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Free Radical Mediated Formation of 3-Monochloropropanediol (3-MCPD) Fatty Acid Diesters

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Supporting Information

ABSTRACT: The present study was conducted to test the hypothesis that a free radical was formed and mediated the formation of 3-monochloropropanediol (3-MCPD) fatty acid diesters, a group of food contaminants, from diacylglycerols at high temperature under a low-moisture condition for the first time. The presence of free radicals in a vegetable oil kept at 120 °C for 20 min was demonstrated using an electron spin resonance (ESR) spectroscopy examination with 5,5-dimethylpyrroline-*N*-oxide (DMPO) as the spin trap agent. ESR investigation also showed an association between thermal treatment degree and the concentration of free radicals. A Fourier transform infrared spectroscopy (FT-IR) analysis of *sn*-1,2-stearoylglycerol (DSG) at 25 and 120 °C suggested the possible involvement of an ester carbonyl group in forming 3-MCPD diesters. On the basis of these results, a novel free radical mediated chemical mechanism was proposed for 3-MCPD diester formation. Furthermore, a quadrupole-time of flight (Q-TOF) MS/MS investigation was performed and detected the DMPO adducts with the cyclic acyloxonium free radical (CAFR) and its product MS ions, proving the presence of CAFR. Furthermore, the free radical mechanism was validated by the formation of 3-MCPD diesters through reacting DSG with a number of organic and inorganic chlorine sources including chlorine gas at 120 and 240 °C. The findings of this study might lead to the improvement of oil and food processing conditions to reduce the level of 3-MCPD diesters in foods and enhance food safety.

KEYWORDS: *3-MCPD diester, formation mechanism, ESR, Q-TOF MS/MS, FT-IR*

INTRODUCTION

Fatty acid esters of 3-monochloro-1,2-propanediol (3-MCPD), a known food contaminant with nephrotoxicity and carcinogenic potential, have been detected in many food products, including cakes, breads, biscuits, cheeses, refined vegetable oils, and infant formulas.[1−4] It was reported that 3-MCPD esters might be formed during oil deodorization.[5,6] In 2008, human breast milk was reported to contain 30−2195 μg/kg 3-MCPD esters on a per fat content basis, indicating that 3-MCPD esters could be absorbed and distributed to human organs and tissues.[7] A few studies examined the potential toxicity of selected 3-MCPD diesters.[8−10] In 2012, our laboratory reported for the first time that the LD_{50} values of 3-MCPD mono- and dipalmitic esters were 2676.81 and >5000 mg/kg, respectively, in Swiss mice, as well as their cytotoxicity in NRK-52E rat kidney cells.[10] The 3-MCPD monopalmitic esters showed greater acute kidney and testes toxicities, including renal tubular necrosis, protein casts, and spermatids decrease, in a single seminiferous tubule than the 3-MCPD dipalmitic ester.[10] In 2011, a 90 day toxicity study using Wistar rats suggested a dose-dependent kidney and testes toxicity for 3-MCPD dipalmitate.[8] In addition, Tee et al. reported cellular toxicity for 3-MCPD esters.[9] These previous studies indicated a food safety concern of 3-MCPD esters.

Recently, a few studies have investigated the factors influencing the level of 3-MCPD esters in refined oils[2,4,6] and the possible mechanism of 3-MCPD ester formation[11−15] for potential improvement of the food processing approaches to reduce the level of 3-MCPD esters in oils and foods. In 2011, Rahn and others summarized the four proposed pathways of 3-MCPD ester formation.[15] Two of the proposed mechanisms involved direct nucleophilic substitution by the chlorine anion at the glycerol carbon replacing either an ester group or a hydroxyl group.[12,15] Another proposed mechanism involved the formation of an epoxide ring, followed by nucleophilic ring-opening reaction with a chloride anion.[14] The other proposal postulated the formation of an intermediate acyloxonium cation[12,13] and a nucleophilic substitution reaction with a chlorine anion to open the cation ring. In 2011, Rahn et al. tried to monitor the acyloxonium cation formation using a Fourier transform IR (FT-IR) spectroscopy and concluded that C-2 carbonyl group was involved in forming the cation intermediate for 3-MCPD diester synthesis.[11] However, the presence of cyclic acyloxonium cations was not directly

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supported with the available evidence regarding the shifting of the IR absorption from the ester carbonyl groups. In other words, the FT-IR data from Rahn and others confirmed the involvement of ester carbonyl groups in 3-MCPD diester formation, but did not directly support the formation of the acyloxonium cation intermediate.

On the other hand, thermal treatment of rapeseed oils at 230 °C time-dependently increased the level of 3-MCPD diesters.[4] Furthermore, heating triglycerides with an organic chloride compound at 235 °C significantly increased the formation of 3-MCPD diesters.[16] These findings raised the question of whether free radicals might have been generated and involved in the 3-MCPD diester formation. A preliminary study was performed in our laboratory to examine the possible presence of free radicals under the thermal treatment conditions. A mixture of commercial vegetable oil and 5,5-dimethylpyrroline-*N*-oxide (DMPO), a radical trapping agent, heated to 80 °C resulted in a detectable level of electron spin resonance (ESR) signals, whereas the mixture kept at ambient temperature had no corresponding ESR signals (Supporting Information, Figure 1S), indicating that free radicals were formed during the thermal treatment of oil. Together, these data suggested a possible involvement of free radical intermediate(s) during the formation of 3-MCPD diesters.

