulting from fat flocculation in a 0.2% level of OML and 1-ML were 0.16 and 1.13 mm/h, respectively, implying that OML showed higher efficiency and emulsion stability in preventing fat flocculation than 1-ML due to the selective oxidation of primary alcohol.

KEYWORDS: Selective oxidation; 1-monolaurin; NMR; water solubility; emulsion stability

INTRODUCTION

1-Monolaurin (1-ML), a kind of emulsifier, has been warranted as GRAS (generally recognized as safe) in food additives since 1977. However, the unique physical properties of 1-ML such as a high melting point and low solubility in water limit its applications to food industries (*1*).

Water solubility of emulsifiers can be determined by hydrophile and lipophile balance (HLB) number, the concept of which was introduced by Griffin in 1949 (*2*) and defined as the relative percentage of hydrophilic to hydrophobic groups within the surfactant molecule. Lipophilic emulsifiers having low HLB numbers (below 6) usually facilitate the formation of water-in-oil (W/O type) emulsions, whereas hydrophilic emulsifiers with high HLB numbers (above 8) prefer to form oil-in water (O/W type) emulsions (*3–7*).

The applicability of low HLB emulsifiers in food industry is somewhat limited due to the low solubility in water. For example, monoacyl glycerol is a typical emulsifier used for the emulsion of cream product with low water solubility. To increase the water solubility of low HLB emulsifiers, organic acids such as acetic, lactic, citric, and oxalic acids can be esterified to the hydroxyl group in the monoacyl glycerol under high-temperature, around 250 °C, and high-pressure conditions using inorganic catalysts. This process is energy intensive, and the end products are usually colorized with an undesirable acidic taste; thus, its applications to food processes are very limited (*8–10*).

DeNooy et al. reported 2,2,6,6-tetramethyl-1-piperidine oxoammonium ion (TEMPO)/hypobromite-mediated oxidation of the primary alcohol groups in methyl-α-D-glucopyranoside, methyl-β-D-glucopyranoside, α-trehalose, potato starch, and pullulan (*11, 12*). Chang and Robyt showed that selective oxidation of primary alcohol groups in polysaccharides and cyclomaltodextrins using TEMPO/hypobromite improved the water solubility significantly (*13, 14*). Also, Chang and Cho revealed that selective oxidation of primary alcohol groups in polysaccharides did not occur at the catalytic condition of sodium hypochlorite (NaOCl) without TEMPO (*15*).

In previous TEMPO-mediated oxidation studies, TEMPO was an actual oxidant regenerated by NaOBr, whereas NaOBr was regenerated from NaBr by NaOCl. However, NaBr was not only known as a toxic compound to lungs (*16, 17*), but it was also reported that continuous exposure of NaBr to rats led to a decrease in fertility (*18*). Therefore, TEMPO-mediated oxidation without NaBr was attempted in this study.

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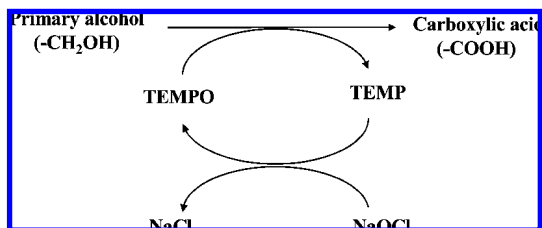

Figure 1. Principle of selective oxidation of primary alcohol by TEMPO/NaOCl. TEMPO is reduced by the selective oxidation of primary alcohol into carboxylic acid, and then, TEMPO is regenerated by the reduction of NaOCl.

The objectives of this study were to oxidize 1-ML selectively into oxidized 1-monolaurin (OML) using TEMPO/NaOCl without NaBr, to confirm the structure of OML through ^{13}C NMR and IR spectrometry, and to determine the water solubility of OML and emulsion stability.

MATERIALS AND METHODS

Materials. 1-ML was obtained from Ilshin Wells Co. (Seoul, Korea). TEMPO was purchased from Sigma-Aldrich (Milwaukee, WI), and NaOCl solution (12%, w/v) was purchased from Yakuri Pure Chemicals Co. (Kyoto, Japan). All other chemicals were of extra pure grade.

