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# Prooxidative and antioxidative properties of β-carotene in chlorophyll and riboflavin photosensitized oil-in-water emulsions

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ABSTRACT

Effects of β-carotene on the oxidative stability of chlorophyll or riboflavin photosensitized oil-in-water (O/W) emulsions were determined by analysing the depleted headspace oxygen content, lipid hydroperoxides, fluorescence intensity, and headspace volatiles. As the concentration of β-carotene increased from 0 to 10, 100, and 1000 μM, the oxidative stability of samples containing chlorophylls decreased in a concentration-dependent manner under light, indicating that β-carotene acted as a prooxidant. However, in riboflavin photosensitized O/W emulsions, 100 and 1000 μM β-carotene inhibited lipid oxidation. Although β-carotene protected both photosensitisers in O/W emulsions, β-carotene displayed antioxidative or prooxidative properties, which depended on the polarity of the photosensitisers. The development of *t*-2-heptenal and 1-octen-3-ol increased greatly in chlorophyll sensitised O/W emulsions with an increase in β-carotene concentration, implying that more singlet oxygen oxidation occurred due to the higher levels of remaining chlorophylls. However, the content of *t*-2-heptenal and 1-octen-3-ol in the riboflavin sensitised O/W emulsions was not significantly correlated with the β-carotene concentration, which indicates that β-carotene in the lipid particles effectively quenched singlet oxygen.



## 1. Introduction

β-Carotene is a precursor of vitamin A and has long been recognised as an efficient singlet oxygen quencher (Burton & Ingold, 1984; Valgimigli, Lucarini, Pedulini, & Ingold, 1997; Woodall, Lee, Weesie, Jackson, & Britton, 1997). Carotenoids can quench singlet oxygen at the rates ranging from $5.72 \times 10^9$ to $9.79 \times 10^9$ M/s in chlorophyll photosensitized soybean oil (Lee & Min, 1990) and one mole of β-carotene can quench 250–1000 molecules of singlet oxygen at a rate of $1.3 \times 10^{10}$ M/s (Foote, 1976). However, β-carotene has been reported to display prooxidative properties under certain environments, such as high oxygen tension and high concentration of β-carotene in foods and model systems. Generally, β-carotene acts as a prooxidant in thermally oxidised bulk oil systems or cells, while it serves as an antioxidant in photosensitized systems due to its singlet oxygen quenching ability (Ha, Park, Kim, & Lee, 2012; Lee, Ozcelik, & Min, 2003; Palozza, Serini, Nicuolo, Piccioni, & Calviello, 2003; Zhang & Omaye, 2001).

Light irradiation plays important roles on the oxidative stability in foods containing photosensitisers and influences the nutritional quality and flavor attributes in foods (Min & Boff, 2002). Chlorophylls and riboflavin, which are non-polar and polar photosensitisers, respectively, can harvest light energy and induce chemical reactions through type I and/or type II pathways. In the type I pathway, photosensitisers themselves can abstract electrons or hydrogen atoms from substrates to generate radicals and superoxide anion. Photosensitisers in the type II pathway can transfer its high energy to triplet oxygen to form singlet oxygen (Min & Boff, 2002; Foote, 1976).

Oil-in-water (O/W) emulsions are composed of an aqueous phase and the dispersed lipid particles surrounded by the emulsifier. The interface of the lipid and aqueous phases are the areas where lipid oxidation can initiate in O/W emulsions (McClements & Decker, 2000; Chaiyasit, Elias, McClements, & Decker, 2007). The presence of metal chelating agents and lipophilic free radical scavengers, interfacial and droplet characteristics, and ingredient interactions are critical factors influencing the rates of lipid oxidation in O/W emulsions (McClements & Decker, 2000; Schwarz et al., 2000; Chaiyasit, McClements, & Decker, 2005).

Riboflavin and chlorophyll photosensitisation greatly reduces the oxidative stability of O/W emulsions (Kim, Decker, & Lee, 2012; Lee & Decker, 2011). However, their oxidative mechanisms are not the same. For example, addition of EDTA, a well known metal chelator, significantly decreased lipid oxidation in riboflavin photosensitisation, while accelerating lipid oxidation in chlorophyll systems, which implies polar and non-polar photosensitisers cause different effects on the oxidative stability in O/W emulsions under visible light irradiation (Kim et al., 2012; Lee & Decker, 2011).

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Although the antioxidative or prooxidative effects of β-carotene have been studied in thermally treated or photosensitized bulk oil systems, there have been no studies that examine the effect of β-carotene on the rates of lipid oxidation in chlorophyll or riboflavin photosensitized O/W emulsions.

The objective of this study was to determine the effects of β-carotene on the oxidative stability in non-polar chlorophyll and polar riboflavin photosensitized O/W emulsions. The oxidative stability of emulsions was analysed based on the headspace oxygen content, lipid hydroperoxides, and profiles of headspace volatiles.

