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ORIGINAL PAPER

Effects of Curcumin on the Oxidative Stability of Oils Depending on Type of Matrix, Photosensitizers, and Temperature

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Abstract The photosensitizing activity of curcumin was tested in corn oil and oil-in-water (O/W) emulsion systems under visible light irradiation. In addition, the antioxidative/prooxidative properties of curcumin were evaluated in corn oil at 100 °C and in O/W emulsion at room temperature under riboflavin photosensitization or at 60 °C in the dark. Curcumin acted as a photosensitizer in corn oil and O/W emulsions . The oxidative stability of corn oil samples containing curcumin (0–5.0 mmol/kg oil) were not significantly different ($p > 0.05$) at 100 °C, implying curcumin did not act as an antioxidant nor a prooxidant in corn oil. However, curcumin inhibited lipid oxidation in O/W emulsions under riboflavin photosensitization at room temperature and 60 °C in the dark. The photosensitization and antioxidant abilities of curcumin were greatly influenced by matrix types and presence of riboflavin. Therefore, antioxidative or prooxidative characteristics of curcumin should be evaluated considering matrix type including bulk oil or O/W emulsions and presence of visible light irradiation.

Keywords Curcumin · Photosensitizer · Antioxidant · Matrix · Corn oil · Oil-in-water emulsion

Introduction

Curcumin (diferuoyl methane) is a naturally occurring yellow pigment found in turmeric (*Curcuma longa*), a major ingredient for curry [1]. Curcumin has been known to possess health benefits to fight cancer, diabetes, cardiovascular diseases, arthritis, and Alzheimer's disease [1]. Curcumin is present in tautomeric forms such as a 1,3-diketo, two equivalent enols, but predominantly as a keto-enol tautomer in diverse solvents [2]. Curcumin has antioxidant properties in acidic solution through hydrogen transfer mechanisms. However, the electron donating ability of curcumin increases in higher pH [3]. Curcumin possesses diverse antioxidant properties including radical scavenging ability, hydrogen peroxide scavenging, power to reduce ferric ion (Fe^{3+}), and ferrous ion (Fe^{2+}) chelating activity [4]. Also, it is known as an electron-transfer photosensitizer in the range of 340–535 nm light irradiation [5]. However, Banerjee *et al*. [6] reported that at low concentration, curcumin showed antioxidant properties whereas at higher concentration, prooxidant activity of curcumin was observed in 2,2′-azobis(2-amidinopropane) hydrochloride (AAPH) induced hemolysis in human erythrocytes models.

The matrix of foods such as bulk oil and oil-in-water (O/W) emulsions are important factors to determine antioxidant or prooxidant properties of compounds [7–9]. Lipophilic compounds like α-tocopherol and ascorbyl palmitate retarded the rates of lipid oxidation in O/W emulsions under riboflavin photosensitization whereas hydrophilic compounds like trolox and ascorbic acid decreased the oxidative stability in the same system [10]. In the case of bulk oil systems, hydrophilic compounds showed higher antioxidant properties than lipophilic compounds under thermal oxidation [11]. Therefore, it is necessary to determine the true antioxidative properties of compounds considering the effects of the matrix. Also, antioxidant mechanisms including hydrogen donating or singlet oxygen quenching are important factors to determine the oxidative stability of systems. Some compounds with singlet oxygen quenching

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ability may accelerate the rates of lipid oxidation during thermal oxidation. For example, β-carotene is a good singlet oxygen quencher, but accelerates the rates of lipid oxidation in thermally oxidation systems [11, 12].

A photosensitizer is a chemical compound, which can harvest light energy and transfer the energy to other chemical compounds [13]. If a chemical compound possess photosensitizing ability, the chemical compound can accelerate chemical reactions including lipid oxidation through generation of singlet oxygen and superoxide anion or abstraction of electron or hydrogen from other components [13]. Riboflavin or chlorophylls are representative photosensitizers, which can greatly reduce the oxidative stability in bulk oil and O/W emulsions [10, 14].

Although antioxidant capacities and the photosensitizing ability of curcumin have been seen, the effects of the food matrix, including bulk oil or O/W emulsions, on the antioxidant or photosensitizing abilities of curcumin have not been reported.