The present study was designed to test the hypothesis that a free radical was formed and mediated the formation of 3-MCPD diesters at high temperature under a low-moisture condition. The results from this study may be used to reduce the level of 3-MCPD diesters in refined edible oils and other food products.

MATERIALS AND METHODS

Materials. Commercial vegetable oil was a gift from Wilmar International Limited, China. *sn*-1,2-Stearoylglycerol (DSG), 1,2-distearoyl-3-MCPD, iron chloride, MS grade sodium acetate, DMPO, and *N*-*tert*-butyl-α-phenylnitrone (PBN) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Iron dichloride and hexadecane were brought from Aladdin Reagent (Shanghai, China). Lindane was purchased from J&K Scientific (Beijing, China). LC-MS grade methanol and 2-propanol were obtained from Merck (Darmstadt, Germany). HCl gas was generated by heating concentrated hydrochloric acid (Ambrosia Pharmaceuticals, Shanghai, China). Toluene, copper chloride, calcium chloride, potassium chloride, sodium chloride, zinc chloride, and sodium hydroxide were purchased from Ambrosia Pharmaceuticals (Shanghai, China). GHP membrane was brought from Pall Corp. (New York, NY, USA). Chlorine gas was a gift from the East China University of Science and Technology (Shanghai, China). Ultrapure water was prepared by a Millipore ultra-Genetic polishing system with $<5 \times 10^{-9}$ TOC and resistivity of 18.2 mΩ (Millipore, Billerica, MA, USA) and used for all experiments.

ESR Determination of Radical Signals. To select a spin trap agent, PBN and DMPO were examined and compared for their potential use in detecting possible radicals under the thermal treatment and low-moisture conditions. Vegetable oil was diluted 100-fold with toluene. The oil solution without a spin trap and those with PBN or DMPO were heated to 80 °C for 20 min and measured on a Bruker EMX-8/2.7C spectrometer equipped with a variable-temperature system (Bruker Optics, Karlsruhe, Germany) in a 0.1 mL quartz tube, respectively, for their ESR signals. The final concentration in the detection mixture was 20 mmol/L for both PBN and DMPO.

To determine the effect of thermal treatments on the generation of radicals, DSG solution (0.5 mg/mL in toluene) containing DMPO was kept at ambient temperature and 40, 60, 80, 120, and 160 °C, respectively, for 20 min and measured for their ESR signals. The final DMPO concentration was 20 mmol/L in the mixture. The ESR conditions were as follows: microwave power, 2.011 mW; modulation amplitude, 1.00 G; receiver gain, 6.32×10^4; sweep time, 41.943 s.

FT-IR Measurements. After the baseline was corrected with an empty pellet holder, DSG was directly applied onto the attenuated total reflectance (ATR) crystal and scanned immediately at 25 °C. Then the temperature was raised to 120 °C and scanned immediately. The temperatures were detected by the temperature-controlled single-bounce diamond equipment. FT-IR spectra were recorded on a Spectrum 100 FT-IR spectrometer (Perkin-Elmer, Waltham, MA, USA) equipped with a pressure application device for solid samples, a deuterated triglycine sulfate (DTGS) detector, a temperature-controlled single-bounce diamond, and ATR crystal. Data were analyzed using Spectrum FT-IR software (PerkinElmer, Waltham, MA, USA).

Q-TOF MS/MS Examination of DMPO Radical Adducts. A Waters Xevo G_2 quadrupole−time of flight (Q-TOF) mass spectrometer (Milford, MA, USA) was used to obtain all high-resolution mass spectra in a positive-ion mode. DSG solutions (0.5 mg/mL in toluene) with and without DMPO were heated to 120 °C and kept for 20 min. The final concentration of DMPO in the DSG solutions was 20 mmol/L. After cooling to ambient temperature in the fume hood, the DSG solution was further diluted 100-fold with methanol and injected directly into the mass spectrometer. MS conditions were as follows: ESI positive mode with lockspray; flow rate, 10 μL/min; cone voltage, 30 V; capillary voltage, 3 kV; source temperature, 120 °C; desolvation temperature, 450 °C; desolvation gas flow, 800.0 L/h; and cone gas flow, 50.0 L/h. A MS^E method was used, and conditions were as follows: mass range, m/z 50−1500; ramp collision energy, set from 20 to 35 V. Data analysis was carried out with Waters MassLynx v4.1 software.

UPLC-Q-TOF MS Analysis of the Reactions between DSG and Selected Chlorine-Containing Compounds. DSG was dissolved in hexadecane to a final concentration of 0.5 mg/mL. For the solid chloride agents, 2 mg of KCl, NaCl, $CaCl_2$, $FeCl_2$, $FeCl_3$, $ZnCl_2$, $CuCl_2$ or lindane was added into 10 mL of 0.5 mg/mL DSG solution, respectively. For dry HCl or Cl_2 gas reaction, HCl and Cl_2 gas were dried through concentrated sulfuric acid and introduced into 10 mL of DSG solution (0.5 mg/mL). Each reaction was conducted at ambient temperature and 120 and 240 °C for 20 min, respectively. After the reaction, the mixtures were cooled to ambient temperature and diluted 100-fold with methanol, filtered through a GHP 0.2 μm membrane, and subjected for UPLC-Q-TOF MS analysis (Waters Xevo G_2 Q-TOF MS).

