Food Sci. Biotechnol. Vol. 18, No. 2, pp. 413 ~ 418 (2009)

Food Science and Biotechnology

Effects of Photooxidation and Chlorophyll Photosensitization on the Formation of Volatile Compounds in Lard Model Systems

JaeHwan Lee[1] and David B. Min*

Department of Food Science and Technology, The Ohio State University, 2015 Fyffe Road, Columbus, OH 43210, USA
[1]Department of Food Science and Technology, Seoul National University of Technology, Seoul 139-743, Korea

Abstract Effects of chlorophyll and visible light exposure on the volatile formations and headspace oxygen content were studied in lard model systems at 55°C. Samples with or without addition of chlorophyll under light underwent photosensization or photooxidation, respectively. Total volatiles (TI) in lard with 5 ppm chlorophyll photosensization were 19 times higher than those in visible light photooxidized samples for 48 hr while TI in lard with chlorophyll in the dark were not significantly different from those in photooxidized samples ($p>0.05$). Headspace oxygen content in photosensitized lard decreased from 21 to 15% for 48 hr but that in photooxidized lard or that in lard with chlorophyll in the dark did not change significantly ($p>0.05$), which indicates that lard system used in this study is a photosensitizer-free model system and the presence of chlorophyll accelerated the lipid oxidation only under visible light. Oxidation mechanisms of photooxidation with or without presence of photosensitizers under visible light were not the same based on the difference of oxidized volatile profiles and headspace oxygen depletion.

Keywords: photooxidation, photosensitization, chlorophyll, volatiles, lard

Introduction

Lipid oxidation in foods causes deterioration of sensory qualities and nutritional values during food manufacturing and storage. Autoxidation, lipoxygenase catalyzed oxidation, and light-induced oxidation are major lipid oxidation mechanisms in foods (1,2).

Autoxidation or triplet oxygen oxidation of lipids is a free radical chain reaction of unsaturated fatty acids. Autoxidation of lipids can be initiated many factors including metal catalysis, heat energy, or ultraviolet irradiation, and produce hydroperoxides of unsaturated fatty acids. The decomposition of lipid hydroperoxides produces volatile compounds, including aldehydes, alcohols, ketones, and hydrocarbons, which are responsible for the increase of off-odor and the decrease of food qualities (2,3).

Light-induced lipid oxidation can be either photooxidation or photosensitization depending on the absence or presence of photosensitizers in samples, respectively (1). Unsaturated fatty acids do not absorb the energy of visible light (4) but ultraviolet irradiation can produce free radicals in oils (2,4,5). Several authors have reported the acceleration of lipid oxidation through light exposure in foods and model systems, which may be due to the presence of photosensitizers such as chlorophylls and riboflavin (6-8).

Photosensitizers can accelerate lipid oxidation either by Type I or II mechanisms. Singlet state of photosensitizers can absorb light energy upon the irradiation and become excited singlet state photosensitizers. The excited singlet state photosensitizers change into the excited triplet state photosensitizers by intersystem crossing mechanisms. The excited triplet state photosensitizers can react with triplet oxygen to form singlet oxygen (Type II mechanism) or abstract electron or hydrogen atom from substrates to generate radicals (Type I mechanism) depending on the solubility and availability of triplet oxygen and substrates (9,10).

Study of photooxidation on lipid is not easy due to the difficulty of removing photosensitizers completely in foods and model systems. A combination of adsorbents including activated alumina, activated silica gel, Celite, powder sugar, and activated charcoal has been used to remove the impurities such as antioxidant compounds, colorants, free fatty acids, mono- and diacylglycerols from triacylglyceros of vegetable oils under nitrogen gas (11-13). However, it is very difficult to remove photosensitizers completely from foods. Stripped vegetable oil under light exposure may undergo photosensitization instead of photooxidation.

Lard is an animal lipid and has been used in deep fat frying and emulsified cake shortening (14). Even though myoglobin in muscle-based foods can induce lipid oxidation as a photosensitizer, myoglobin could not accelerate oxidation in lard due to the low solubility of myoglobin in lard (15,16). Therefore, lard could be a suitable substrate for the study of mechanism differences in photooxidation and photosensitization.