Selective Oxidation. TEMPO (0.1 mmol, 16 mg) and two different levels (18.7 mmol, 11.6 mL; and 32.2 mmol, 20 mL) of NaOCl solution (12%, w/v) were dissolved in 100 mL of distilled water by vigorous stirring. The starting pH was adjusted to 10.8 with 0.5 N HCl and maintained with 0.5 N NaOH solution by a pH-Stat (Metrohm Ltd., Hensau, Switzerland) as the oxidation reaction proceeded. Oxidation was initiated by adding 10 mmol of 1-ML (2.744 g) and was conducted at 25 and 35 °C, respectively. The schematic representation for the reaction principle is shown in **Figure 1**. The volume (mL) of consumed 0.5 N NaOH solution was recorded every 10 min as the oxidation proceeded. Reaction products were sampled at different oxidation degrees by adding 2.5 mL of ethanol/100 mL of solution, followed by neutralization with 4 N HCl.

Product Recovery. OML was precipitated by addition of 2 or 3 volumes of ethanol. After storage for 1 h, the supernatant was removed after centrifugation at 5000*g* for 20 min, and the precipitate was washed with acetone three or four times to remove TEMPO and other catalysts. The precipitate was dried at 45 °C in vacuo and followed by nitrogen gas flushing at ambient temperature.

Degree of Oxidation. The degree of oxidation reaction from primary alcohol group in 1-ML to carboxyl group in OML was defined as:

$$\text{degree of oxidation(DO, \%)} = \frac{\text{mol of NaOH consumed to maintain the initial pH of 10.8}}{\text{initial mol of the primary alcohol group in 1-ML}} \times 100 \quad (1)$$

Samples were prepared in triplicate.

^{13}C NMR Analysis. Proton-coupled ^{13}C NMR spectra were obtained to determine the structure of the oxidation product of 1-ML using a Bruker AMX-500 NMR instrument (Bruker Co., Bremen, Germany). An oxidized sample (2.5 mg/mL) was dissolved using D_2O in a capillary tube, and deuterated dimethyl sulfoxide was used as an internal standard.

IR Analysis. IR analysis was performed on 1-ML and TEMPO OML. Dry powder was applied with potassium bromide pellet as a control. Spectra were collected using a FTS-135 (Bio-Rad Co., Cambridge, MA) to elucidate the change of 1-ML structure and confirm the formation of carboxyl group in OML.

Water Solubility. Two grams of sample precipitated by ethanol at each degree of oxidation was dispersed in 200 mL of distilled water. The sample mixture was stirred at 25 °C for 24 h and centrifuged at 5000*g* for 20 min. Fifteen milliliters of ethanol was added to 5.0 mL of clear supernatant solution and centrifuged at 5000*g* for 20 min again. The supernatant was removed, and then, ethanol was evaporated at 45
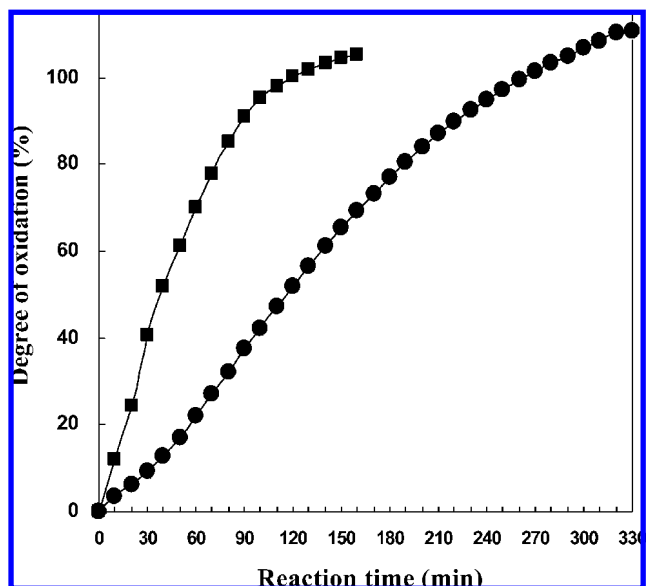

Figure 2. Time courses of TEMPO-mediated oxidation of 1-ML at two different conditions: ●, 0.1 mmol of TEMPO and 18.7 mmol of NaOCl at 25 °C; ■, 0.1 mmol of TEMPO and 32.2 mmol of NaOCl at 35 °C.

°C in vacuo for 12 h. The collected product was dried under nitrogen gas flushing and then weighed to determine the amount of soluble material. Samples were taken after 0, 25, 50, 75, and 100% degrees of oxidation.