Ultra-performance liquid chromatography (UPLC) was performed using a Waters Acquity UPLC BEH Phenol column (50 × 2.1 mm i.d., 1.7 μm). The temperature of the column oven was set at 40 °C. Mobile phase A contained methanol and water at a ratio of 1:9 (v/v) with 1 mmol/L NaOAc; mobile phase B consisted of methanol and 2-propanol at a ratio of 4:1 (v/v) with 1 mmol/L NaOAc. The elution gradient was as follows: 0−0.3 min, 35% B; 0.3−3.0 min, 35−72% B; 3.0−5.0 min, 72% B; 5.0−6.0 min, 72−80% B; 6.0−9.0 min, 80−95% B; 9.0−11.0 min, 95−35% B; and 11.0−13.0 min, 35% B. The flow rate was 0.4 mL/min, and the injection volume was 2 μL. MS conditions were as follows: ESI positive mode with lockspray; capillary voltage for positive ion mode, 3.0 kV; sampling cone voltage for negative mode, 30.0 kV; source temperature, 120 °C; desolvation temperature, 450 °C; desolvation gas flow, 800.0 L/h; cone gas flow, 50.0 L/h; scan range, m/z 50−1200; scan time, 0.2 s. Data analysis was carried out with MassLynx 4.1 software. The amount of 1,2-distearoyl-3-MCPD was determined using the total ion counts in the extracted ion chromatogram.

RESULTS AND DISCUSSION

The present study was performed to test if free radicals are formed and served as intermediates for 3-MCPD diester formation at high temperature under a low-moisture condition, similar to that during the oil refining process. First, the correlation between free radical formation and thermal treatment was examined. Second, the investigation by FT-IR

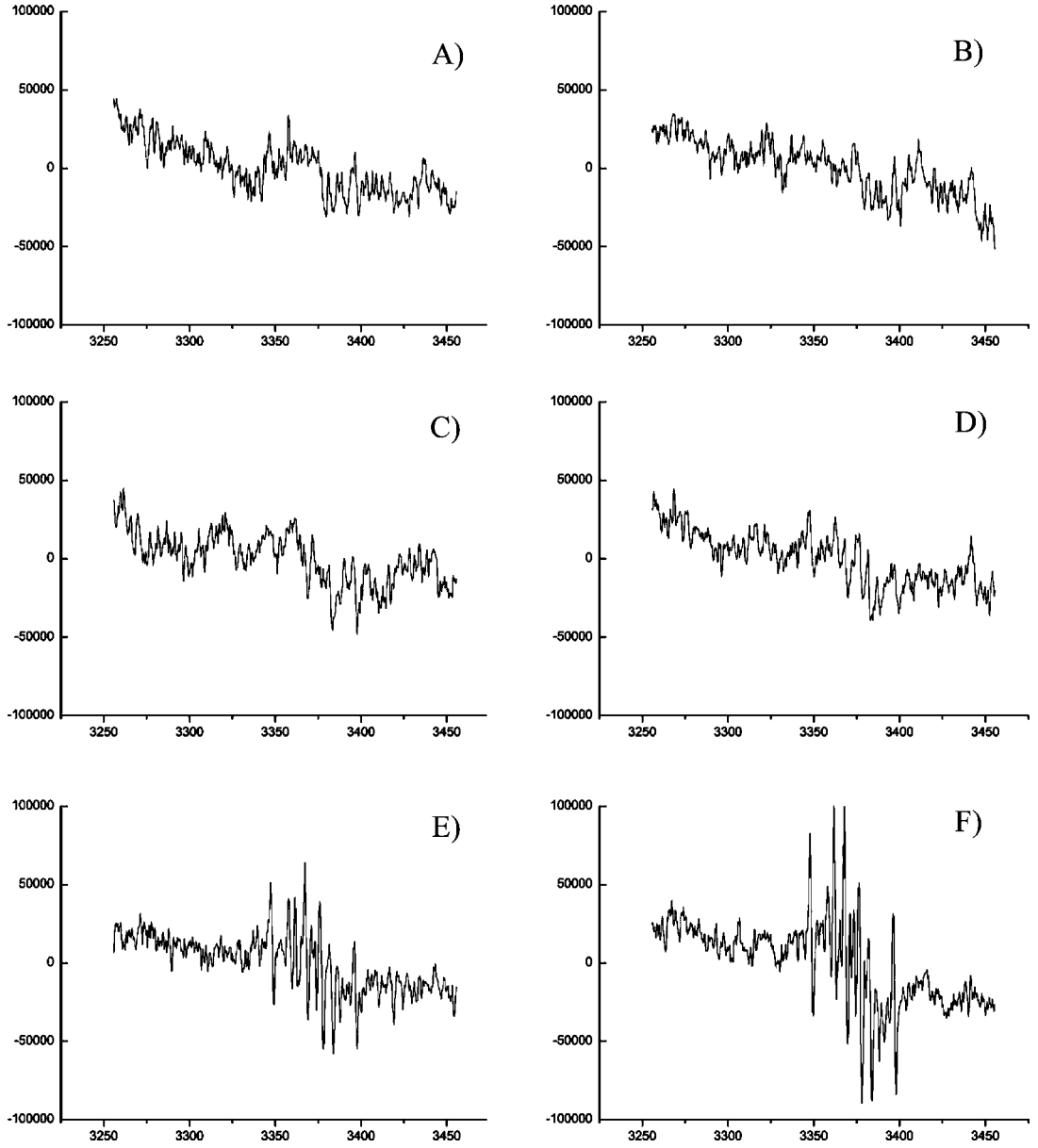

Figure 1. ESR spectra of 0.5 mg/mL of DSG dissolved in toluene with 20 mmol/L of DMPO kept at (A) ambient temperature after 20 min, (B) 40 °C after 20 min, (C) 60 °C after 20 min, (D) 80 °C after 20 min, (E) 120 °C after 20 min, and (F) 160 °C after 20 min. The *X*-axis is the magnetic-field intensity, and the *Y*-axis is ESR signal intensity.