The objectives of this study were to develop a photosensitizer-free lipid model system using lard and to study the differences in the profiles of volatile compounds from photooxidation and chlorophyll photosensitization in lard model systems.

Materials and Methods

Materials Pork meat with lard was purchased from a local grocery store (Meijer, Columbus, OH, USA). Chlorophyll *b*, 1-pentanol, hexanal, 2-hexenal, heptanal, 2-heptenal, 1-

*Corresponding author: Tel: +1-614-292-7801; Fax: +1-614-292-0218
E-mail: Min.2@osu.edu
Received September 11, 2008; Revised November 11, 2008;
Accepted November 19, 2008

octene-3-ol, 2,4 heptadienal, 2-nonenal, octanoic acid, and 2,4 decadienal were purchased from Sigma-Aldrich (St. Louis, MO, USA). Teflon-coated rubber septa, aluminum caps, serum bottles, glass liners, the solid phase microextraction (SPME) fiber assembly holder, and 65-mm polydimethylsiloxane/divinylbenzene (PDMS/DVB) were purchased from Supelco, Inc. (Bellefonte, PA, USA). 2-Pentylfuran was purchased from Karl Industries Inc. (Aurora, OH, USA).

Sample preparation for the light and dark storage of lard Lard was carefully separated from pork meat to avoid containing red colored muscle tissues. Thirty g of lard was cut into small pieces of 0.5 cm long and put into a 100-mL serum bottle sealed airtight with a Teflon-coated rubber septum and an aluminum cap. Headspace air in the bottles of lard was replaced with nitrogen gas. Bottles of lard was put in a 70°C oven to melt lard and the oils drained from lard was collected in a 10-mL serum bottle (25×40 mm, 20 mm diameter from Supelco, Inc.) wrapped with aluminum foil under nitrogen gas flow.

Chlorophylls were dissolved in the oils from lard in a 10-mL bottle (25×40 mm, 20 mm diameter) with a magnetic stirring bar (10×3 mm) to obtain 0 and 5 ppm (w/v) in a 55°C water bath while magnetic stirring. Chlorophyll was selected due to its ubiquitous prevalence in food materials with photosynthetic ability, edible characteristics, and high solubility in hydrophobic solvent like fats and oils. The temperature of 55°C was chosen to maintain lard sample in liquid state.

Samples of 0.1 g lard with 0 or 5 ppm chlorophyll were put in 10-mL serum bottles and sealed airtight with Teflon-coated rubber septa and aluminum caps. Sample bottles were kept at 55°C in a tungsten light box. Light source was 2 tungsten light bulbs of 100 W with 2,000 Lux light intensity from Topco Association (Smokie, IL, USA) (11). One set of sample bottles with 5 ppm chlorophyll was wrapped with aluminum foil to make a dark condition. Volatile compounds and headspace oxygen content in sample bottles were analyzed at 0, 6, 12, 24, 36, and 48 hr. Sample bottles were prepared in triplicate at each sample analysis.

Analysis of volatile compounds by SPME The headspace volatile compounds in airtightly sealed sample bottles were isolated by 65 mm PDMS/DVB of SPME solid phase. Sample bottles of lard were put in a 55°C water bath for 30 min while 65 mm PDMS/DVB of SPME solid phase was exposed to the headspace of sample bottles. The isolated volatile compounds by SPME solid phase were separated in a gas chromatograph equipped with a flame ionization detector (Agilent Technologies, Palo Alto, CA, USA). The isolated volatile compounds in solid phase of SPME were desorbed at 250°C of gas chromatography (GC) injector for 2 min.

Headspace oxygen analysis The depleted headspace oxygen was determined by injecting 100 mL headspace gas of sample bottles into a gas chromatograph equipped with a thermal conductivity detector. A stainless steel column (1.8 m×0.32 cm) packed with 60/80 Molecular Sieve 13× (Alltech Assoc., Inc. Deerfield, IL, USA) was used. The flow rate of helium gas was 20 mL/min. Temperatures of oven, injector, and thermal conductivity detector were 40, 120, and 150°C, respectively.