## 2. Materials and methods

### 2.1. Materials

β-Carotene, chlorophyll *a*, riboflavin, tween 20, ferrous sulfate, barium chloride, ammonium thiocyanate, hexanal, *t*-2-heptenal, and 1-octen-3-ol were purchased from Sigma–Aldrich. (St. Louis, MO, USA). The solid phase microextraction (SPME) fibre of 50/30 μm DVB/Carboxen/PDMS StableFlex was purchased from Supelco, Inc. (Bellefonte, PA, USA). Corn oil was purchase from a local grocery market (Seoul, Korea). Other reagent grade chemicals were obtained from Daejung Chemical Co. (Seoul, Korea).

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

Oil-in-water emulsions were prepared according to the method described by Kim et al. (2012). Chlorophyll *a* and β-carotene were dissolved in chloroform first and then added to corn oil. The solvent was removed under nitrogen gas flow. Tween 20 was added to deionised water at a concentration of 0.25% (w/w) and then corn oil was combined with deionised water containing tween 20 at a concentration of 2.5% (w/w). A coarse emulsion was made by homogenising the mixture for 3 min using a DE/T 25 homogenizer (Ika® werke, Staufen, Germany) and ultrasonication for 5 min with a vibra cell sonicator (Sonics & Materials, CT, USA) was used to make O/W emulsions. Riboflavin was added to the O/W emulsions at a final concentration of 11 μM and mixed using a magnetic stirrer. The concentrations of chlorophyll *a* in O/W emulsions were 11 μM and those of β-carotene were 10, 100, and 1000 μM, respectively. One millilitre of each sample was placed in a 10 mL vial and sealed under an air-tight condition. Sample vials were stored under visible light irradiation at 1333 Lux light intensity for 32 h and sampled at 0, 4, 8, 16, and 32 h. Samples wrapped with aluminium foil were prepared as dark controls. Samples were prepared in triplicate at each sampling.

### 2.3. Headspace oxygen analysis

The degree of oxidation was determined by the depletion of headspace oxygen in air-tight samples. The headspace oxygen in the air-tight sample bottles was analysed according to methods described by Kim et al. (2012). A headspace gas volume of 20 μL was removed from a sample bottle using an air-tight syringe and the oxygen content were determined using a gas chromatograph (GC)-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., USA) and a TCD from Agilent Technologies (Palo Alto, CA, USA) was used. The flow rate of helium gas was 20 ml/min. The temperatures of the oven, injector, and a thermal conductivity detector were 60, 180, and 180 °C, respectively.

### 2.4. Lipid hydroperoxides

Lipid hydroperoxides were determined using the method described by Kim et al. (2012). Briefly, 0.2 mL of the sample 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*. 0.1 mL of the upper layer was collected and mixed with 1.4 mL of methanol/1-butanol (2:1, v:v). 30 μL of a thiocyanate/$Fe^{2+}$ solution was added to the mixture and vortex-mixed for 10 s. The thiocyanate/$Fe^{2+}$solution was made by mixing an equal volume of 3.94 M thiocyanate solution with 0.072 M $Fe^{2+}$ solution (obtained from the supernatant of a 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 the 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 gas chromatograph with a flame ionisation detector (FID) according to the method described by Kim et al. (2012). The solid phase used for extracting and concentrating the headspace was 50/30 μm DVB/Carboxen/PDMS. Sample vials were incubated for 5 min to equilibrate the headspace volatiles and extracted for 10 min at 35 °C and volatiles in the fibre were removed at the GC injection port for 2 min. Volatiles were separated over a gradient temperature on an HP-5 column (30 m × 0.32 mm i.d., 0.25 μm film thickness). The oven temperature was initially set at 40 °C for 2 min, increased at a rate of 10 °C/min to 160 °C, and then maintained at this temperature for 1 min. The temperature of the injector and detector was 250 and 300 °C, respectively. The flow rate of the helium carrier gas was 1.0 mL/min and the GC was operated in the splitless mode. Concentrations were determined from the peak areas using standard curves made from authentic hexanal, *t*-2-heptenal, and 1-octen-3-ol in the same O/W emulsion matrix.

### 2.6. Fluorescence analysis

The chlorophylls and riboflavin content in O/W emulsions was measured based on fluorescence intensity. The 0.2 mL of sample was mixed with 1.5 mL of isooctane/2-propanol (3:2, v:v) and the mixture was centrifuged for 3 min at 2000×*g*. 1.0 mL of the upper layer was collected and mixed with 4.0 mL of isooctane/2-propanol (3:2, v:v). Chlorophylls and riboflavin in the upper layer and lower layer were recovered and the fluorescence intensity was measured using a fluorescence spectrometer (Model LS-55, Perkin Elmer, Liantrisant, UK). The excitation wavelengths for the chlorophylls and riboflavin were 410 and 270 nm, respectively, whereas the emission wavelengths for detecting chlorophylls and riboflavin were 670 and 515 nm, respectively. The chlorophylls and riboflavin concentrations were calculated using a calibration curve made from chlorophyll *a* and riboflavin, respectively.

### 2.7. Statistical analysis

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