The objectives of this study were to determine the photosensitizing ability of curcumin and to evaluate the antioxidant or prooxidant properties of curcumin under visible light irradiation or thermal oxidation in both corn oil and O/W emulsions. Corn oil was used as a representative bulk oil system.

Materials and Methods

Materials

Curcumin, riboflavin, Tween 20, ferrous sulfate, barium chloride, and ammonium thiocyanate were purchased from Sigma-Aldrich. (St. Louis, MO). Corn oil was purchased from a local grocery market (Suwon, Gyeonggi, Korea). Glass vials, seals, and septa were purchased from Supelco, Inc. (Bellefonte, PA). Dichloromethane and other reagent grade chemicals were purchased from Daejung Chemical Co. (Seoul, Korea).

Sample Preparation

Corn oil was mixed with 0, 0.2, 1.0, and 5.0 mmol curcumin/kg oil, which had been dissolved in dichloromethane, and the oil was flushed with nitrogen gas at room temperature to remove the solvent. One gram of corn oil containing from 0 to 5.0 mmol curcumin/kg oil was put in 10-mL sample bottles and the bottles were sealed air-tight with Teflon-coated rubber septa and aluminum caps. For the photosensitization study, samples were placed in a custom-made light box with a white fluorescent light source. The light intensity was 1333 Lux. Samples were prepared in triplicate and analyzed at 0, 2, 3, and 4 days. The temperature of the light box was maintained at 20 ± 5 °C. For the thermal oxidation studies, corn oil containing 0, 0.2, 1.0, and 5.0 mmol curcumin/kg oil were placed in air-tight conditions in a convection oven (HYSC, Seoul, Korea) at 100 °C. Samples were prepared in triplicate and analyzed at 0, 10, 14, and 18 h.

The photosensitizing effects of curcumin were evaluated in O/W emulsions. The O/W emulsions were prepared according to previous reports [10]. Briefly, deionized water was mixed with Tween 20 and then combined with corn oil. The O/W emulsion had 2.5 % (w/w) oil concentration and 0.25 % (w/w) emulsifier concentration. A coarse emulsion was made by homogenizing the mixture for 3 min using an HB501 instrument (Tepal, Rumilly, Haute-Savoie, France) and the coarse emulsion was passed three times through a two valve high pressure homogenizer (Taewon Chemical, Suwon, Korea) at 4000 psi. Curcumin was added to the O/W emulsion at a final concentration of 0.2, 1.0, and 5.0 mM and mixed overnight in the dark. Two milliliters 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 and analyzed at 0, 24, 36, and 48 h.

The antioxidant properties of curcumin in O/W emulsions were tested two ways: under riboflavin photosensitization and thermal oxidation. Once O/W emulsion was prepared, 0.13 μM riboflavin was added and mixed overnight. Riboflavin is a well known photosensitizer and accelerates the rates of lipid oxidation in O/W emulsion [14]. Samples containing both curcumin and riboflavin were stored in the light box and analyzed at 0, 24, 36, and 48 h.

The O/W samples containing 0.2, 1.0, and 5.0 mM were stored at 60 °C in the oven (HYSC) and analyzed at 0, 1, 3, and 5 days. All the samples in corn oil and O/W emulsions were prepared in triplicate at each sampling time.

Headspace Oxygen Content Analysis

The depleted headspace oxygen was determined by injecting 30 μL headspace gas from the sample bottles into a gas chromatograph (7890A, Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with a thermal conductivity detector. Stationary phase was a stainless steel column (1.8 m × 0.32 cm) packed with 60/80 Molecular Sieve 13× (Alltech Assoc., Inc. Deerfield, IL). The flow rate of helium gas was 20 mL/min. The temperatures of the oven, injector, and thermal conductivity detector were 60, 180, and 180 °C, respectively [11].

CDA Analysis

CDA determines the diene conjugation of unsaturated linkages present, which are typical primary oxidation products

from polyunsaturated fatty acids. The CDA of the samples was determined according to AOCS method Ti la-64 [15].

