Volatile profiles and involvement step of moisture in bulk oils during oxidation by action of deuterium oxide (D₂O)

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Received: 14 February 2018 / Revised: 23 March 2018 / Accepted: 8 April 2018 / Published online: 21 June 2018
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Abstract Volatile formation is an inevitable result of lipid oxidation, which impact the quality of lipid rich foods. In this study, moisture role on the formation of volatiles were evaluated using deuterium oxide (D₂O) and possible steps of moisture involvement were suggested. Moisture content in corn oil with deuterium free water (H₂O) was significantly ($p < 0.05$) higher than that in corn oil with D₂O. The contents of some volatiles including pentane, hexanal, 2-hexenal, and *t*-2-heptenal in corn oil with D₂O were higher than those in corn oil with H₂O for the first 8 days. Volatiles containing deuterium appeared in the order of pentane, *t*-2-pentenal, and *t*-2-heptenal during oxidation. Deuterium incorporated volatiles could be formed after the β -scission of lipid hydroperoxides. Therefore, moisture plays important roles in the formation of volatiles as well as the locations of oxidation in bulk oils.

Keywords Deuterium oxide · Headspace volatile · Moisture · Lipid oxidation

Introduction

Lipid oxidation is one of the main reasons causing quality deterioration of lipid based foods because oxidation products can induce undesirable flavor, change color and

texture, and lower nutrition values of foods (Frankel, 1998; Halliwell et al., 1995; Nawar, 1996). Major factors involved in lipid oxidation include the degree of unsaturation in fatty acids and oxygen molecules such as triplet or singlet oxygen. Minor components present in oils including transition metals, pigments, moisture, phospholipids (PLs), sterols, and antioxidants can also greatly affect lipid oxidation rate (Chaiyasit et al., 2007; Choe and Min, 2006; McClements and Decker, 2000).

Lipid oxidation mechanisms in bulk oils and oil-in-water (O/W) emulsion systems have been extensively evaluated (Abdalla and Roozen, 1999; Frankel et al., 1994; Fritsch, 1994; Halliwell et al., 1995; McClements and Decker, 2000). Generally, lipid oxidation rate is higher in O/W emulsion than that in bulk oil, indicating that moisture might play significant roles in oxidative stability of lipids (Frankel et al., 1994). The interface of water–oil has been proposed as lipid oxidation site in O/W emulsion and bulk oil (Laguerre et al., 2015; McClements and Decker, 2000; Schwarz et al., 2000). Commercial bulk oil contains small amount of moisture (in the range of 300–800 ppm). These oils also contain free fatty acids (FFAs), monoacylglycerols (MAGs), diacylglycerols (DAGs), PLs, and oxidation products such as lipid hydroperoxides that are mostly amphiphilic compounds. Moisture and amphiphilic molecules can form association colloids which may provide a local environment for lipid oxidation. These association colloids could alter physical locations of chemical compounds with prooxidative and/or antioxidative properties.

In previous studies, Kim et al. (2014) have shown that deuterium oxide (D₂O) is involved in the formation of headspace volatiles during oxidation of linoleic acid-D₂O. Among volatile compounds produced from oxidation of linoleic acid, fragmented mass to charge ratio (m/z) of

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(molecular weight + 1)/(molecular weight) which is 73.1/72.1 for *d*-pentane/pentane and 57.0/56.0 for *d*-2-propenal/2-propenal has been increased significantly in the presence of D₂O compared to that in samples with deuterium free water (H₂O) during oxidation. This implies that moisture might be involved in the formation of volatiles during oxidation. Such trend has also been confirmed in the oxidation of corn oil containing different ratios of D₂O (Kim et al., 2014b).

Various volatiles can be formed from different pathways during lipid oxidation depending on singlet or triplet oxygen oxidation or types of monounsaturated or polyunsaturated fatty acid (Frankel, 1985; Frankel, 1998; Frankel et al., 1981). To prevent oxidative deterioration that causes off-aroma associated with rancidity of lipid rich foods, understanding those formation processes are very important.