HLB Number. HLB numbers of 1-ML and OML were calculated according to Griffin's method (*2*), that is

$$\text{HLB number} = \frac{\text{molecular mass of the hydrophilic portion* of the molecule}}{\text{molecular mass of the whole molecule}} \times 20 \quad (2)$$

Here, *hydrophilic portion was determined by Griffin's definition.

Emulsion Stability. Anhydrous olive oil was obtained from Dong Suh Oil & Fat Co. Ltd. (Changwon, Korea) to form a water−emulsifier−oil emulsion solution. The concentration of 1-ML or OML was varied from 0.2 to 0.4% (w/w), whereas the mixing ratio of water and olive oil was changed from 9:1 to 5:5 (w/w). The emulsion was prepared by a Ultraturrax T25 (Janke & Kunkel Co., Staufen, Germany) at 3000 rpm and 55 °C for 1 min. The emulsion stability was monitored using an optical scanning instrument (Turbiscan LAb Expert, Formulaction, France). This instrument measured the backscattering of an emulsion as a function of the height of the test tube and time at 55 °C, and transmission and backscattering data were acquired at every 10 min for 2 h; thereafter, the creaming velocities of the fat globules for each sample were determined. Emulsions were placed into flat-bottomed cylindrical glass tubes (70 mm height, 25 mm internal diameter). The destabilization phenomena of the emulsion sample such as creaming, flocculation, and sedimentation can be characterized by the backscattering pattern over time. From this result, time-dependent behavior of the emulsion could be monitored and analyzed quantitatively (*19−21*).

RESULTS AND DISCUSSION

TEMPO-Mediated Oxidation of 1-ML. Time courses of TEMPO-mediated oxidation of 1-ML at two different conditions are shown in **Figure 2**. It took 4.3 h to complete the oxidation under the condition of 0.1 mmol of TEMPO and 18.7 mmol of NaOCl, pH 10.8 at 25 °C, and the degree of oxidation vs reaction time showed almost linear correlation. In contrast, when the reaction was conducted at 35 °C and 32.2 mmol of NaOCl, a different oxidation pattern was observed. The reaction was completed in 2 h after the initiation of oxidation, with 52% of total oxidation occurring within 40 min. The faster reaction rate
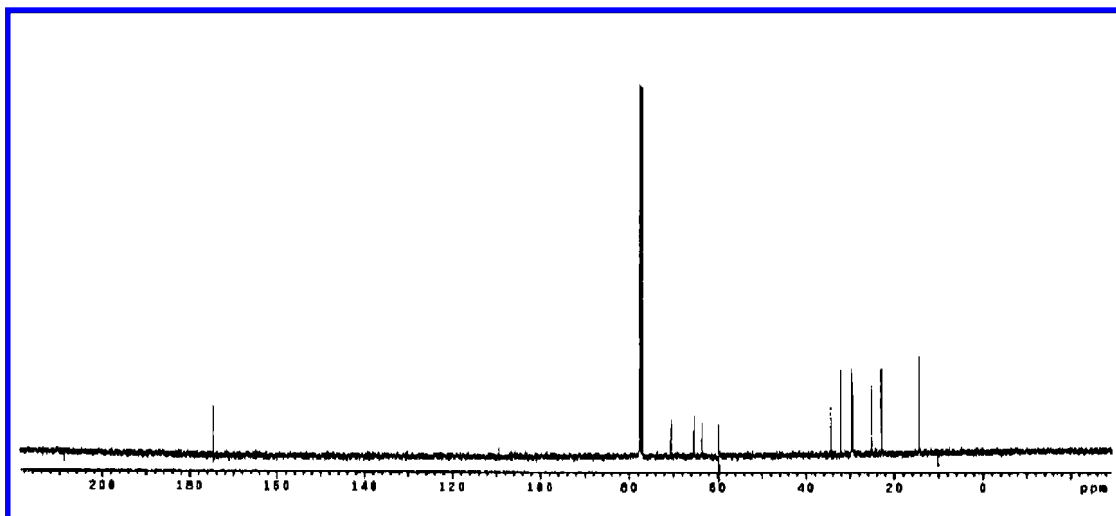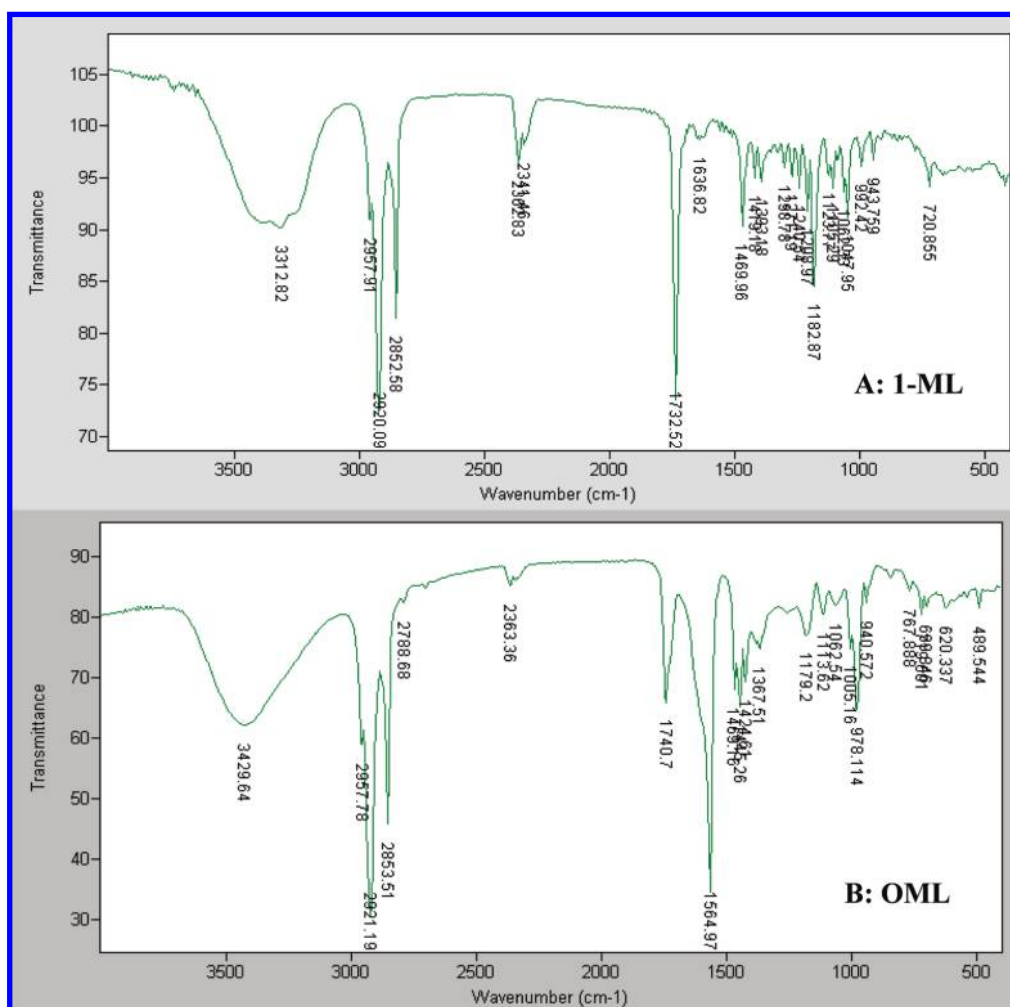
Figure 3. ^{13}C NMR spectra of OML.