Lipid Hydroperoxide Analysis

Lipid hydroperoxides were determined using a modified method of Kim *et al*. [10]. Samples (0.2 mL) were mixed with 1.5 mL of isooctane/2-propanol (3:1, v/v), vortex mixed three times for 10 s each time, and centrifuged for 2 min at 2000 g. The upper layer (0.2 mL) was collected and mixed with 2.8 mL of methanol/1-butanol (2:1, v/v). 30 μL of thiocyanate/Fe^{2+} solution was added to the mixture and vortex mixed 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 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 20 min at room temperature and the absorbance (at 510 nm) was measured using a UV/VIS-spectrometer (Jenesis 10UV, Thermo, Waltham, MA). The concentration of lipid hydroperoxide was calculated using a cumene hydroperoxide standard curve.

Statistical Analysis

All data of headspace oxygen content, CDA, and lipid hydroperoxides were analyzed statistically by ANOVA and an LSD (least significant difference) test using SPSS software program (SPSS Inc., Chicago, IL). A p value < 0.05 was considered significant.

Results and Discussion

Photosensitization of Curcumin in Corn Oil

The effects of curcumin concentration on headspace oxygen content (a) and CDA (b) in corn oil at room temperature under visible light irradiation are shown in Fig. 1. As the concentration of curcumin increased from 0 to 0.2, 1.0, and 5.0 mmol/kg oil, more headspace oxygen was depleted for 4 day visible light irradiation, indicating curcumin accelerated the consumption of oxygen significantly by oils ($p < 0.05$). The headspace oxygen content in corn oils containing 0, 0.2, 1.0, and 5.0 mmol curcumin/kg oil after 4 day were 18.78, 17.85, 15.31, and 10.58 %, respectively (Fig. 1a). However, samples in the dark did not show any significant difference in headspace oxygen content ($p > 0.05$) after 4 days of storage ranging from 0 to 5.0 mmol curcumin/kg oil (data not shown). This trend can be observed in the CDA results (Fig. 1b). Corn oil samples containing curcumin had significantly higher CDA values than those without added curcumin ($p < 0.05$). Samples with higher concentrations of curcumin had higher CDA values than those with lower curcumin concentration

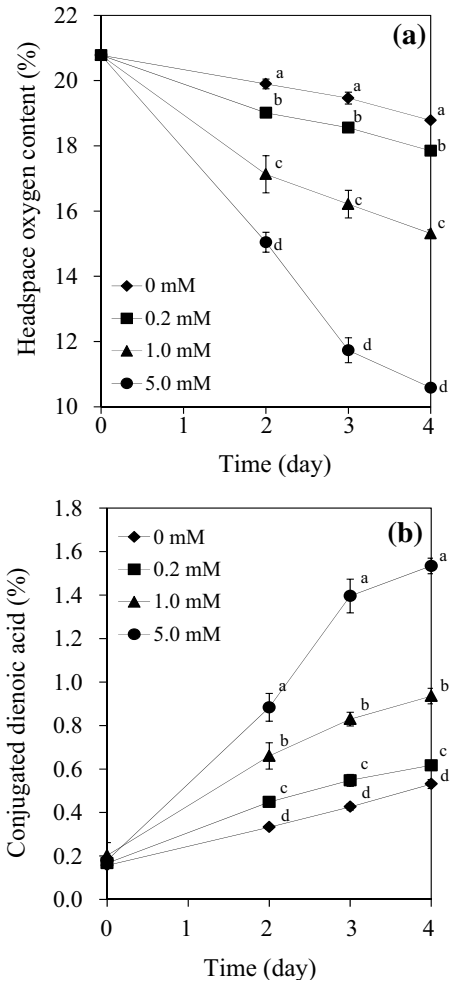

Fig. 1 Effects of curcumin concentration on headspace oxygen content (**a**) and CDA (**b**) in corn oil at room temperature under visible light irradiation. *Different small letters* are indicate significant differences with the same treatment time ($p < 0.05$)

(Fig. 1b), which agrees with the results of headspace oxygen analysis. In the dark condition, CDA values in samples were not significantly different after 4 days of irradiation, irrespective of the concentration of curcumin ($p > 0.05$) (data not shown). It is clearly observed that curcumin may act as a photosensitizer in corn oils under visible light irradiation based on headspace oxygen analysis and CDA values (Fig. 1).