spectroscopy revealed the involvement of the ester carbonyl groups in 3-MCPD diester formation, which led to a proposed new free radical mechanism. Furthermore, Q-TOF MS/MS examination detected the DMPO radical adducts and the product MS ions, supporting the formation of a free radical intermediate during 3-MCPD diester generation. Additionally, the radical mechanism was confirmed by the formation of the 3-MCPD diester through the reaction between DSG and chlorine gas and the other selected organic and inorganic chlorine-containing compounds. To our knowledge, this is the first report of a free radical mechanism for 3-MCPD diester formation.

Selection of a Spin-Trapping Agent. ESR spectroscopy directly measures the chemical species with unpaired electrons. Free radicals are a group of such chemical species. The ESR determinations generally include direct ESR measurement and the ESR spin-trapping and spin-labeling techniques.[17] The ESR spin-trapping technique uses a spin-trapping agent, which is an exogenous molecule capable of interacting with short-lived radicals such as hydroxyl radicals, to form relatively stable secondary radicals. The radical spin trap adducts could be used for qualitative and quantitative ESR detections.

To confirm the presence of free radicals during the formation of 3-MCPD diesters, vegetable oils containing either DMPO or PBN were heated at 80 °C for 20 min and monitored using ESR. PBN is a nitric oxide spin trap reagent, whereas DMPO is a spin trap for superoxide-, O-, C-, S-, and N-centered free radicals. As shown in Figure 1S in the Supporting Information, the ESR spectrum of vegetable oil without spin traps and that with PBN did not show detectable level of ESR signal under the experimental conditions. Interestingly, heating the vegetable oil with DMPO resulted in a strong ESR signal under the same experimental conditions (Figure 1S), indicating the radical formation. The results also suggested that DMPO might serve

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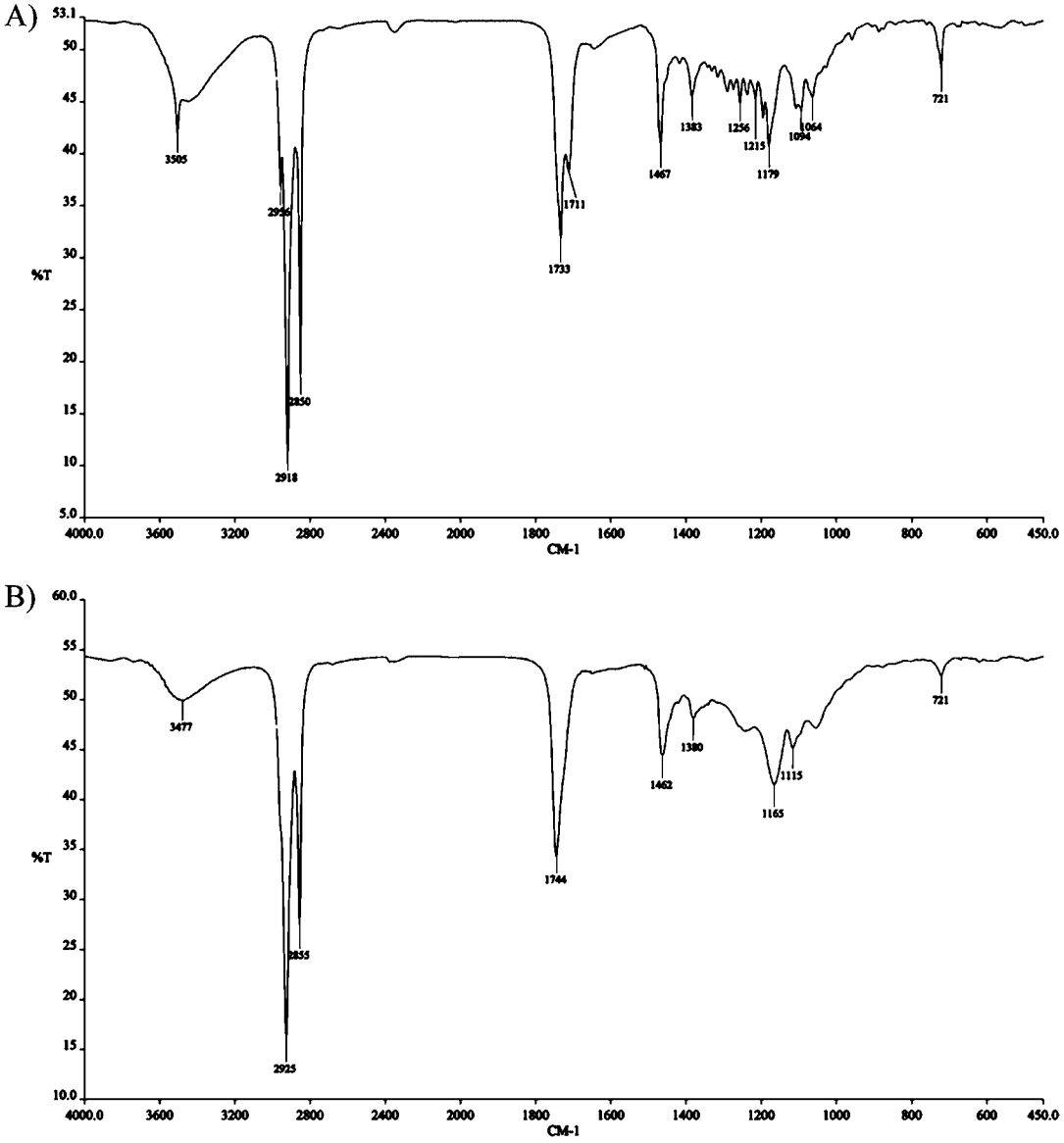