Figure 4. IR spectra of 1-ML (**A**) and OML (**B**).

at higher temperature and higher concentration of NaOCl can be explained in two ways. First, as the temperature was closer to the melting point of 1-ML, the solid fat of 1-ML would be gradually reduced to liquid oil much like the tendencies of margarine and cooking fats exerted by heating (*22*). Therefore, the collision frequency between 1-ML and the other components for the oxidation reaction would be increased. Second, NaOCl was able to regenerate TEMP (reduced form of TEMPO) to TEMPO, and the regenerated TEMPO favorably facilitated the

oxidation reaction (*11, 12*). The rate of oxidation reaction was decreased as the reaction proceeded (**Figure 2**), which might be due to the limitation of available NaOCl (*23, 24*).

^{13}C NMR Spectrometric Analysis. Proton-coupled ^{13}C NMR spectra of 1-ML and OML are shown in **Figure 3**. The spectra of OML showed new resonance in 175 ppm, which resulted from the formation of carboxylic acid, while no resonance was observed in the region of 198−205 ppm, indicating that the keto group was not formed during the oxidation reaction (**Figure**
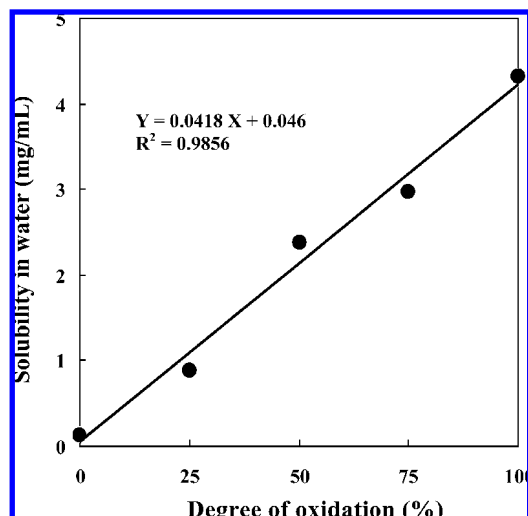
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Figure 5. Effects of degree of oxidation on the water solubility of 1-ML.