GC condition A Hewlett-Packard 5890 gas chromatograph was equipped with a 0.75-mm i.d. glass injection liner, a flame ionization detector, and a 30 m ×0.32 mm i.d., 0.25 mm film, HP-5, from Agilent Technologies. The oven temperature was held at 40°C for 2 min and increased from 40 to 160°C at 6°C/min and from 160 to 220°C at the rate of 10°C/min. The temperatures of injector and detector were 250 and 300°C, respectively. The flow rate of nitrogen carrier gas was 1.0 mL/min and GC was operated in splitless mode.

Identification of volatile compounds The identification of compounds was made by the combination of NIST Mass Spectra and gas chromatographic retention times of standard compounds. A Hewlett-Packard 5971A mass selective detector (MS) equipped with a Hewlett-Packard 59822 B ionization gauge controller (Agilent Technologies) was used. All mass spectra were obtained at 70 eV and 220°C ion source temperature. Helium carrier gas at 0.9 mL/min and a HP-5 column (30 m×0.25 mm i.d., 0.25 mm film thickness) were used. The GC conditions for GC-MS were the same as the gas chromatographic analysis conditions described previously.

Statistical analysis One-way analysis of variance (ANOVA), Tukey's multiple-comparisons method, and general linear model were used to analyze the data. A p value of 0.05 or less was considered significant. All statistical analyses were conducted with Minitab 12.1 (Minitab Inc., State College, PA, USA)

Results and Discussion

Effect of photooxidation and chlorophyll photosensitization on the headspace volatile compounds in lard Gas chromatograms of headspace volatile compounds in lard containing 0 and 5 ppm chlorophyll stored under light at 55°C for 24 hr are shown in Fig. 1. Gas chromatogram of lard with 5 ppm chlorophyll is different quantitatively and qualitatively from that of lard with 0 ppm chlorophyll under light for 24 hr. Headspace volatile compounds in the photooxidized and chlorophyll photosensitized lard samples increased from 0.3 to 0.5 and 0.3 to 8.2 (1×10^5), respectively, in electronic counts at 55°C for 24 hr. New peaks of number 2, 4, 6, 8, 9, 10, 11, 12, 13, 14, 16, 17, 18, and 19 were formed only in the lard with chlorophyll photosensitization (Fig. 1).

Changes of headspace volatile compounds in total GC peak areas from lard with 0 and 5 ppm chlorophyll at 55°C for 48 hr are shown in Fig. 2. Total volatile compounds in lard with 0 ppm chlorophyll under light are not significantly different from those in lard in the dark for 48 hr ($p>0.05$), which indicates that lard with 0 ppm chlorophyll under light was oxidized to the similar degree compared to the lard with 5 ppm chlorophyll in the dark. As storage time increased from 0 to 6, 12, 24, 36, and 48 hr, total volatiles in lard with 5 ppm chlorophyll stored under light increased by 0.46, 0.82, 1.47, 2.15, and 1.93 times, respectively,