Antioxidant or Prooxidant Properties of Curcumin in Corn Oil

Changes of headspace oxygen content and CDA in corn oil heated at 100 °C are shown in Table 1. As heat treatment time increased from 0 to 18 h, headspace oxygen content in corn oils without addition of curcumin decreased from 20.77 to 14.41 %. Addition of curcumin from 0 to 5.0 mmol/kg oil did not make a significant difference in headspace oxygen content among

Table 1 Changes of headspace oxygen content and CDA in corn oil containing different concentration of curcumin heated at 100 °C

Time (h)	Headspace oxygen content (%)				Conjugated dienoic acid value (%)			
	0 mmol/kg oil	0.2 mmol/kg oil	1.0 mmol/kg oil	5.0 mmol/kg oil	0 mmol/kg oil	0.2 mmol/kg oil	1.0 mmol/kg oil	5.0 mmol/kg oil
0	20.78 ± 0.06a	20.78 ± 0.06a	20.78 ± 0.06a	20.78 ± 0.06a	0.16 ± 0.01a	0.17 ± 0.01a	0.20 ± 0.06a	0.18 ± 0.00a
10	19.19 ± 0.03a	18.98 ± 0.47a	18.92 ± 0.53a	19.01 ± 0.03a	0.54 ± 0.04a	0.55 ± 0.08a	0.60 ± 0.12a	0.60 ± 0.04a
14	17.99 ± 0.80ab	17.92 ± 0.35ab	18.42 ± 0.24a	17.39 ± 0.28b	0.78 ± 0.13b	0.89 ± 0.05ab	0.82 ± 0.06ab	0.95 ± 0.03a
18	14.41 ± 2.00a	13.98 ± 1.20a	14.69 ± 0.25a	14.28 ± 0.14a	1.34 ± 0.36a	1.36 ± 0.18a	1.26 ± 0.04a	1.32 ± 0.02a

Mean ± standard deviation ($n = 3$)

Different letters indicate significant differences in the row at $p < 0.05$

Table 2 Effects of curcumin on the changes of headspace oxygen content (%) in O/W emulsions under visible light and in the dark

Time (h)	Headspace oxygen content (%) under visible light				Headspace oxygen content (%) in the dark			
	0 mM	0.2 mM	1.0 mM	5.0 mM	0 mM	0.2 mM	1.0 mM	5.0 mM
24	20.89 ± 0.02a	20.76 ± 0.06b	20.80 ± 0.03ab	20.76 ± 0.06b	20.94 ± 0.06a	20.90 ± 0.08a	20.93 ± 0.08a	20.93 ± 0.06a
36	20.68 ± 0.05a	20.54 ± 0.05b	20.43 ± 0.01c	20.16 ± 0.03d	20.82 ± 0.02b	20.93 ± 0.05a	20.96 ± 0.03a	20.96 ± 0.01a
48	20.25 ± 0.12a	20.01 ± 0.06ab	20.29 ± 0.15a	20.04 ± 0.06d	20.85 ± 0.05b	20.91 ± 0.02a	20.92 ± 0.02a	20.93 ± 0.00a

Mean ± standard deviation ($n = 3$)

Different letters indicate significant differences in the row at $p < 0.05$

samples after 18 h ($p > 0.05$), which implies that curcumin did not act as an antioxidant nor prooxidant in corn oils at 100 °C (Table 1). Although CDA values in corn oils with 0, 0.2, 1.0, and 5.0 mmol curcumin/kg oil increased as heating time increased from 0 to 18 h, there were no significantly differences in CDA values among samples added curcumin after 18 h ($p > 0.05$), which agrees with the results of headspace oxygen results (Table 1). Curcumin does not act as an antioxidant nor a prooxidant in corn oils under 100 °C heat treatment for 18 h treatment.

Photosensitization of Curcumin in O/W Emulsion

Effects of curcumin on the changes of headspace oxygen content in O/W emulsions under visible light and in the dark at room temperature are shown in Table 2. As light irradiation time increased from 0 to 48 h, headspace oxygen content in O/W emulsions with addition of curcumin was significantly lower than those in samples without addition of curcumin ($p < 0.05$), which implies that curcumin acted as a photosensitizer (Table 2). However, in the dark condition, addition of curcumin inhibited the consumption of headspace oxygen significantly ($p < 0.05$), implying antioxidant properties of curcumin.

