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# Effects of chlorophyll photosensitisation on the oxidative stability in oil-in-water emulsions

Tae Soo Kim [a], Eric A. Decker [b], JaeHwan Lee [c,*]

[a] Department of Food Science and Technology, Seoul National University of Science and Technology, Republic of Korea
[b] Department of Food Science, University of Massachusetts, Amherst, MA 01002, United States
[c] Department of Food Science and Biotechnology, Sungkyunkwan University, Suwon, Republic of Korea

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ABSTRACT

Effects of chlorophyll photosensitisation on the oxidative stability of oil-in-water (O/W) emulsions were determined by analysing headspace oxygen content, lipid hydroperoxides, and headspace volatiles. The roles of transition metals and singlet oxygen were tested by adding ethylenediaminetetraacetic acid (EDTA) and sodium azide, respectively. Emulsions with chlorophylls and visible light irradiation had significantly high lipid hydroperoxides and headspace volatiles and low headspace oxygen content ($p < 0.05$) after 32 h while samples without light irradiation did not show any significant changes ($p > 0.05$). Sodium azide did not show clear antioxidant capacities in O/W emulsion systems rather showed prooxidant properties at some concentration. Addition of EDTA, a metal chelator, accelerated the rates of lipid oxidation in a concentration dependent manner. EDTA may enhance the stability of chlorophylls in O/W emulsions and the resulting higher chlorophyll concentrations may generate more singlet oxygen thus accelerating the rates of lipid oxidation.



## 1. Introduction

Lipid oxidation is one of chemical reactions influencing the nutritional quality and sensory attributes in lipid-rich foods. Major mechanisms and factors influencing oxidative stability are diverse depending on the food matrix such as bulk oil or emulsions (Chaiyasit, Elias, McClements, & Decker, 2007; Choe & Min, 2006; McClements & Decker, 2000). The rates of lipid oxidation in oil-in-water (O/W) emulsions, which are composed of aqueous continuous phase, dispersed lipid particles, and emulsifiers, are much faster than those of bulk oil systems (McClements & Decker, 2000; Schwarz et al., 2000). Interfaces between lipid particles and aqueous solutions in O/W emulsions are a major location for lipid oxidation reactions which are influenced by many factors including antioxidants in lipid droplets and continuous phase, and transition metals in the aqueous phase (Chaiyasit et al., 2007; McClements & Decker, 2000). Prooxidant transition metals are clearly involved in O/W emulsion under both autoxidation (Mei, Decker, & McClements, 1998) and riboflavin photosensitisation (Lee & Decker, 2011).

Photosensitised oxidation is one of the most important factors accelerating the rates of lipid oxidation in foods containing photosensitisers (Boff & Min, 2002; Foote, 1976). Chlorophylls and riboflavin are representative non-polar and polar photosensitisers found in foods, respectively and can induce oxidative reactions through type I and/or type II pathways. Photosensitisers in type I pathway can abstract electrons or hydrogen atoms from substrates to generate radicals whereas those in type II pathway can transfer its high energy to triplet oxygen to form singlet oxygen. Riboflavin is reported to prefer type I pathway to type II pathway while chlorophylls act as type II photosensitisers (Boff & Min, 2002; Foote, 1976).

Lee and Decker (2011) showed that riboflavin photosensitisation can accelerate lipid oxidation in O/W emulsions and addition of EDTA, a well known metal chelator, significantly decreased the rates of lipid oxidation. These results showed that transition metals were important prooxidants in both riboflavin photosensitised reactions as well as autoxidation. An, Lee, and Choe (2011) reported the prooxidative effects of chlorophyll photosensitisation on the oxidative stability in O/W emulsions under acidic conditions. Trace amounts of chlorophylls and transition metals can be found in refined oils and in emulsion-type foods containing vegetable, fruits, and herbs as ingredients.

The objectives of this study were to determine the effects of chlorophyll photosensitisation on the lipid oxidation in O/W emulsions and to monitor the effects of transition metals and sodium azide on the oxidative stability of the chlorophyll photosensitised O/W emulsions.

* Corresponding author. Tel.: +82 31 290 7809; fax: +82 31 290 7882.
*E-mail address:* s3hun@skku.edu (J. Lee).

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## 2. Materials and methods

### 2.1. Materials

Chlorophyll *a*, ethylenediaminetetraacetic acid (EDTA), sodium azide, hexanal, 2-heptenal, 1-octen-3-ol, Tween 20, ferrous sulphate, barium chloride, and ammonium thiocyanate were purchased from Sigma–Aldrich (St. Louis, MO, USA). The 50/30 μm DVB/Carboxen/PDMS solid phase microextraction (SPME) fibre, aluminium seals, and Teflon-coated rubber septa were purchased from Supelco, Inc. (Bellefonte, PA, USA). Corn oil was purchase from a local grocery market (Seoul, Korea). Hydrochloric acid and other reagent grade chemicals were obtained from Daejung Chemical Co. (Seoul, Korea).

