Effects of α -Tocopherol on the Oxidative Stability and Incorporation of Deuterium in Volatiles from a Linoleic Acid-deuterium Model System

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Received November 17, 2015
Revised January 19, 2016
Accepted January 29, 2016
Published online June 30, 2016

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pISSN 1226-7708
eISSN 2092-6456

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Abstract The effects of α -tocopherol on the oxidative stability and incorporation of deuterium in volatiles were evaluated in linoleic acid-water model systems treated at 60°C by analyzing headspace oxygen depletion, formation of lipid hydroperoxides, and profiles of headspace volatiles. Deuterium oxide accelerated the rates of linoleic acid oxidation compared to samples in deuterium-free water. As the concentration of α -tocopherol increased from 0 to 1500 ppm, the consumption of headspace oxygen and the formation of volatiles decreased, whereas the contents of lipid hydroperoxides did not decrease in the linoleic acid-water system. The mass to charge ratios (m/z) of volatiles in linoleic acid-deuterium oxide were significantly higher than those with deuterium oxide-free water. Generally, the presence of α -tocopherol decreased the mass to charge ratios (m/z) of volatiles including pentanal, hexanal, *t*-2-heptenal, and 2-octenal, implying that α -tocopherol may be involved in the aldehyde formation from lipid oxidation.

Keywords: α -tocopherol, deuterium oxide, lipid oxidation, headspace volatile

Introduction

Lipid oxidation is a chemical reaction the results of which greatly reduce nutritional values and consumer acceptance. Moisture in bulk oil plays important roles in the rates of lipid oxidation through forming association colloids with amphiphilic compounds including free fatty acids, monoacylglycerols, diacylglycerols, and phospholipids (1-3). Bulk oils possess about 200-800 ppm moisture content although the water activity of bulk oils is reported to be 0 (1,3-5). Reverse micelles are typical structures of association colloids in bulk oils and amphiphilic compounds that, like phospholipids, can form reverse micelles with moisture in oils (5).

Lipid oxidation is believed to occur largely at the oil-water interface in association colloids or reverse micelles (6). An increase in moisture content was observed in the bulk oil as the degree of oxidation increased due to influx of moisture from the atmosphere (7). The moisture content in the bulk oil affects the antioxidant efficiency of lipophilic α -tocopherol and hydrophilic ascorbic acid. Stripped bulk oils having the same concentrations of α -tocopherol and ascorbic acid had different antioxidant properties under the different moisture conditions (8,9), which implies that the antioxidant properties and stability of hydrogen-donating compounds are closely associated

with moisture in bulk oils. The moisture content of bulk oil with phospholipids like phosphatidylcholine influenced the oxidative stability of the bulk oil (10). Oils containing phosphatidylcholine showed strong synergistic antioxidant effects with α -tocopherol, which could be because the association colloids formed by phospholipids provided an advantageous location for antioxidants to be effective in the reverse micelles formed in this system (10).

Moisture may participate in the formation of volatile compounds from linoleic acid oxidation that end up in the headspace of the storage vessel (11,12). Natural water possesses 152 ppm deuterium, which has one more neutron and is therefore heavier than hydrogen by 1 atomic mass unit. Oh *et al.* (12) reported that the presence of deuterium oxide accelerated the consumption of headspace oxygen and volatile formation in linoleic acid as compared to samples with deuterium-free water. However, the effects of deuterium on the antioxidant properties of α -tocopherol have not been reported in the literature.

The objectives of this study were to determine the effects of deuterium oxide on the antioxidant properties of lipophilic α -tocopherol and to find out whether α -tocopherol influences the incorporation of deuterium in volatiles from oxidized linoleic acid-water model systems.

Materials and Methods

Materials Deuterium free water (H_2O), D_2O , linoleic acid, and α -tocopherol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Fibers for solid phase microextraction (SPME) (i.e., 50/30 mm DVB/Carboxen/Polydimethylsiloxane (PDMS) StableFlex) were purchased from Supelco, Inc. (Bellefonte, PA, USA). Vials, magnetic seals, septa, and aluminum caps were purchased from Agilent Technologies, Inc. (Santa Clara, CA, USA). Other reagent grade chemicals were purchased from Daejung Chemical Co. (Seoul, Korea).

Sample preparation α -Tocopherol was dissolved in hexane and then added to linoleic acid at a concentration of 1500 ppm. Solvent in the mixture was removed by nitrogen gas flushing. Samples were serially diluted to make solutions with 250, 500, 750, and 1,000 ppm α -tocopherol in linoleic acid. Then, 0.2 g of the linoleic acid-containing α -tocopherol was added to 0.6 g of D_2O or H_2O . The sample vials were sealed in air-tight containers with rubber septa and aluminum caps and were treated at 60°C in a drying oven (HYSC, Seoul, Korea) for 7 h. Linoleic acid without added α -tocopherol was prepared as a control. Linoleic acids with H_2O or D_2O were designated as D0 or D1, respectively. Samples were prepared in triplicate and each experiment was performed three times.