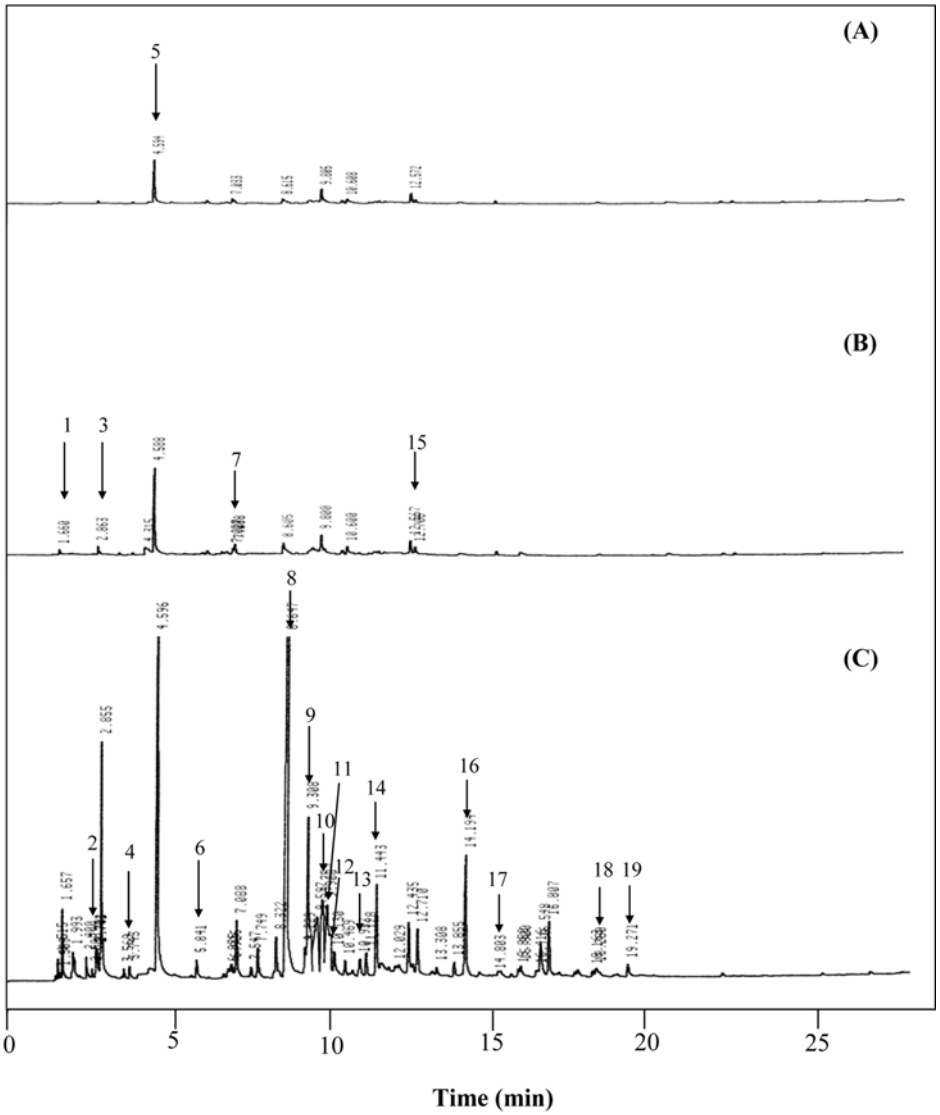

Fig. 1. Gas chromatograms of headspace volatile compounds in lard before treatment (A), lard without addition of chlorophyll under light (B), and lard with 5 ppm chlorophyll under visible light (C) for 24 hr at 55°C. Numbered volatiles are shown in Table 1.

compared to those in lard with 0 ppm chlorophyll under light. Total volatiles in lard with 5 ppm chlorophyll under light were significantly different from those in lard with 0 ppm chlorophyll under light from 6 to 48 hr ($p<0.05$), which shows that chlorophyll and light exposure accelerated lipid oxidation in lard.

Effect of photooxidation and chlorophyll photosensitization on the headspace oxygen content Changes of headspace oxygen content in lard sample bottles with 0 and 5 ppm chlorophyll at 55°C for 48 hr are shown in Fig. 3. Headspace oxygen analysis has been used to determine the degree of oxidation in oils (11). Headspace oxygen content in lard sample bottles with 0 ppm chlorophyll under light was not significantly different from that in lard sample bottles 5 ppm chlorophyll in the dark at 55°C for 48 hr ($p>0.05$).

Headspace oxygen content in photosensitized lard samples was significantly different from that in lard samples with 5 ppm chlorophyll in the dark and that in photooxidized samples at 55°C for 48 hr ($p<0.05$). As the storage time increased from 0 to 6, 12, 24, 36, and 48 hr, headspace oxygen content in photosensitized lard was lower than that in lard sample bottles with 0 ppm chlorophyll by 0.5, 1.0, 2.5, 4.4, and 6.2%, respectively, which shows that chlorophyll and light accelerated the headspace oxygen depletion and the lipid oxidation of lard.