### 2.2. Sample preparation for the emulsion and photosensitised oxidation

The O/W emulsions were prepared according to the method of Lee and Decker (2011) with a slight modification. Chlorophyll *a* was dissolved in chloroform and mixed with corn oil and then solvent was removed under nitrogen gas flow. Tween 20 was added to deionised water at the concentration of 0.25% (w/w) and then combined the mixture with 2.5% (w/w) corn oil containing chlorophyll *a*. A coarse emulsion was made by homogenising the mixture for 3 min using a DE/T 25 homogenizer (Ika® werke, Staufen, Germany) followed by ultrasonication for 5 min using a cell sonicator (Sonics & Materials, CT, USA) to further reduce lipid droplet size. The final concentration of chlorophyll *a* in O/W emulsion was 11.2 μM. Powder of sodium azide and EDTA were directly added to O/W emulsions containing chlorophyll *a* at 10, 100, and 1000 μM, respectively and mixed for overnight in the dark. One millilitre of each sample was put in a 10-ml vial with an air-tight seal. Sample vials were stored under fluorescent light at 1333 Lux light intensity for 32 h and analysed at 0, 4, 8, 16, and 32 h. Samples were prepared in triplicate at each sampling time. Samples maintained stable O/W emulsion states during 32 h treatment.

### 2.3. Headspace oxygen analysis

The headspace oxygen in air-tight sample bottles was analysed according to the method of Lee and Min (2009). Twenty microlitre of headspace gas was removed from a sample vial with an air-tight syringe and oxygen content was determined using a gas chromatograph (GC) equipped with a thermal conductivity detector (TCD). A Hewlett–Packard 7890 GC (Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with a 60/80 packed column (3.0 m × 2 mm ID, Restek Ltd., Bellefonte, PA, USA) and a TCD from Agilent Technologies (Palo Alto, CA, USA) was used. The flow rate of helium gas was 20 ml/min. Temperatures of oven, injector, and a thermal conductivity detector were 60, 180, and 180 °C, respectively.

### 2.4. Lipid hydroperoxides

Lipid hydroperoxides were determined using a modified method of previous reports (Lee & Decker, 2011;Shanta & Decker, 1994). The sample (0.2 ml) was mixed with 1.5 ml of isooctane/2-propanol (3:2, v/v), vortex-mixed three times for 10 s each, and centrifuged for 3 min at 2000*g*. The upper layer (0.1 ml) was collected and mixed with 1.4 ml of methanol/1-butanol (2:1, v/v) and 30 μl of thiocyanate/$Fe^{2+}$ solution by vortex-mixing for 10 s. The thiocyanate/$Fe^{2+}$solution was made by mixing equal volumes of 3.94 M thiocyanate solution with 0.072 M $Fe^{2+}$ solution (obtained from the supernatant of the mixture of one part of 0.144 M $FeSO_4$ and one part of 0.132 M $BaCl_2$ in 0.4 M HCl). The samples were incubated for 30 min at room temperature and absorbance at 510 nm was measured using an UV/VIS-spectrometer (Model UV-1650PC, Shimadzu, Kyoto, Japan). The concentration of lipid hydroperoxide was calculated using a cumene hydroperoxide standard curve.

### 2.5. Headspace volatile analysis

Headspace volatiles in O/W emulsion samples were determined using a Hewlett–Packard 7890 GC with a flame ionisation detector (FID). Solid phase for extracting and concentrating headspace volatiles was a 50/30 μm DVB/Carboxen/PDMS SPME fibre. Sample vials were incubated for 5 min to equilibrate the headspace volatiles and extracted for 10 min at 35 °C. Volatiles on the fibre were removed in the GC injection port for 2 min at 250 °C. Volatiles were separated using gradient temperatures on an HP-5 column (30 m × 0.32-mm i.d., 0.25-μm film thickness). Oven temperature started at 40 °C for 2 min, increased at the rate of 10 °C/min to 160 °C, and stayed for 1 min. The detector temperature was 300 °C. The flow rate of helium carrier gas was 1.0 ml/min and GC was operated in splitless mode. Concentrations of volatiles were determined from the peak areas using standard curves made from authentic hexanal, 2-heptenal, and 1-octen-3-ol in the same O/W emulsion matrix.

### 2.6. Fluorescence analysis

The concentration of chlorophylls was indirectly determined by measuring fluorescence intensity in O/W emulsion samples. The sample of 0.2 ml was mixed with 1.5 ml of isooctane/2-propanol (3:2, v/v) and centrifuged for 3 min at 2000*g*. The upper layer (1.0 ml) was collected and mixed with 4.0 ml of isooctane/2-propanol (3:2, v/v). The chlorophyll content was measured at the excitation wavelength of 410 nm and the emission wavelength of 670 nm using a fluorescence spectrometer (Model LS-55, Perkin Elmer, Liantrisant, UK). Concentrations of chlorophylls were calculated using a calibration curve made from chlorophyll *a*.

### 2.7. Statistical analysis

Data of headspace volatiles, headspace oxygen content, lipid hydroperoxides and fluorescent intensity were analysed statistically by ANOVA and Duncan's multiple range test using SPSS software program (SPSS Inc., Chicago, IL). A $p$ value <0.05 was considered significant.

## 3. Results and discussion

### 3.1. Oxidative stability of chlorophyll photosensitised O/W emulsions

The effects of chlorophylls and visible light irradiation on the oxidative stability in O/W emulsion are shown in Fig. 1. The samples with addition of chlorophylls and light irradiation showed significant changes in headspace oxygen content (a), lipid hydroperoxides (b) and total volatiles (c) compared to samples without visible light irradiation ($p < 0.05$). Preliminary studies showed that O/W emulsions without chlorophylls stored under light or in the dark did not show any significant changes in headspace oxygen content, lipid hydroperoxides, and total volatile over the same time period (data not shown). Lee and Decker (2011) confirmed that oxidative stability of O/W emulsions without addition of photosensitisers were not significant for 22 h under light or in the dark conditions.

Chlorophylls and its derivatives may provide antioxidant activities in methyl linoleate and linoleic acid/water emulsions in the dark (Endo, Usuki, & Kaneda, 1985). However, chlorophyll