Figure 2. FT-IR spectra of DSG at (A) 25 °C and (B) 120 °C.

as a spin-trapping agent to investigate the role of radicals in 3-MCPD diester formation. In addition, the shape of the ESR spectrum for the oil containing DMPO at the elevated temperatures suggested that both O- and C-centered radicals might have been generated under the thermal treatment conditions.

Effect of Thermal Treatment on Free Radical Generation. The mixtures of DSG and DMPO kept at ambient temperature and 40, 60, 80, 120, and 160 °C for 20 min might differ in their ESR signal intensities (Figure 1). No significant ESR signal was detected in the oil−DMPO mixtures kept at ambient temperature or 40−80 °C (Figure 1A−D). The ESR signal was observed in the oil−DMPO mixtures treated at 120 and 160 °C (Figure 1E,F), and a greater intensity of the ESR signal was associated with a higher temperature treatment.

FT-IR Determination of DSG at 25 and 120 °C. In the present study, DSG kept at 25 and 120 °C was measured for its FT-IR spectra using a temperature-controlled single-bounce diamond ATR crystal. As shown in Figure 2A, DSG exhibited two carbonyl absorbance bands at 25 °C, one band centered at 1733 cm^{-1} and the other at 1711 cm^{-1}, indicating the presence of two chemically different environments for the ester carbonyl groups. The ester carbonyl group at the C-1 position might contribute to the absorbing band at 1733 cm^{-1}, and the ester group at the C-2 position might be responsible for the absorbance at 1711 cm^{-1}.[11] Upon heating to 120 °C, only a single peak at 1744 cm^{-1} was observed, reflecting a hypsochromic shift of carbonyl group (Figure 2B). The IR data suggested the possible direct involvement of an ester carbonyl group in the free radical formation and the significant change of the chemical environment for the other ester carbonyl group.

In 1991, Collier et al. first proposed the acyloxonium cation as the intermediate in the formation of 3-MCPD diester in the presence of acetic acid.[12] In 2011, Rahn et al. heated tripalmitin at temperatures above 60 °C and monitored the changes in the FT-IR spectrum.[11] The disappearance of a peak at 1728 cm^{-1} was observed, but was taken as evidence for the formation of a cyclic acyloxonium ion intermediate. The FT-IR determination results from the present study and those from the previous studies[11,12] suggested the possible involvement of ester carbonyl groups in 3-MCPD diester formation, possibly

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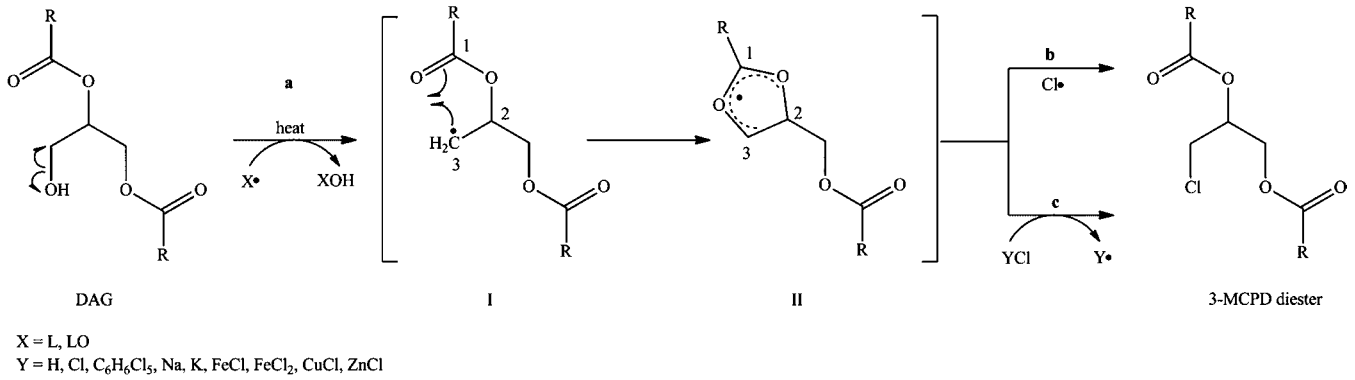

Figure 3. Proposed mechanism for the formation of 3-MCPD diesters from DAG. L represents lipid.

through a free radical intermediate and the direct chemical reaction on one of the carbonyl groups in forming the radicals under the low-moisture and high-temperature conditions. The ESR and FT-IR studies led to a proposed mechanism for 3-MCPD diester formation under the high-temperature and low-moisture conditions similar to those for deodorization during the oil refining process.