Headspace oxygen analysis The headspace oxygen content in air-tight vials was analyzed to find out the degree of oxidation based on the method of Kim *et al.* (13) by using a Hewlett-Packard 7890 gas chromatograph (GC) (Agilent Technologies) equipped with a thermal conductivity detector (TCD) from Agilent Technologies. The stationary phase was a 60/80 packed column (3.0 mm $\times$ 2 mm ID, Restek Ltd., Bellefonte, PA, USA) and the mobile phase was helium gas. The flow rate of helium was 20 mL/min. The temperatures of the oven, the injector, and the detector in the GC-TCD were 60, 180, and 180°C , respectively (13).

Analysis of lipid hydroperoxides by HPLC Lipid hydroperoxides (LOOHs) in the mixtures of linoleic acid-water were determined using a high pressure liquid chromatograph (HPLC) with an ultraviolet detector (Hitachi, Tokyo, Japan). We carefully separated 0.05 g of linoleic acid from the sample mixtures, and this was mixed with acetonitrile and filtered through a $0.45\ \mu\text{m}$ hydrophilic polypropylene membrane. The stationary phase of the HPLC was a LiChrosorb C_{18} reverse phase column (4 μm , 3.9 mm $\times$ 150 mm), and the mobile phase was a mixture of acetonitrile, ultra-pure water, and acetic acid (60:40:0.12, v:v:v) under isocratic conditions. The flow rate of the mobile phase was 1.5 mL/min and the injection volume was 10 μL . LOOHs in the eluent were monitored at 234 nm, and the HPLC-oven temperature was kept at 25°C . The four major peaks in the HPLC chromatograms were tentatively identified by comparing retention time and peak patterns with those of previous reports (14,15). Peak areas of the four major peaks in the HPLC chromatograms were

added together to determine the contents of LOOHs.

Analysis of headspace volatiles by SPME Analysis conditions for volatile compounds by SPME were based on the methods of Oh *et al.* (12). Sample vials were placed in a water bath (RW-0525G; Lab Camp, Incheon, Korea) at 30°C for 30 min to create an equilibrium between the headspace and samples. Then, a 50/30 mm DVB/Carboxen/PDMS StableFlex SPME fiber was inserted to the headspace of the sample vials to extract headspace volatiles for 30 min at 30°C .

The SPME fiber was inserted into the injector of the GC at 250°C for 3 min. Volatiles were separated and identified using a Hewlett-Packard 6890 GC with a 5971A mass selective detector (MS) (Agilent Technologies). The stationary phase was a DB-5ms column (30 m $\times$ 0.25 mm i.d., 0.25 mm film thickness, Agilent J & W, Folsom, CA, USA). The mobile phase was helium gas with a flow rate of 1.0 mL/min. The GC oven temperature started at 40°C for 3 min and increased to 150°C at $4^\circ\text{C}/\text{min}$ and from 150 to 220°C at a rate of $15^\circ\text{C}/\text{min}$. All mass spectra were obtained at 70 eV and a 220°C ion source temperature. Volatile compounds were identified by a comparison with NIST mass spectra and the retention times of some standard compounds.

Detection of volatiles containing deuterium The presence of deuterium in volatiles was determined by comparing m/z (mass to charge ratios) of each volatile (10,11). The incorporation of a $\text{D}^\bullet$ in a molecule will increase the m/z by 1 compared to the m/z in a deuterium free molecule. The molecular ion (M) of each volatile was used for the incorporation of $\text{D}^\bullet$. Comparison of m/z ($M+1$) to m/z (M) may show the presence of $\text{D}^\bullet$ in the volatile. For example, the m/z of 113.17 ($M+1$) from d_1 - t -2-heptenal (deuterated t -2-heptenal) was compared with the m/z of 112.17 (M) from t -2-heptenal at each sampling time using the selected ion monitoring (SIM) mode of the GC/MS ion chromatograms (11,12).

Statistical analysis Data of headspace oxygen content, content of LOOH, and headspace volatiles were analyzed statistically by ANOVA and Duncan's multiple range test using SPSS software (SPSS Inc., Chicago, IL, USA). A p value <0.05 was considered significant.