The objectives of this study were to determine the effect of moisture on the formation of volatile compounds in corn oil with D₂O or H₂O and suggest possible pathways for moisture to participate in the formation of volatiles during lipid oxidation.

Materials and methods

Materials

Standard compounds of volatiles, H₂O, and D₂O were purchased from Sigma-Aldrich (St. Louis, MO, USA). Solid phase microextraction (SPME) solid phase [50/30 mm DVB/Carboxen/Polydimethylsiloxane (PDMS) StableFlex] was purchased from Supelco, Inc. (Bellefonte, PA, USA). Vials, magnetic seals, and septa were purchased from Agilent technologies, Inc. (Santa Clara, CA, USA). Other reagent grade chemicals were purchased from Daejung Chemical Co. (Seoul, Korea).

Sample preparation

D₂O or H₂O (0.3 g) was placed in 10-mL vials and 2-mL vials filled with 0.5 g of corn oil were placed inside the 10-mL vials as a double vial system. Such double vials were sealed air-tight with rubber septa and aluminum caps. Samples were stored in a dry oven (HYSC Co, Ltd, Seoul, Korea) at 60 °C for 0, 4, 8, 12, and 18 days. Samples without moisture were used as controls. All samples were prepared in triplicates at each sampling point.

Moisture content analysis

Moisture content in oil was determined using a coulometric KF titrator (C20, Mettler-Toledo Intl., Columbus, OH, USA) according to the manufacturer's instructions.

Analysis of headspace volatile by SPME

Analysis condition of volatile compounds using SPME was adapted from the method of Kim et al. (2014a). Sample vials were placed in a water bath (RW-0525G; Lab Camp, Incheon, Korea) at 30 °C for 30 min and then transferred to a multipurpose sampler (Gerstel, Inc., Baltimore, USA). Headspace volatiles were isolated and extracted for 30 min using a 50/30 mm DVB/Carboxen/PDMS StableFlex solid phase at 30 °C.

Volatiles were separated and identified using a Hewlett-Packard 6890 GC equipped with a 5971A mass selective detector (MS) (Agilent Technology) and a DB-5 ms column (30 m × 0.25 mm i.d., 0.25 µm film thickness, Agilent J & W, Folsom, CA, USA). Solid phase of SPME was exposed to an injector for 3 min to separate volatiles attached to the SPME. Carrier helium gas was flowed at 1.0 mL/min. The oven temperature was held at 40 °C for 3 min, then increased from 40 to 150 °C at rate of 4 °C/min and from 150 to 220 °C at rate of 15 °C/min. All mass spectra were obtained at 70 eV with ion source temperature of 220 °C. The identification of compounds was made by NIST Mass Spectra combined with gas chromatographic retention times of standard compounds.

Detection of volatiles containing deuterium

The presence of deuterium (D·) in volatiles was analyzed by comparing *m/z* (mass to charge ratio) of each volatile (Kim et al., 2014a; 2014b; Oh et al., 2015). If a *m/z* of sample in deuterium oxide was increased by one mass unit compared to that of sample in deuterium free water, deuterium might have been incorporated in the formation of volatiles. Comparison of *m/z* (molecular weight + 1)/(molecular weight) can show the presence of D· in each volatile. For example, in case of pentane (molecular weight of 72.1) and *t*-2-heptenal (molecular weight of 112.17), the *m/z* of 73.1/72.1 for *d*₁-pentane/pentane and 113.17/112.17 for *d*₁-*t*-2-heptenal/*t*-2-heptenal was determined by GC/MS ion chromatogram in SIM mode.

Statistical analysis

Data of moisture content and headspace volatiles were analyzed statistically by one-way analysis of variance (ANOVA) and Duncan's multiple range test using SPSS

Fig. 1 Moisture content in corn oil at double vial system, containing H₂O or D₂O for 18 days at 60 °C. CON, H₂O, and D₂O are corn oil without moisture, with deuterium free water, and with deuterium oxide, respectively. Different small letters are significantly different at 0.05 among samples

software program version 22 (SPSS Inc., Chicago, IL). A p value < 0.05 was considered statistically significant.