The significant difference of total volatiles and headspace oxygen content between lard samples of photooxidation and photosensitization indicates that singlet oxygen, which is formed in the presence of photosensitizers and light exposure, was involved in oxidation steps and lard does not contain any photosensitizers in it. Therefore, lard can be used as a photosensitizer-free model system without tedious and laborious photosensitizer removing steps using various adsorbents including activated silica gel, Celite, powder sugar, and activated charcoal. Lard can be a proper and useful substrate for oxidation study to determine the effects of fat-soluble photosensitizers such as chlorophylls in photooxidation of foods.

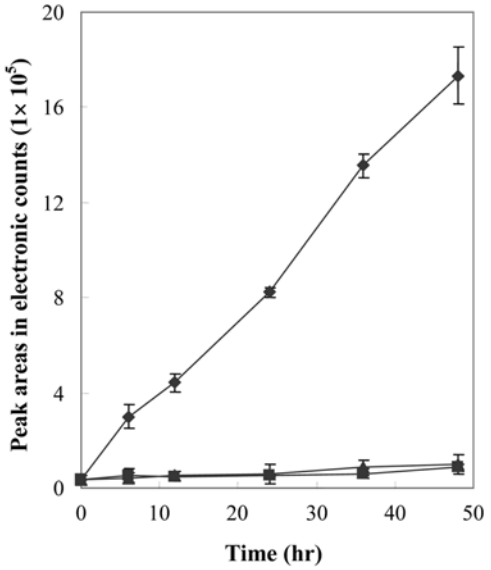

Fig. 2. Effects of chlorophyll on the peak areas of volatile compounds in lard under visible light and in the dark at 55°C for 48 hr. -◆- Lard with 5 ppm chlorophyll under light; -■- lard with 0 ppm chlorophyll under light; -▲- lard in the dark.

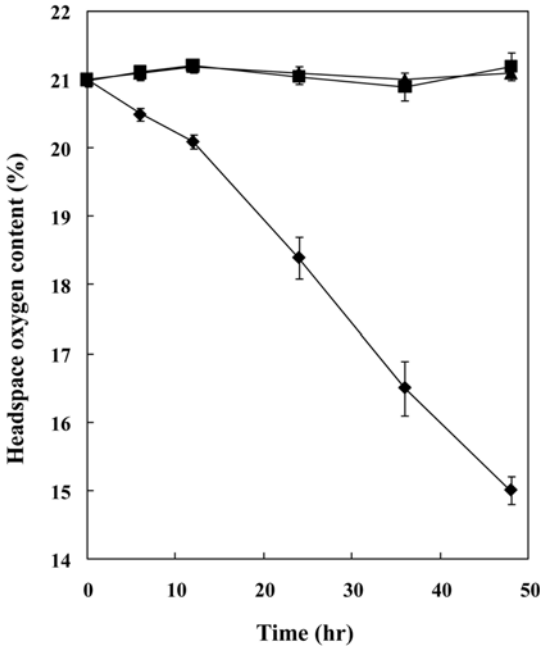

Fig. 3. Effects of chlorophyll on the headspace oxygen content in lard stored under light and in the dark at 55°C for 48 hr. -◆- Lard with 5 ppm chlorophyll under light; -■- lard with 0 ppm chlorophyll under light; -▲- lard in the dark.