Results and Discussion

Effects of α -tocopherol on oxidative stability in linoleic acid-deuterium oxide Changes in (a) headspace oxygen content, (b) total volatiles, and (c) the content of lipid hydroperoxides in linoleic acid containing α -tocopherol at 60°C after 7 h of storage in oil-water systems (0, 250, 500, 750, 1000, and 1500 ppm) are shown in Fig. 1. The presence of deuterium oxide (D1) significantly accelerated the consumption of headspace oxygen, the formation of lipid hydroperoxide, and the formation of volatiles in linoleic acid model

Fig. 1. Effects of deuterium oxide or deuterium free water on the changes of headspace oxygen content (A), the content of lipid hydroperoxides (B), and total volatiles (C) in linoleic acid containing α -tocopherol at 60°C after 7 h treatment in oil-water systems. D0 and D1 are linoleic acid with deuterium free H_2O or D_2O , respectively.

systems compared to the samples with deuterium free water (D0) ($p < 0.05$) when α -tocopherol was not added (Fig. 1). The prooxidant properties of deuterium oxide have been reported previously (12,16). Deuterium oxide may stabilize prooxidative components like oxygen molecules or transition metals as compared to normal water. For example, the half-life of singlet oxygen can be extended to 65 s while that in H_2O is 3–4 μ s (17). Further studies are needed to elucidate the prooxidant properties of deuterium oxide.

As the concentration of α -tocopherol increased from 0 to 1,500 ppm, the consumption of headspace oxygen decreased significantly ($p < 0.05$) in both D0 and D1 systems compared to those in control samples (Fig. 1A). Clearly α -tocopherol inhibited the consumption of headspace oxygen by linoleic acid. The difference in depleted headspace oxygen molecules when D0 and D1 were used without added α -tocopherol was 2.22%, whereas those from samples with 250, 500, and 1,000 ppm α -tocopherol were 1.04, 0.93, and 0.29%, respectively. Although the deuterium oxide accelerated the rates of linoleic acid oxidation regardless of the presence of α -tocopherol in the model systems, the antioxidant efficiency of α -tocopherol seemed to decrease under deuterium oxide based on the depletion of headspace oxygen.

Total volatiles in D1 were higher than those in D0 at each

concentration of α -tocopherol (Fig. 1B), which is in agreement with the results of headspace oxygen content (Fig. 1A). Total volatiles in D0 and D1 with 1500 ppm α -tocopherol decreased by 32.5 and 14.3%, respectively, compared to those in samples with 0 ppm α -tocopherol, which implies that deuterium oxide may hinder the antioxidant activity of α -tocopherol.

However, a somewhat different result was observed in the content of lipid hydroperoxides, which are the typical oxidation products. The contents of lipid hydroperoxide in D0 and D1 with 0 ppm α -tocopherol were 4.17 and 5.70 (1×10^8 pA) respectively, which implies that deuterium oxide accelerated the formation of lipid hydroperoxide (Fig. 1C). When α -tocopherol was added to the D0 system at concentrations from 250 to 1500 ppm, the content of lipid hydroperoxide showed an increasing trend compared to those of samples without added α -tocopherol, although significant changes were not observed ($p > 0.05$). In the case of D1 samples, the added α -tocopherol did not significantly change the content of lipid hydroperoxide compared to samples without added α -tocopherol ($p > 0.05$).

Currently, studies on the influence of deuterium oxide on the antioxidant properties of α -tocopherol are rare in the literature. The prooxidant properties of deuterium oxide were confirmed by analyzing

Fig. 2. Profiles of selected headspace volatiles including pentanal (A), hexanal (B), *t*-2-heptenal (C), 2-octenal (D), 2,4-octadienal (E) and 2,4-decadienal (F) in linoleic acid-deuterium oxide or linoleic acid-deuterium free water system containing α -tocopherol at 60°C after 7 h treatment. D0 and D1 are linoleic acid with deuterium free H₂O or D₂O, respectively.

loss of headspace oxygen content (Fig. 1A) and the formation of primary oxidation products like lipid hydroperoxides (Fig. 1C) and secondary oxidation products including total volatiles (Fig. 1B). Also, the presence of deuterium oxide seemed to impede the antioxidant activity of α -tocopherol in a linoleic acid-water model system. However, the content of lipid hydroperoxides in the presence of α -tocopherol did not decrease compared to those in samples without added α -tocopherol (Fig. 1C).

Autoxidation, which is one of basic lipid oxidation mechanisms, is known to proceed as a free radical chain reaction with initiation, propagation, and termination steps. The initial step is the formation of lipid radicals (L•) by the loss of a hydrogen atom from an unsaturated lipid (LH). Lipid radicals (L•) reacting with oxygen molecules can make peroxy radicals (LOO•), which can abstract hydrogen atoms from LH to form lipid hydroperoxide (LOOH) and another L•. Lipid hydroperoxide can again be cleaved into an alkoxy radical (LO•) and a hydroxyl radical (•OH) by β -scission, and the LO• are precursors for the formation of volatiles including alcohols, aldehydes, and ketones (2). α -Tocopherol can efficiently donate its hydrogen atom to the peroxy radical (LOO•) resulting in the

formation of more lipid hydroperoxides (18). However, β -scission of lipid hydroperoxides did not occur efficiently in the presence of α -tocopherol and relatively few volatiles were formed. Therefore, lipid hydroperoxides remained high, whereas volatiles did not increase (Fig. 1B and 1C). Currently, the major reasons for the decrease in the antioxidant properties of α -tocopherol by deuterium oxide are not fully understood yet, and therefore further research is required in this area.