Effects of curcumin on the changes of lipid hydroperoxides in O/W emulsions under visible light (a) and in the dark (b) are shown in Fig. 2. As visible light irradiation time increased to 36 h, samples containing 5.0 mM had the highest lipid hydroperoxide values followed by 1.0, 0.2, and 0 mM, indicating curcumin accelerated the formation of lipid hydroperoxides in a concentration dependent manner (Fig. 2a). However, results of lipid hydroperoxides in O/W emulsions stored in the dark did not agree with those under light conditions. Lipid hydroperoxides in samples containing 5.0 mM were the lowest followed by 1.0, 0.2, and 0 mM, implying that curcumin inhibited the formation of lipid hydroperoxides under dark conditions (Fig. 2b), which is in agreement with the headspace oxygen content (Table 2). Curcumin acted as a photosensitizer in corn oil and in the O/W emulsion while curcumin acted as an antioxidant in the O/W emulsion in the dark. Photosensitizing abilities of curcumin in corn oil were higher than those in O/W emulsions. For example, headspace oxygen content in corn oil or O/W containing 5.0 mM after 48 h light irradiation was 15.04 and 20.04 %, respectively. Lower amounts of oil content or a high percentage of moisture in O/W emulsion may influence the photosensitizing ability of curcumin.

Photosensitizing ability of curcumin has been reported in diverse systems and the phototoxicity of curcumin has been used to control the growth of microorganisms including *Escherichia coli* and cell systems. The phototoxicity of curcumin could be due to the production of singlet oxygen and superoxide anion radicals through photosensitization [16]. Therefore, the prooxidant properties of curcumin may be due to the generation of reactive oxygen species including singlet oxygen and superoxide anion. Less amount of

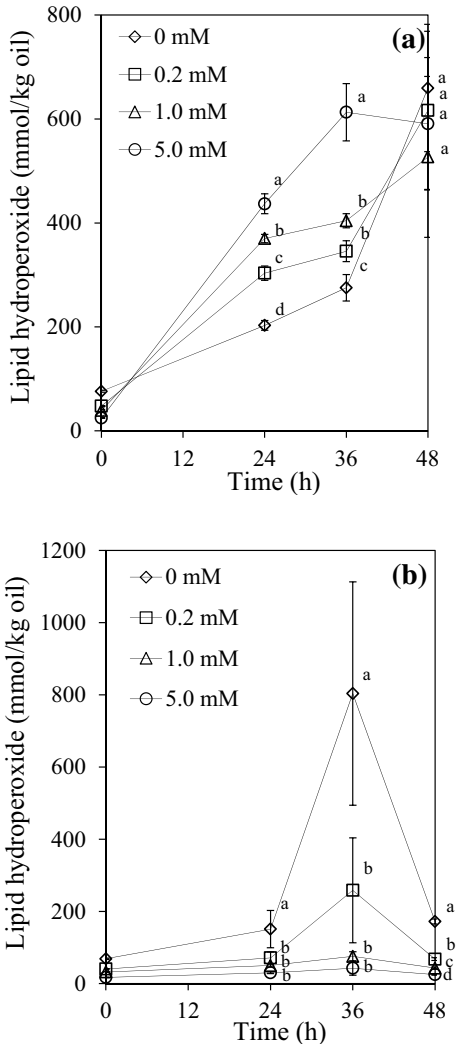

Fig. 2 Effects of curcumin on the changes of lipid hydroperoxides in O/W emulsions under visible light (**a**) and in the dark (**b**). *Different small letters* indicate significant differences at the same treatment time ($p < 0.05$)