Proposed Mechanism for the Formation of 3-MCPD Diesters. A new free radical mediated mechanism was proposed for 3-MCPD diester formation under a high-temperature and low-moisture condition (Figure 3). The reaction might be initiated by elimination of a hydroxyl radical on C-3 of glycerol in the DAG to a thermally generated radical such as those formed during lipid peroxidation ($L^{\bullet}$ or $LO^{\bullet}$) or even a breakdown of the covalent bond between the hydroxyl group and the C-3 of glycerol due to thermal treatment; the carbon-centered radical (**I**) might immediately attack the ester carbonyl group located at the C-2 and form a cyclic acyloxonium free radical (**II**, CAFR); CAFR could be a relatively stable radical as the unpaired electron could be dislocated among the two cyclic carbon (C-2 and C-3) and two oxygen atoms (**II**); CAFR could react with a Cl radical ($^{\bullet}Cl$) to form a 3-MCPD diester through path b; or CAFR could extract a $^{\bullet}Cl$ from the chlorine-containing agents (YCl) to form a 3-MCPD diester and release a new radical species ($^{\bullet}Y$) through path c (Figure 3). The other chlorine sources might include organic chlorine compounds such as chlorine-containing pesticides, Cl_2, and inorganic chlorine compounds including hydrogen chloride (HCl) and $FeCl_2$. It has been demonstrated that metal halide salts, such as $FeCl_2$ and $CuCl_2$, could take part in the radical chain reaction through an inner-sphere electron transfer.[18] This previous paper supported the proposed radical mechanism for 3-MCPD diester formation under high-temperature and low-moisture conditions. Both organic and inorganic chlorine-containing substances have been detected in the edible oils contaminated through the oil production chain.[19] Controlling the level of chlorine-containing agents though the oilseed production and oil-processing chain could be critical for reduction of 3-MCPD esters in the final oil products and other foods.

As other diacylglycerols (DAG) might have a chemical environment similar to that of DSG, this proposed radical-mediated mechanism could be applied to the formation of 3-MCPD diesters from all DAGs. To further confirm the proposed radical mechanism, Q-TOF MS/MS was employed for possible determination of the radical intermediates.

Q-TOF MS/MS Determination of Cyclic Acyloxonium Free Radical (CAFR)−DMPO Spin Adducts. DSG was heated with or without DMPO to 120 °C for 20 min, and the reaction mixtures were examined using Q-TOF MS/MS. The observation of $[CAFR]^+$ (m/z 607.5629, calcd 607.5665) (Figure 4A) in the absence of DMPO confirmed the formation of CAFR (**II**) as proposed (Figure 3). Analysis of the DSG and DMPO mixture observed two sodiated peaks, $[M + Na]^+$ at m/z 760.6448 representing $[CAFR + DMPO + O + Na]^+$ (calcd 760.6431) and 761.6494 for $[CAFR + DMPO + OH + Na]^+$ (calcd 761.6509) (Figure 4B). Additionally, no $[CAFR]^+$ was detected in the mixture of DSG and DMPO (Figure 4B), indicating that the generated CAFR (**II**) were all trapped by DMPO at 120 °C.

Upon collisional activation, the ions at m/z 760.6423 for $[CAFR + DMPO + O + Na]^+$ and 761.6468 for $[CAFR + DMPO + OH + Na]^+$ (Figure 5B) decomposed to an abundant product ion at m/z 607.5660 for $[CAFR]^+$, which was formed by elimination of [DMPO + O + Na] or [DMPO + OH + Na]. Also, ions at m/z 738.6608 for $[CAFR + DMPO + O + H]^+$ with calculation values of 738.6612 and 739.6674 for $[CAFR + DMPO + OH + H]^+$ with a calculation value of 739.6690 were also detected. In addition, $[DSG + Na]^+$ was found in all spectra (Figures 4 and 5). Finally, the ion at m/z 341.3052 formed by breaking down the *sn*-1 stearic acid side chain from DSG was detected (Figures 4A and 5A). Together, these product ions further confirmed the presence of DMPO adducts and the formation of CAFR, supporting the proposed free radical mechanism for 3-MCPD diester formation from DSG or DAG at high-temperature and low-moisture conditions.

Formation of 3-MCPD Diesters from DSG with Different Chlorine Donors. To further confirm the radical mechanism, DSG was reacted with a group of selected chlorine donors, including KCl, NaCl, $FeCl_2$, $ZnCl_2$, $CuCl_2$, $FeCl_3$, $CaCl_2$, lindane, and dry HCl gas. Lindane served as an organic chloride agent, whereas the others contained inorganic chlorine. The reactions with each of the chloride agents were performed at ambient temperature and 120 and 240 °C, respectively. At 240 °C, significant amounts of 3-MCPD diesters were detected in the reaction mixtures except that for $CaCl_2$, possibly due to its poor solubility in hexadecane. Typically extracted ion chromatograms for the reactions are provided in the Supporting Information (Figure 2S), demonstrating the formation of 3-MCPD diester from DSG in the presence of chloride agents. Interestingly, at ambient temperature only HCl gas and at 120 °C only HCl gas, $FeCl_2$, and $FeCl_3$ resulted in detectable amounts of 3-MCPD diesters. These results directly