Figure 6. Structural representation of 1-ML and OML for the calculation of HLB number.

3). Therefore, ^{13}C NMR spectra of OML provided good evidence that only the primary alcohol in 1-ML was oxidized to carboxylate in OML during TEMPO-mediated oxidation. This result was consistent with reports on the regioselective oxidation of primary alcohol in polysaccharides and cyclomaltodextrins, which was conducted by De Nooy et al. (*11*) and Chang and Robyt (*13, 14*).

IR Spectrometric Analysis. IR spectra of the 1-ML and OML are shown in **Figure 4**. IR spectra of OML showed a sharp peak at $1560-1570$ cm^{-1}, implying the presence of a C=O bond in OML. Spectra showing bell-shaped peaks are responsible for the presence of OH group in the range of $3200-3600$ cm^{-1}. If the secondary alcohol in 1-ML is oxidized during the reaction, the peak in the range of $3200-3600$ cm^{-1} is expected to disappear. However, the bell-shaped peak observed in the spectrum of the oxidized product revealed the presence of a secondary alcohol ($-$CHOH$-$). Because 1-ML is an ester compound, it has a group with the formula $-$OC$-$O$-$. This ester group was not affected by the oxidation, which is proved by the presence of the peak near 1730 cm^{-1}. In addition, both 1-ML (**Figure 4A**) and OML (**Figure 4B**) showed the same peaks between 1200 and 1500 cm^{-1}, which signify twisting and rocking of $-$CH$_2$ groups in the lauric acid. Therefore, the information obtained from IR spectra confirmed that the selective oxidation was performed at only primary alcohol in 1-ML without modifications of secondary alcohol, ester, and methylene groups in the same substrate.

Oxidation Effects on Water Solubility. Water solubility of the products at different degree of oxidation is shown in **Figure 5**. As the degree of oxidation increased from 0 to 100%, water solubilities were proportionally increased from 0.13 mg/mL up to 4.32 mg/mL. Regression analysis showed that the water solubility was changed according to an equation of $Y = 0.0418X + 0.046$ ($R^2 = 0.9856$), where Y is the water solubility (mg/mL) and X is the degree of oxidation (%). This change could be derived from the strong hydrophilicity exerted by the carboxyl group. Similarly, Chang et al. (*25*) reported that the water solubility of 1-monostearin was increased from 0.5 to 7.3 mg/mL by TEMPO-mediated oxidation.

Oxidation Effects on HLB Number. Hydrophilic moieties of 1-ML are shown in **Figure 6**. The molecular weights of 1-ML and OML are 274 and 288 g/mol, respectively, and the molecular weights of the hydrophilic portions in 1-ML and OML are 61 and 75 g/mol, respectively. Thereafter, calculated HLB
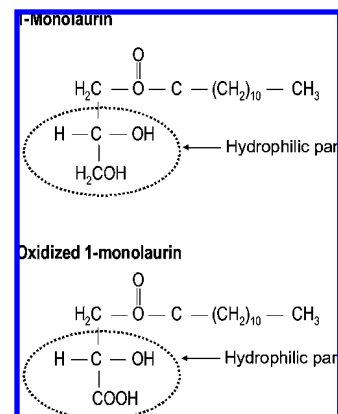