Volatile analysis and presence of deuterium The contents of volatiles in the headspace including (A) pentanal, (B) hexanal, (C) *t*-2-heptenal, (D) 2-octenal, (E) 2,4-octadienal, and (F) 2,4-decadienal in linoleic acid containing α -tocopherol at 60°C after 7 h treatment in oil-water systems are shown in Fig. 2. Pentanal, hexanal, *t*-2-heptenal, 2-octenal, and 2,4-decadienal are typical volatiles formed from the thermal decomposition of methyl linoleate hydroperoxide (19,20). Generally, the peak areas of volatiles from D1 were higher than D0 without added α -tocopherol except for hexanal (Fig. 2). As the concentration of α -tocopherol increased to 1500 ppm, peak areas of all the volatiles decreased, which implies efficient antioxidant

Fig. 3. Ratios of m/z (M+1) to m/z (M) from pentanal (A), hexanal (B), *t*-2-heptenal (C), and 2-octenal (D) in linoleic acid-deuterium oxide or linoleic acid-deuterium free water system containing α -tocopherol at 60°C after 7 h treatment. D0 and D1 are linoleic acid with deuterium free H₂O or D₂O, respectively.

behavior of α -tocopherol. The content of *t*-2-heptenal was highest followed by hexanal and 2-octenal (Fig. 2). Jeong *et al.* (21) reported a correlation of headspace volatiles and fatty acids during thermal oxidation, and *t*-2-heptenal had the highest correlation based on changes in linoleic acid content.

Ratios of m/z (M+1) to m/z (M) from (A) pentanal, (B) hexanal, (C) *t*-2-heptenal, and (D) 2-octenal in linoleic acid containing α -tocopherol at 60°C after 7 h storage in oil-water systems are shown in Fig. 3. The ratios of m/z (M+1) to m/z (M) of pentanal, *t*-2-heptenal, and 2-octenal in D1 were higher than those in D0, whereas those of hexanal in D1 were not different from those in D0 when α -tocopherol was not added. Based on the above observation, the mechanism for the formation of hexanal may not be the same as those of pentanal, *t*-2-heptenal, and 2-octenal. As the concentration of α -tocopherol increased from 0 to 1500 ppm, the ratio of m/z (M+1) to m/z (M) of pentanal and hexanal decreased in both D0 and D1 systems, which implies that the presence of α -tocopherol inhibited the incorporation of deuterium in the D1 system (Fig. 3A

and 3B). For *t*-2-heptenal and 2-octenal, the ratio of m/z (M+1) to m/z (M) started to decrease as the concentration of α -tocopherol increased from 0 to 1,500 ppm (Fig. 3C and 3D), although the decreasing patterns of the ratio of m/z (M+1) to m/z (M) from *t*-2-heptenal and 2-octenal were different from those from pentanal and hexanal. Kim *et al.* (10) reported an increase in the ratios of m/z (M+1) to m/z (M) of pentane in the deuterium oxide compared to those in a deuterium-free water system. However, the ratios of m/z (M+1) to m/z (M) of hexanal did not match those of pentane, which implies that the formation mechanisms of pentane and hexanal may not be the same (11).

The effects of deuterium oxide on the antioxidant properties of hydrophilic ascorbic acid were also conducted, and the results revealed that added ascorbic acid (0 to 1,500 ppm) did not significantly change the oxidative stability between D0 and D1, while added ascorbic acid showed antioxidant properties in a concentration dependent manner (data not shown). Also, incorporation of deuterium in oxidized volatiles was not influenced by the presence of

ascorbic acid, which agrees with the results of α -tocopherol addition (data not shown).

In conclusion, antioxidant properties of α -tocopherol were evaluated in linoleic acid containing deuterium oxide or deuterium-free water. The presence of deuterium oxide seemed to reduce the antioxidant capacities of α -tocopherol. The mass to charge ratios (m/z) of headspace volatiles showed decreasing trends, which implies that the presence of α -tocopherol inhibited the incorporation of deuterium in oxidized volatiles (specifically aldehydes) in the deuterium oxide system. This study can help provide an understanding of the role of moisture in lipid oxidation and an understanding of the antioxidant properties of lipophilic compounds including α -tocopherol.

Acknowledgment This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIP) (No. 2014R1A2A1A11050047).

Disclosure The authors declare no conflict of interest.

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