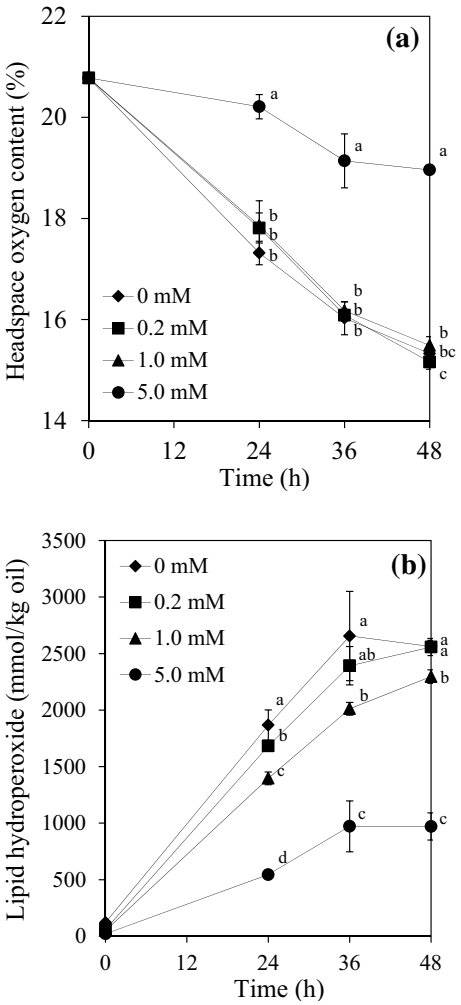

Fig. 3 Changes of headspace oxygen content (**a**) and lipid hydroperoxides (**b**) in O/W emulsions containing curcumin under riboflavin photosensitization. *Different small letters* indicate significant differences at the same treatment time ($p < 0.05$)

oils in O/W emulsions and poor solubility of curcumin in aqueous solution may limit the photosensitizing ability of curcumin. Generation of singlet oxygen by curcumin was observed in relatively non-polar solvent systems including benzene, toluene, and acetonitrile, but was not detected in more polar solvent systems including ethanol, isopropanol, sodium dodecyl sulfate, and TX-100 micelles in deuterium oxide [16, 17]. Lipophilic environments may be required to show the high photosensitizing ability of curcumin properly.

Antioxidant Properties of Curcumin in O/W Emulsion

Further research was conducted in O/W emulsions under riboflavin sensitization or 60 °C heat treatment. Changes of headspace oxygen content (a) and lipid hydroperoxides (b) in O/W emulsions containing curcumin under riboflavin photosensitization are shown in Fig. 3. Riboflavin is a well known hydrophilic photosensitizer and can accelerate the rate of lipid oxidation in bulk oils [18] and O/W emulsions [14]. As irradiation time increased to 48 h, headspace oxygen content in samples without curcumin decreased significantly compared to samples before oxidation ($p < 0.05$) (Fig. 3a). Samples containing 0, 0.2, and 1.0 mM did not have significantly different headspace oxygen content ($p > 0.05$) whereas samples containing 5.0 mM had significantly higher headspace oxygen content ($p < 0.05$), implying the high concentration of curcumin acted as an antioxidant under riboflavin photosensitization. This trend was observed more clearly in the results of lipid

hydroperoxides (Fig. 3b). As oxidation time increased to 48 h, samples containing 5.0 and 1.0 mM significantly inhibited the formation of lipid hydroperoxides compared to control samples (Fig. 3b).

Changes of headspace oxygen content (a) and lipid hydroperoxides (b) in O/W emulsions containing curcumin in the dark at 60 °C for 5 days are shown in Fig. 4.

Headspace oxygen content in samples containing 0, 0.2, 1.0, and 5.0 mM after 5 days of storage at 60 °C were 6.71, 10.15, 20.47, and 20.66 %, respectively. The presence of curcumin inhibited the consumption of headspace oxygen significantly depending on the concentration (Fig. 4a). In the case of lipid hydroperoxides, a similar trend was observed. Lipid hydroperoxides in samples with 1.0 and 5.0 mM were significantly lower than those with 0 and 0.2 mM ($p < 0.05$) (Fig. 4b).

The antioxidant properties of curcumin were observed in O/W emulsions in the dark (Fig. 2b) and under riboflavin photosensitization (Fig. 3). Riboflavin is a well known photosensitizer, which can accelerate the rate of lipid oxidation through type I and type II pathways [13, 19]. The excited triplet state of riboflavin can react with triplet oxygen to form singlet oxygen (type II pathway) or can extract an electron or hydrogen atom from substrates to generate free radicals (type I pathway) [13, 19]. Curcumin has known to possess dual roles on singlet oxygen generation of singlet oxygen by the activity as a photosensitizer and scavenging properties of singlet oxygen as an antioxidant. Curcumin can generate singlet oxygen with a 0.12 quantum yield [20] whereas curcumin can react with singlet oxygen at the rates ranging from 1.3 to 7 $\times$ 10^6 M^{-1} s^{-1} depending on the solvent systems [16].