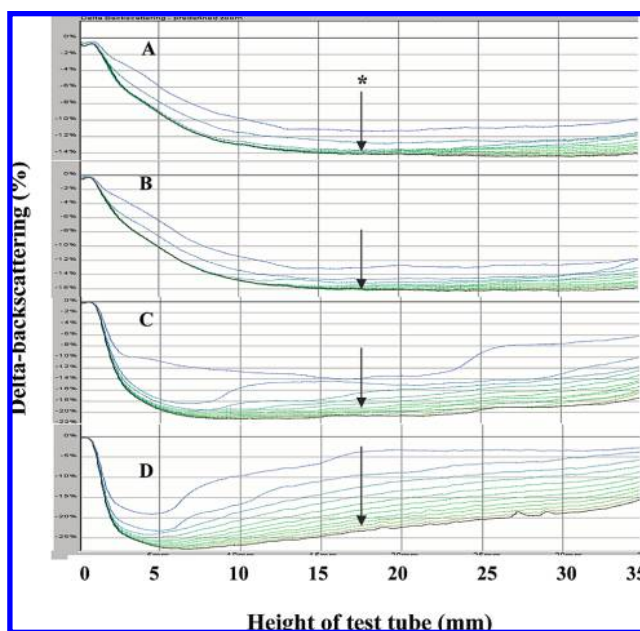**Figure 7.** Diagrammatical representation for the δ-backscattering in an O/W type emulsion (90% water, 10% oil). (**A**) OML, 0.2%; (**B**) OML, 0.4%; (**C**) 1-ML, 0.2%; and (**D**) 1-ML, 0.4% (w/w). *δ-Backscattering was determined at 17.5 mm height of a Turbiscan test tube.

numbers of 1-ML and OML by Griffin's method are 4.45 and 5.21, respectively. OML had a 3.8% higher HLB number as compared to 1-ML in the 20-point scale of HLB. It is well-known that emulsifiers with HLB between 4 and 8 are optimal for W/O emulsions, whereas emulsifiers with HLBs between 10 and 16 are suitable for O/W emulsions (*26*). However, it was found out that this small increase in the HLB number of OML is able to function more efficiently as an emulsifier in an O/W emulsion through emulsion stability analysis below.

Oxidation Effects on Emulsion Stability. δ-Backscattering of emulsions prepared by changing the ratio of water from 90 to 50% (w/w) using two different levels of 1-ML and OML concentration, 0.2 and 0.4% (w/w), respectively, is shown in **Figure 7**. A gradual decrease of δ-backscattering in the middle part of the test tube over time was observed in every sample, which demonstrated that fat flocculation and coalescence by emulsion destabilization occurred (**Figure 7**). Because the emulsion solution comprising water$-$emulsifier$-$oil is thermo-dynamically unstable, a drastic change in backscattering occurred 30 min after preparing the emulsion. Therefore, the

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Table 1. Effects of Selective Oxidation on the Creaming Velocity Resulting From Fat Flocculation Depending on the Ratio of Water and Oil

emulsifier concentration (%, w/w)	distilled water: olive oil (w/w)	migration velocity (mm/h)	
		OML	1-ML
0.2	9:1	0.16 ± 0.03^a	1.13 ± 0.20
7:3		0.30 ± 0.04	0.96 ± 0.20
5:5		0.53 ± 0.08	0.60 ± 0.15
0.4	9:1	0.13 ± 0.02	0.59 ± 0.09
7:3		0.43 ± 0.07	0.95 ± 0.25
5:5		0.51 ± 0.09	0.31 ± 0.04

^{a} Average $\pm$ standard deviation ($n = 3$).

creaming velocity caused by the fat flocculation could be determined from the overall change within 30 min.

The creaming velocities resulting from fat flocculation computed by a Turbiscan are shown in **Table 1**. The creaming velocity of the emulsion comprised of 90% water and 10% oil at 0.2% (w/w) of OML was 0.16 mm/h, whereas that of 1-ML was 1.13 mm/h (**Table 1**). This implies that OML, a selectively oxidized product of 1-ML, became more an efficient surfactant in the O/W emulsion than the native 1-ML. This trend can be observed in the level of 0.4% (w/w) surfactants. The creaming velocities at the 0.4% (w/w) level of 1-ML and OML were 0.13 and 0.59 mm/h, respectively, at 90% water and 10% oil. However, the efficacy of OML as an emulsifier decreased as the oil content increased up to 50%. This phenomenon was repeated at the 0.4% (w/w) level of surfactant. Therefore, even though the change of HLB number by TEMPO-mediated oxidation is small from 4.45 to 5.21, this change made OML a more efficient emulsifier in preventing fat flocculation in the O/W emulsion due to its higher hydrophilicity exerted by the selective oxidation.

In conclusion, OML was obtained by selective oxidation of 1-ML by TEMPO/NaOCl, and its chemical structure was characterized by ^{13}C NMR and IR spectrometry. OML showed 33.2 times more water solubility and 7.0 times more emulsion stability when comprised of a 90% water and 10% oil model system at the level of 0.2% (w/w) than 1-ML.

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