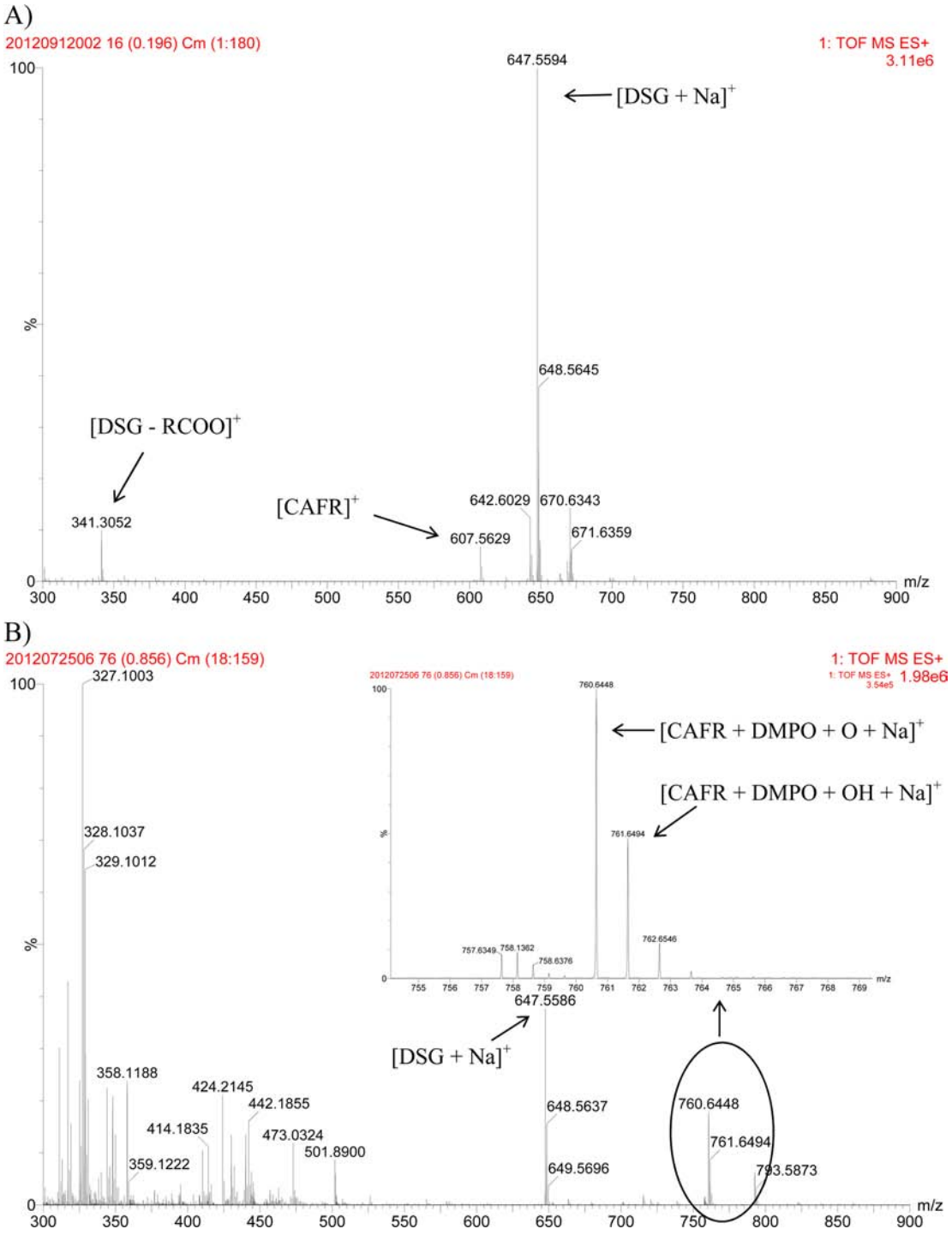

Figure 4. HR ESI-MS spectra of 0.5 mg/mL of DSG at 120 °C (A) in the absence of DMPO and (B) in the presence of 20 mmol/L of DMPO.

supported radical mechanism proposed, because acyloxonium cation intermediates could have been generated at 120 °C, and a nucleophilic substitution reaction between the chloride anion and the reported acyloxonium cation would have formed 3-MCPD diester.[11,12] In other words, most inorganic chlorine-containing compounds would have ionized to provide chloride anions and form 3-MCPD diesters through the nucleophilic substitution reaction at 120 °C, if the acyloxonium cation mechanism was correct. It is widely accepted that radical formation requires higher temperature to generate chloride radicals from both organic and inorganic chlorine-containing compounds,[20] which was in excellent agreement with the observations in the present study. In addition, previous studies have shown that the formation of 3-MCPD diesters was dramatically increased when the temperature was raised above 200 °C, especially above 240 °C.[16] These data further supported our hypothesis that the radical intermediates were involved in the formation of 3-MCPD diesters.