Volatile profiles in photosensitized and photooxidized lard Volatile compounds identified from lard containing 0 and 5 ppm chlorophyll under visible light at 55°C for 0 and 48 hr are shown in Table 1. Pentane, pentanal, hexanal, heptanal, and nonanal were identified in lard both with 0 and 5 ppm chlorophyll stored under light, which indicates that these volatile compounds can be formed in lard in the absence of photosensitizers. Frankel (2) reported that pentane, pentanal, and hexanal can be formed from methyl linoleate,

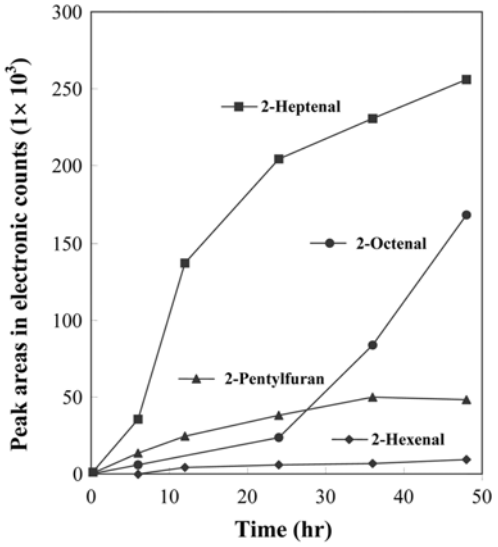

Fig. 4. Effects of chlorophyll on the changes of 2-hexenal, 2-heptenal, 2-pentylfuran, and 2-octenal in lard with 5 ppm chlorophyll under light at 55°C for 48 hr.

and heptanal and nonanal from methyl oleate by autoxidation. These volatile compounds identified in photooxidized lard, were also found in lard stored at 55°C in the dark, which underwent autoxidation (data not shown). Lipid oxidation mechanism of photooxidation under visible light seems to be a free radical chain reaction like autoxidation based on the profile comparison of volatile compounds.

Hexanal had the highest volatile peak area in both photooxidized and photosensitized lard samples. GC peak area of pentane, pentanal, hexanal, heptanal, and nonanal in photosensitized lard for 48 hr was higher than that in lard with 0 ppm chlorophyll by 11.0, 22.0, 3.0, 4.8, and 2.3 times, respectively (Table 1), which shows that chlorophyll photosensitization accelerated the formation of pentane, pentanal, hexanal, heptanal, and nonanal.

1-Penten-3-ol, 1-pentanol, 2-hexenal, 2-heptenal, 1-octene-3-ol, 2-pentylfuran, octanal, 2,4 heptadienal, 3-octene-2-one, 2-octenal, octanoic acid, 2,4 decadienal, and 2-undecenal, which were not identified in photooxidized lard, were identified in lard with 5 ppm chlorophyll at 55°C under visible light for 48 hr (Table 1). The changes of 2-hexenal, 2-heptenal, 2-pentylfuran, and 2-octenal in photosensitized lard are shown in Fig. 4. As storage time increased from 0 to 48 hr, 2-hexenal, 2- heptenal, 2-pentylfuran, and 2-octenal in photosensitized lard were formed and increased. Among volatiles only detected in photosensitized lard samples, peak area of 2-heptenal was the highest and 2-octenal, 2-pentylfuran, and 2-nonenal followed. It was reported that 2-heptenal was formed in soybean oil only in the presence of chlorophyll and light (17), which agrees with the results of in this study. Min *et al.* (17) identified 2-pentylfuran, a reversion flavor compound, in soybean oil containing 5 ppm chlorophyll during storage under light. However, 2-pentylfuran was not identified in soybean oil without chlorophyll or without light under current experimental conditions.

The photosensitizing effects of chlorophyll can explain the significant increase of the volatile compounds and the

Table 1. Volatile compounds from lard with 0 and 5 ppm chlorophyll under visible light at 55°C for 0 and 48 hr in GC peak areas (1×10^4)