Results and discussion

Moisture content analysis

Moisture content in corn oil with H₂O or D₂O are shown in Fig. 1. Moisture contents in corn oil with H₂O or D₂O were significantly higher than those in control corn oils ($p < 0.05$) for 18 days. Relatively high moisture content in corn oil compared to control could be pro-oxidative factor in lipid oxidation which can increase the number of association colloids, the possible sites for lipid oxidation. Higher number of association colloids may provide more surface areas in bulk oils, which are the places for lipid oxidation (Kim et al., 2014a; Laguerre et al., 2015). Interestingly, moisture content in corn oil with H₂O (2500 ppm) was significantly ($p < 0.05$) higher than that in corn oil with D₂O (2200 ppm). Lower moisture in corn oil with D₂O than H₂O could be due to the different affinity among water and amphiphilic compounds present in bulk oil or difference in the depletion of moisture during lipid oxidation, which needs further researches.

Volatile analysis

Changes of selected major volatile compounds in corn oil with H₂O or D₂O during oxidation at 60 °C for 18 days in the dark are summarized in Table 1.

Some volatiles including 2-hexenal and *t*-2-heptenal were not detected in corn oil before oxidation. At 4 and 8 days of storage, all the volatiles including pentane, hexanal, 2-hexenal, and *t*-2-heptenal in corn oil with D₂O were higher than other samples, which implies that presence of D₂O accelerated the formation of some volatiles. However, only 2-hexenal and *t*-2-heptenal in corn oil with D₂O were higher than those in corn oil with H₂O at the 18 day of storage. After 18 days of storage, peak areas of hexanal was the highest followed by *t*-2-heptenal and pentane in corn oil with D₂O whereas those of pentane was the highest followed by hexanal and *t*-2-heptenal in corn oil with H₂O and control samples (Table 1). Although moisture content in corn oil with D₂O was lower than those in corn oil with H₂O (Fig. 1), higher degree of oxidation, that is more peak areas of headspace volatiles, was found in corn oil with D₂O, which agrees with the prooxidative properties of D₂O (Kim et al., 2014b).

Pentane, hexanal, and *t*-2-heptenal are typically generated from oxidation of linoleic acid (Frankel, 1985). *t*-2-Pentenal and 2-hexenal can be formed from linolenic acid oxidation (Frankel et al., 1981). Corn oil contained 53.6% linoleic acid and 1.6% γ -linolenic acid in total fatty acid composition. Generally, hexanal and pentane were the most abundant two volatile in corn oil–water system (Table 1). Pentane and hexanal can be produced from scission of 13-hydroperoxides in linoleic acid (Frankel, 1998; Oh et al., 2015). Especially, hexanal has been used for an indicator to determine the rate of lipid oxidation in bulk oil or O/W emulsions (Chen et al., 2012; Frankel et al., 1981; Frankel et al., 1994; Lee and Decker 2011). Presence of D₂O might have accelerated the formation of headspace volatiles at relatively early stages of oxidation such as 4 and 8 days of storage compared to 12 and 18 days of storage. Although clear difference among peak areas of some volatiles were observed, total peak areas of volatiles showed no significant ($p > 0.05$) differences among samples at each oxidation time.

The m/z of $(M + 1)/(M)$ for pentane, *t*-2-pentenal, and *t*-2-heptenal from oxidation of corn oil containing H₂O or D₂O at 60 °C in the dark for 18 days are shown in Fig. 2. Incorporation of deuterium into volatile compounds can be determined from the m/z of $(M + 1)/(M)$. Increases in mass to charge ratio of $(M + 1)/(M)$ for volatiles from oils with D₂O could be used as clues for involvement of deuterium in volatile formation. Increases in mass to charge ratio of $(M + 1)/(M)$ have been used to indicate the participation of moisture in volatile compounds (Kim et al., 2014a; 2014b). After corn oil was oxidized for 4 days, the m/z of $(M + 1)/(M)$ for pentane appeared in corn oil with H₂O and D₂O. Mass to charge ratio of $(M + 1)/(M)$ from corn oil with H₂O could be due to the presence of isotopic carbon rather than the added D₂O. The isotope ratio of ¹³C