Formation of 3-MCPD Diesters from DSG and Chlorine Gas. To provide additional evidence of the radical mechanism for 3-MCPD diester formation, chlorine gas was employed as chlorine donor. Chlorine gas was flowed into a

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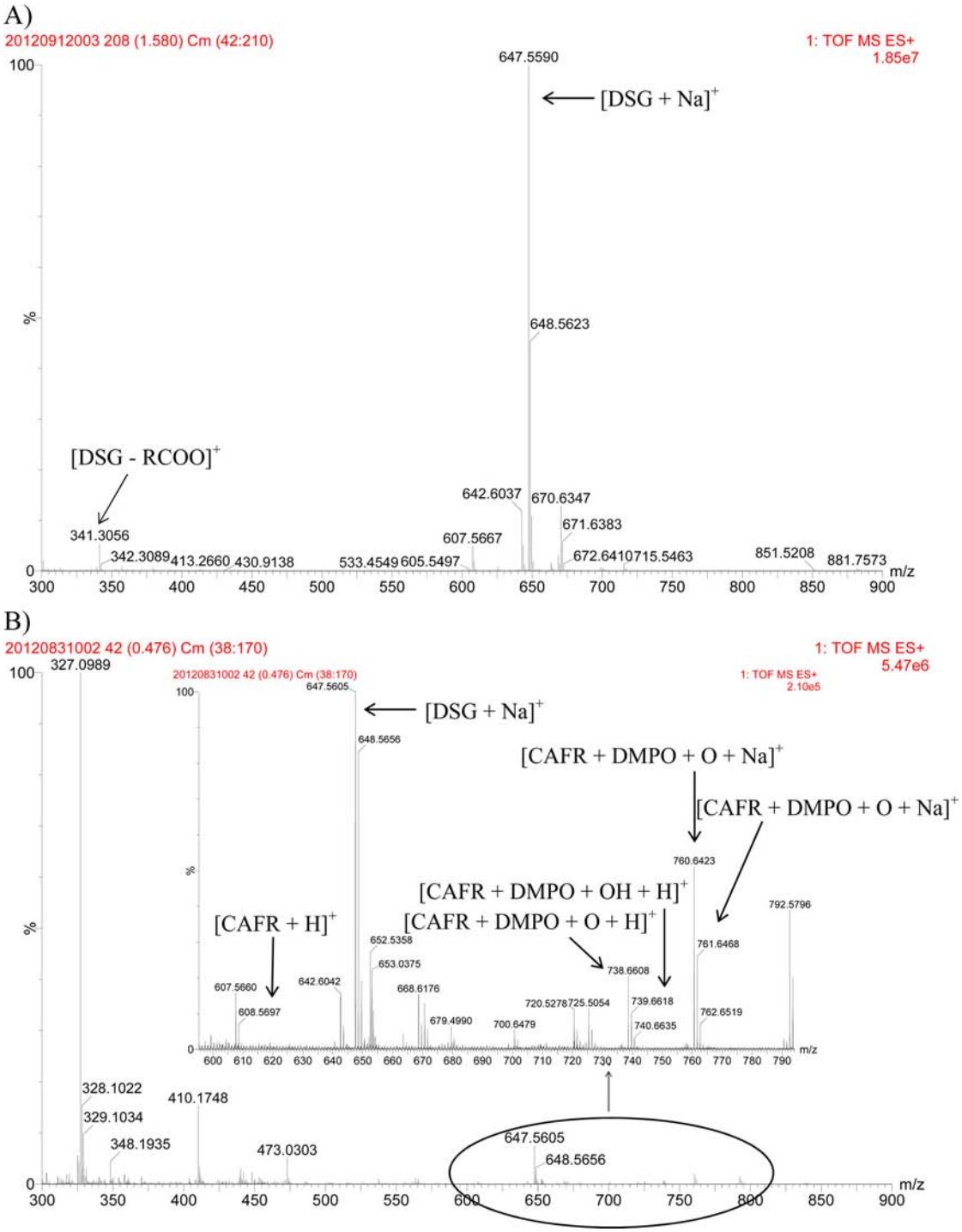

Figure 5. HR ESI-MS/MS spectra of 0.5 mg/mL of DSG at 120 °C (A) in the absence of DMPO and (B) in the presence of 20 mmol/L of DMPO.

hexadecane solution containing 0.5 mg/mL DSG at ambient temperature and 120 and 240 °C for 20 min, respectively. Little 1,2-distearoyl-3-MCPD was detected in the reaction mixture kept at ambient temperature, whereas the mixtures kept at 120 and 240 °C had significant levels of 1,2-distearoyl-3-MCPD. Furthermore, the mixture kept at 240 °C had a much greater level of 3-MCPD diesters according to a UPLC-Q-TOF MS analysis of the same two reaction mixtures under the same conditions (Supporting Information, Figure 2S). It is well-known that chlorine gas undergoes a hemolytic cleavage to form chlorine radicals at high temperature. The formation of 1,2-distearoyl-3-MCPD indicated that chlorine radicals could participate in the reaction (Figure 3), supporting the free radical mechanism.

In summary, this study indicated a free radical mediated reaction mechanism for 3-MCPD diester formation from DSG. This is the first report for the free radical mechanism for 3-MCPD diester formation under high-temperature and low-moisture conditions, and provides important scientific insight for controlling the level of 3-MCPD diesters in refined edible oils.

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■ ASSOCIATED CONTENT

Supporting Information

Additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

3-MCPD, 3-monochloro-1,2-propanediol; DMPO, 5,5-dimethylpyrroline-*N*-oxide; DSG, *sn*-1,2-stearoylglycerol; CAFR, cyclic acyloxonium free radical; FT-IR, Fourier transform infrared; ESR, electron spin resonance; PBN, *N*-*tert*-butyl-α-phenylnitrone; ATR, attenuated total reflectance; DTGS, deuterated triglycine sulfate; UPLC-Q-TOF MS, ultraperformance liquid chromatography−quadrupole−time of flight mass spectrometer; DAG, diacylglycerols

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