No.[1)]	Volatile compounds	0 hr sample	Lard sample with 0 ppm	Lard sample with 5 ppm
1	Pentane	ND[2)]	0.10±0.00[3)]	4.98±0.51
2	**1-Penten-3-ol**[MS]	ND	ND	1.09±0.08
3	Pentanal[MS]	ND	0.51±0.04	10.42±0.91
4	**1-Pentanol**	ND	ND	0.81±0.05
5	Hexanal	1.45±0.14	6.72±0.52	26.84±2.56
6	**2-Hexenal**	ND	ND	0.94±0.12
7	Heptanal	ND	0.73±0.08	4.05±0.41
8	**2-Heptenal**	ND	ND	25.61±2.11
9	**1-Octene-3-ol**	ND	ND	2.05±0.18
10	**2-Pentylfuran**	ND	ND	7.45±0.84
11	**Octanal**	ND	ND	4.62±0.32
12	**2,4 Heptadienal**[MS]	ND	ND	5.42±0.41
13	**3-Octene-2-one**	ND	ND	1.35±0.08
14	**2-Octenal**	ND	ND	16.72±1.01
15	Nonanal	ND	0.82±0.09	2.62±0.12
16	**2-Nonenal**	ND	ND	6.69±0.84
17	**Octanoic acid**	ND	ND	0.77±0.04
18	**2,4 Decadienal**	ND	ND	1.01±0.04
19	**2-Undecenal**[MS]	ND	ND	1.94±0.11

[1)]The numbered volatiles in Fig. 1; Bold character represents a compound, which was detected in lard with 5 ppm chlorophyll under light but not detected in lard without chlorophyll under light; [MS]A compound identified by library of GC-MS only. The other compounds were identified by both GC retention time of standard compounds and library of GC-MS.
[2)]Not detected.
[3)]Mean±SD (n=3).

significant decrease in the headspace oxygen content in lard with 5 ppm chlorophyll under visible light. The major fatty acids of lard are myristic (1-4%), palmitic (20-28%), stearic (5-14%), oleic (41-51%), linoleic (2-15%), linolenic acid (trace-0.1%), and arachidonic (0.3-1.0%) (2). Photosensitizers exposed to light can oxidize unsaturated fatty acids in lard through Type I and Type II mechanisms depending on the solubility and concentration of oxygen and substrates. The solubility of oxygen is higher in lipid and nonpolar solvents than in water. Singlet oxygen generation (Type II mechanism) is preferred in lipid phase due to the high solubility of triplet oxygen (18). Rawls and Van Santen (19) reported that chlorophyll accelerated the oxidation of methyl linoleate approximately 80% through Type II mechanism and 20% through Type I mechanism. Yang *et al.* (20) also showed that formation of volatiles in linoleic acid under riboflavin photosensitization were significantly influenced by addition of sodium azide and D_2O, which can act as a singlet oxygen quencher and a singlet oxygen stabilizer, respectively.

Developed lard model system in this study can help to explain the formation mechanisms of some volatiles which have not been understood using previous conventional model systems. For example, 2-heptenal was formed in trace concentration from autoxidation of methyl linoleate but photooxidation of methyl linoleate produced 9.9 relative percentage of 2-heptenal (2). This study showed that 2-heptenal was formed only in chlorophyll photosensitized lard with relatively high concentration. Therefore, singlet oxygen oxidation is highly responsible for the formation of 2-heptenal in chlorophyll photosensitized lard in current experiment conditions.

However, it is needed to understand that several oxidation mechanisms including singlet oxygen oxidation, free radical chain reaction, and both can be involved in the formation of volatiles depending on the different experimental conditions. Recently, Lee *et al.* (21) reported that 2-heptanal was formed and increased in the thermally oxidized mixtures of free fatty acids at 93°C for 200 min. The authors selected 2-heptanal and total volatiles as possible volatile markers to determine the degree of oxidation and hydrogen donating antioxidant activities.

In conclusion, a photosensitizer-free lipid model system was developed using lard, which can be used to determine the difference of oxidation mechanisms between photosensitized and photooxidized lipid and/or foods containing lipids. Oxidation mechanisms of photooxidation of visible light and photosensitization on lard were different based on the results of volatile profiles and headspace oxygen contents. Oxidation mechanisms of photooxidation with or without photosensitizers under visible light were not the same based on the profile changes of oxidized volatile profiles and changes of headspace oxygen contents. Singlet oxygen oxidation can explain the increases of volatile compounds and of headspace oxygen depletion in the chlorophyll photosensitized lard.