Table 1 Peak areas (1×10^6) of selected volatiles in oxidized corn oil-H₂O or D₂O system for 18 days at 60 °C

Compound	Retention time (min)	Sample before oxidation	Storage time (day)					
			4			8		
			CON ¹	H ₂ O	D ₂ O	CON	H ₂ O	D ₂ O
Pentane	1.82	0.55 ± 0.31 ²	1.68 ± 0.21	3.87 ± 2.28	5.22 ± 0.37	3.81 ± 1.76	3.51 ± 1.05	13.79 ± 5.58
<i>t</i> -2-Pentenal	4.32	0.05 ± 0.00	0.17 ± 0.07	0.52 ± 0.31	0.49 ± 0.03	0.91 ± 0.65	1.70 ± 0.22	3.39 ± 0.23
Hexanal	5.32	0.49 ± 0.17	2.34 ± 0.80	2.32 ± 0.02	9.27 ± 2.21	7.60 ± 4.81	6.83 ± 4.49	12.67 ± 2.30
2-Hexenal	6.66	N.D. ³	N.D.	0.17 ± 0.09	0.40 ± 0.02	0.14 ± 0.09	0.32 ± 0.12	1.02 ± 0.34
<i>t</i> -2-Heptenal	9.59	N.D.	0.12 ± 0.04	0.57 ± 0.28	1.50 ± 0.32	0.58 ± 0.40	1.16 ± 0.13	3.31 ± 1.67
Total volatiles		28.44 ± 6.54	269.46 ± 22.87bc ⁴	264.09 ± 56.09bc	197.12 ± 58.57 ^c	342.80 ± 42.24bc	329.57 ± 11.79bc	422.09 ± 234.65ab
Compound	Retention time (min)	Sample before oxidation	Storage time (day)					
			12			18		
			CON	H ₂ O	D ₂ O	CON	H ₂ O	D ₂ O
Pentane	1.82	0.55 ± 0.31 ²	6.92 ± 1.18	4.74 ± 0.61	6.01 ± 0.51	8.29 ± 3.17	10.07 ± 6.13	7.01 ± 1.08
<i>t</i> -2-Pentenal	4.32	0.05 ± 0.00	2.32 ± 0.49	3.24 ± 0.23	2.87 ± 0.38	2.88 ± 0.70	4.52 ± 0.53	3.88 ± 0.23
Hexanal	5.32	0.49 ± 0.17	12.57 ± 12.95	8.75 ± 5.47	10.64 ± 0.15	5.66 ± 0.58	7.11 ± 1.22	10.76 ± 1.23
2-Hexenal	6.66	N.D. ³	N.D.	0.68 ± 0.04	0.97 ± 0.28	0.52 ± 0.17	0.86 ± 0.19	1.34 ± 0.17
<i>t</i> -2-Heptenal	9.59	N.D.	1.02 ± 0.31	1.74 ± 0.24	3.53 ± 0.79	1.79 ± 0.35	2.16 ± 0.20	7.94 ± 3.31
Total volatiles		28.44 ± 6.54	463.45 ± 181.84ab	588.70 ± 28.20a	591.01 ± 31.87a	454.56 ± 147.90ab	462.82 ± 181.24ab	573.87 ± 12.51a

¹CON, H₂O, and D₂O are corn oil without moisture, with deuterium free water, and with deuterium oxide, respectively²Mean ± standard deviation (n = 3)³N.D.: not detected⁴Different letters are significant in the row at $p < 0.05$

Fig. 2 The mass to charge ratio (m/z) of $(M + 1)/M \times 100$ (percentage) in selected volatiles in corn oil at double vial system containing H_2O or D_2O for 18 days at $60^\circ C$. Abbreviations are listed in the legend of Fig. 1. N.D.: not detected