Antioxidant properties of curcumin in O/W emulsions treated at 60 °C (Fig. 4) could be due to the ability to transfer hydrogen atoms, to scavenge peroxyl radicals and to chelate metal ions [3, 4, 6]. Among the keto-enol-enolate equilibrium of the heptadienone moiety in curcumin, keto forms are dominants in acidic solution (pH 3–7) and hydrogen atom transfer is the major antioxidant mechanism of curcumin [3]. Banerjee *et al.* [6] reported that curcumin can scavenge the peroxyl radicals generated from AAPH. Ak and Gülçin [4] proposed that ferrous ion may be chelated between hydroxyl and methoxyl groups in curcumin. Interestingly, the antioxidative properties of curcumin were not effective in corn oil systems at thermal oxidation at 100 °C (Table 1) compared to those in O/W emulsions at 60 °C. Locations and partition of compounds are important factors determining antioxidant or prooxidant properties of compounds in bulk oil and O/W emulsions. For example, α-tocopherol, which is a lipophilic compound, showed high antioxidant properties in O/W emulsions whereas hydrophilic compounds like ascorbic acid accelerated the rates of

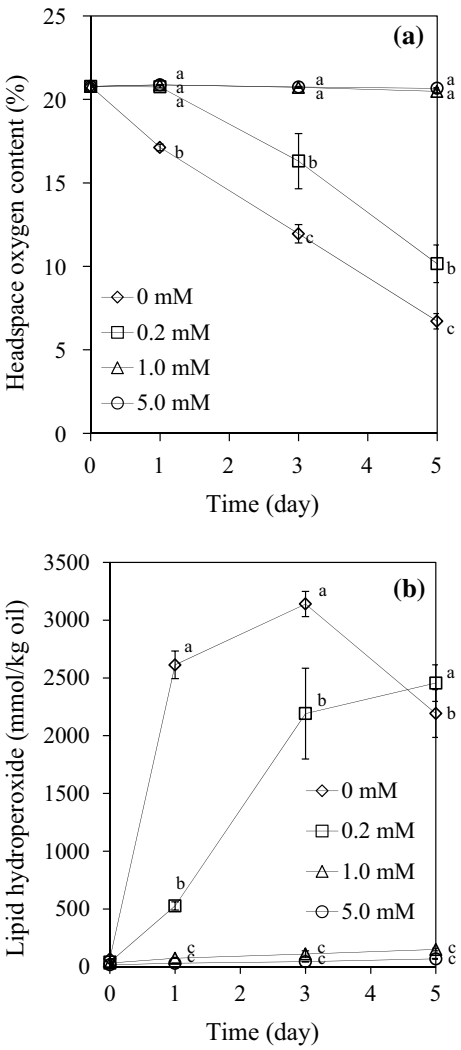

Fig. 4 Changes of headspace oxygen content (**a**) and lipid hydroperoxides (**b**) in O/W emulsions containing curcumin in the dark at 60 °C for 5 days. *Different small letters* indicate significant differences at the same treatment time ($p < 0.05$)

lipid oxidation in O/W emulsions under riboflavin sensitization [10]. Due to the poor solubility in aqueous phase, curcumin may be positioned on the interface of oil–water in O/W emulsions and may quench reactive oxygen species such as singlet oxygen or chelate transition metal ions effectively. Microenvironments, especially content of oil and water, seemed to affect the photosensitization or antioxidant abilities of curcumin—this calls for further research.

Conclusion

Depending on the types of matrix including bulk corn oil and O/W emulsions, curcumin showed different

photosensitizing and antioxidant properties. Curcumin acted as a photosensitizer in corn oil and in O/W emulsions under visible light whereas the photosensitizing ability in O/W emulsions was lower than in corn oil. Curcumin acted as an antioxidant in O/W emulsions with riboflavin but not in corn oil. The oil or water content may play important roles in determining photosensitizing or antioxidant properties of curcumin. Further research is needed to elucidate the major factors influencing antioxidative or photosensitizing properties of curcumin in diverse environments.

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