References

1. Chan HWS. Photo-sensitized oxidation of unsaturated fatty acid methyl esters. The identification of different pathways. J. Am. Oil Chem. Soc. 54: 100-104 (1977)
2. Frankel EN. Chemistry of autoxidation: Mechanism, products, and flavor significance. pp. 1-37. In: Flavor Chemistry of Fats and Oils. Min DB, Smouse TH (eds). AOCS, Champaign, IL, USA (1985)
3. Choe E, Min DB. Mechanisms and factors for edible oil oxidation. Compr. Rev. Food Sci. F. 5: 1-18 (2006)
4. Faria JAF, Mukai MK. Use of gas chromatographic reactor to study lipid photooxidation. J. Am. Oil Chem. Soc. 60: 77-81 (1983)
5. Yang WTS, Min DB. Chemistry of singlet oxygen oxidation of foods. pp. 15-29. In: Lipids in Food Flavors. Ho CT, Hartmand TG (eds). American Chemical Society, Washington, DC, USA (1994)
6. Edwards AM, Silva E. Effect of visible light on selected enzymes, vitamins, and amino acids. J. Photoch. Photobio. B 63: 126-131 (2001)
7. Thron M, Eichner K, Ziegleder G. The influence of light of different wavelengths on chlorophyll-containing foods. Lebensm. -Wiss. Technol. 34: 542-548 (2001)
8. Yang SO, Chang PS, Lee JH. Effects of riboflavin-photosensitized oxidation on the formation of volatile compounds in oleic acid model systems. Korean J. Food Sci. Technol. 37: 717-722 (2005)
9. Foote CS. Photosensitized oxidation and singlet oxygen: Consequences in biological systems. Vol. 2, pp. 85-133. In: Free Radicals in Biology. Pryor WA (ed). Academic Press, New York, NY, USA (1976)
10. Boff JM, Min DB. Chemistry and reaction of singlet oxygen in foods. Compr. Rev. Food Sci. F. 1: 58-72 (2002)
11. Lee EC, Min DB. Quenching mechanism of β-carotene on the chlorophyll sensitized photooxidation of soybean oil. J. Food Sci. 53: 1894-1985 (1988)
12. Kulas E, Ackman RG. Properties of α-, γ-, and σ- tocopherol in purified fish oil triacylglycerols. J. Am. Oil Chem. Soc. 78: 361-367 (2001)
13. Romero N, Robert P, Masson L, Ortiz J, González K, Tapia K,

Dobaganes C. Effect of α-tocopherol, α-tocotrienol, and Rosa mosqueta shell extract on the performance of antioxidant-stripped canola oil (*Brassica* sp.) at high temperature. Food Chem. 104: 383-389 (2007)
14. Kincs FR. Meat fat formulation. J. Am. Oil Chem. Soc. 62: 815-818 (1985)
15. Pokorny J, Zwain H, Pazlar M. Effect of myoglobin on the autoxidation of lard in nonaqueous medium. Nahrung 10: 730-731 (1966)
16. Baron CP, Andersen H. Myoglobin-induced lipid oxidation. A review. J. Agr. Food Chem. 50: 3887-3897 (2002)
17. Min DB, Callison AL, Lee HO. Singlet oxygen oxidation for 2-pentylfuran and 2-pentenylfuran formation in soybean oil. J. Food Sci. 68: 1175-1178 (2003)
18. Bradley DG, Min DB. Singlet oxygen oxidation of foods. Crit. Rev. Food Sci. 31: 211-236 (1992)
19. Rawls HR, Van Santen PJ. A possible role for singlet oxidation in the initiation of fatty acid autoxidation. J. Am. Oil Chem. Soc. 47: 121-125 (1970)
20. Yang SO, Lee JM, Lee JC, Lee JH. Effects of riboflavin-photosensitization on the formation of volatiles in linoleic acid model systems with sodium azide or D_2O. Food Chem. 105: 1375-1381 (2007)
21. Lee JH, Kim DH, Chang PS, Lee JH. Headspace-solid phase microextraction (HS-SPME) analysis of oxidized volatiles from free fatty acids (FFA) and application for measuring hydrogen donating antioxidant activity. Food Chem. 103: 414-420 (2007)