Fig. 3 Proposed formation pathways of pentane, *t*-2-pentenal, and *t*-2-heptenal from oxidized corn oil containing D_2O at double vial systems

to ^{12}C is approximately 1.12% under normal condition (Hofstetter et al., 2008). After storage for 8 days, not only pentane, but also *t*-2-pentenal had detectable m/z of $(M + 1)/(M)$. After corn oil was oxidized for 12 days, m/z of $(M + 1)/(M)$ of pentane and *t*-2-pentenal were detected in controls. Those in corn oil with D_2O were higher than those in corn oil with H_2O . After oxidation for 12 days, only *t*-2-heptenal was detected in corn oil with D_2O . The sequence for formation of volatiles could be highly related to the oxidation rate which was high in corn oil with D_2O but low in controls. D_2O might have been incorporated in headspace volatiles. The formation sequence for volatiles was in order of pentane, *t*-2-pentenal, and *t*-2-heptenal.

Based on results stated above, proposed pathways for the formation of pentane, *t*-2-pentenal, and *t*-2-heptenal are shown in Fig. 3. Kim et al. (2014a) have suggested a

formation pathway for deuterated pentane from linoleic acid oxidation by β -scission of 13-hydroperoxide. Deuterium from D_2O may join pentane radical to form pentane containing deuterium. It has been reported that hexanal without needing hydrogen atom from an outside source does not have significant changes in the m/z of $(M + 1)/(M)$ (Kim et al., 2014a). After pentane is removed from linoleate, the remaining part can go through further oxidation process. Susceptibility to autoxidation depends on the availability of allylic hydrogens for reactions with peroxy radicals (Frankel, 1998). The energy for removing allylic hydrogen is relatively small. Thus, it is easy to become in the state of alkyl radicals. Valence bond structure could be in the state of hybrid with a partial free radical at each end of the allylic system (Frankel, 1998). Diverse hydroperoxides can be generated from the allylic

system. Valence bond structure [Fig. 3(A)] may produce partial free radical of the allylic system [Fig. 3(B)]. If oxidation proceeds on radical of carbon-8, decomposition of hydroperoxide can produce *t*-2-pentenal radical without a proton. Deuterium could join carbon with unpaired electron and form deuterated *t*-2-pentenal. By similar way, if oxygen molecule attacks the radical of carbon-6, deuterated *t*-2-heptenal can be generated (Fig. 3). As shown in Fig. 2, deuterated pentane appeared first and deuterated *t*-2-pentenal then appeared and followed by deuterated *t*-2-heptenal during lipid oxidation. The sequential detection of deuterated volatiles indirectly supports above suggested pathways for volatile formation with deuterium. Results of this study suggested steps of moisture involvement in the formation of volatiles during lipid oxidation for the first time. Oh et al. (2017) could not detect lipid hydroperoxides containing deuterium and reported that moisture could be involved in the lipid oxidation during the decomposition stage of lipid hydroperoxides rather than formation of lipid hydroperoxides, which agrees with the results of this study. Therefore, moisture may participate the lipid oxidation not only providing sites through formation of association colloids with amphiphilic compounds but also involving lipid oxidation as one of substrates for the formation of volatiles.

In conclusion, the role of moisture on the formation of volatiles was tested in corn oil-D₂O system. Major deuterated volatiles were detected in the order of pentane, *t*-2-pentenal, and *t*-2-heptenal and their formation pathways were proposed in this study. Previously only the formation pathway of pentane containing deuterium was suggested and pathways for other volatiles have not been understood clearly (Kim et al., 2014a). Moisture content in corn oil with D₂O was lower than that in corn oil with H₂O. Our results demonstrate that moisture is an important factor that affects the formation of volatiles from lipid oxidation especially after β -scission of lipid hydroperoxide step. Moisture content should be more carefully monitored in lipid rich foods during processing and storage to control the formation of volatiles with rancidity and extend the shelf-life of foods.

Acknowledgements This research was supported by a Grant (NRF-2017R1A2B4002613) of the Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology, Republic of Korea.

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