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ORIGINAL ARTICLE

Effects of antioxidants on the stability of β-Carotene in O/W emulsions stabilized by Gum Arabic

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Abstract The potential of oil-in-water emulsions as a β-carotene delivery system was examined in this study. Oil-in-water (O/W) emulsions containing β-carotene were formed by gum arabic with α-tocopherol, tertiary butyl hydroquinone (TBHQ) and ascorbyl palmitate, respectively. The influence of antioxidants on the chemical degradation of β-carotene in gum arabic stabilized emulsions was investigated at 4, 25, 45 and 65 °C in the dark, respectively. An accelerated photo-oxidation test was carried out at 45 °C (450 W/m^2). Moreover, β-carotene degradation rate constants (k_1-value), activation energy (E_a) and decimal reduction time (D-value) were estimated to interpret the degradation kinetics. The impact of antioxidants on the thermal stability of β-carotene in diluted emulsions was generally in the following order: α-tocopherol>TBHQ>ascorbyl palmitate. α-Tocopherol was found to be the most effective to the antioxidation of β-carotene at the concentration of 0.10 wt% under light exposure. It was concluded that the stability of β-carotene in oil-in-water emulsions could be improved by the presence of different antioxidants.

Keywords Antioxidants · Emulsion · Stability · β-Carotene · Gum Arabic

Introduction

In the past decades, great attentions have been paid to the carotenoids family, such as β-carotene, lycopene, lutein, astaxanthin, due to their provitamin A activity and antioxidant properties. Food products which contain high amounts of these compounds may offer great health benefits and provide new opportunities for the development of food industry. However, carotenoids are insoluble in water and only slightly soluble in oil at room temperature, which greatly limit their applications. Furthermore, carotenoids from vegetables are probably bound in protein complex or exist as crystal form, which may lead to poor bioavailability (West and Castenmiller 1998).

In order to improve the water solubility and bioavailability, carotenoids can be dissolved within the oil phase of O/W emulsions which can be easily incorporated into food products. Factors responsible for the degradation of carotenoids in emulsions include the composition of emulsion systems and environmental conditions, such as emulsifiers, antioxidants, light, heat, singlet oxygen and food systems (Boon et al. 2008, 2010).

Recent studies have been focused on the formation and stability evaluation of carotenoids emulsions. The degradation kinetics and isomerization of lycopene in O/W emulsions were investigated as a function of thermal treatment temperature and oxygen content (K et al. 2003). Moreover, the oxidative stability of carotenoids in the emulsion lipid phase was found to depend on the type of oil used to form the emulsion (Szterk et al. 2013). Chu et al. evaluated the stability of protein-stabilized β-carotene nano-dispersions against heating, salts and pH (Chu et al. 2007). Boon et al. studied the effect of different surfactants (cationic, anionic and non-ionic), oil types, pH, iron and hydroperoxides on the lycopene oxidation in oil-in-water emulsions, and the results suggested that the stability of lycopene in O/W emulsions could be improved by altering the emulsion droplet interface and the presence of tocopherols and EDTA (Boon et al. 2008, 2009, 2010). In our previous work, β-carotene emulsions were prepared and the effects of emulsifiers (Tween series, decaglycerol monolaurate, octenyl succinate starch, SSPS

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and WPI) and processing parameters on the physical and chemical stability of β-carotene were investigated (Yuan et al. 2008a, b; Mao et al. 2009, 2010; Hou et al. 2010; Xu et al. 2013).

Because of the special properties of O/W emulsions as a carotenoids carrier system, they are potential for the application in food systems, such as beverage emulsions and milk products. However, food systems are complex due to the numerous other compounds present, such as proteins, fiber, vitamin C, iron and sugar. In addition, the processing and storage might lead to further loss of carotenoids. There are a number of stability issues that must be overcome before these products come to market. Ribeiro et al. investigated the chemical stability of lycopene emulsions diluted in three different food systems (skimmed milk, orange juice and water) and the result indicated that lycopene stability strongly depended on the food system; it was particularly stable in orange juice (Ribeiro et al. 2003).

Beverage emulsions are unique and different from other food emulsions and they are consumed in a highly diluted form rather than in their original concentrated ones. They are firstly prepared as emulsion concentrates which are later diluted in several hundred times in sucrose solution in order to produce either carbonated or non-carbonated beverages. For beverage emulsions, the most critical criterion is their stability in the finished beverage products. The stability of emulsions in the form of concentrate is easier to be achieved because the viscosities of beverage emulsions are much higher due to the presence of hydrocolloid. The diluted beverage emulsions can almost be described as the concentrates being dispersed in a second water phase, that is, from a gum-solution water phase to a sucrose-solution water phase (Tan 1997). The degradation of carotenoids may be accelerated in the diluted beverage emulsions for the weaker protection from the hydrocolloids.

Oxidation of the carotenoids is one of the major factors that diminish the emulsion stability. Not only do carotenoids emulsions oxidize, but also diluted beverage emulsions are prone to oxidation. It is well known that antioxidants are proper inhibiters of oxidation in oil-in-water emulsions. Early in 1997, Heinonen et al. paid attention to the effects of β-carotene, α-tocopherol, and γ-tocopherol on antioxidation of 10 % oil-in-water emulsions of rapeseed oil triacylglycerols (Heinonen et al. 1997). Different antioxidants were investigated in margarine dispersions: L-ascorbic acid, ascorbyl palmitate and dl-α-tocopherol against the lipid oxidation (Filip et al. 2009). In addition, the antioxidative effects of ascorbic acid and ascorbyl palmitate in the O/W emulsions have been fully studied (Van Ruth et al. 1999; Liu and Yang 2008; Mosca et al. 2008; Watanabe et al. 2010; Yang et al. 2011). Previous studies have also revealed that tert-butyl hydroquinone (TBHQ) acted effectively as the free radical scavenging agents in emulsions (Karamac and Amarowicz 1997; Kishk and Al-Sayed 2007; Alamed et al. 2009).

However, to the best of our knowledge, no information is available concerning the impact of different antioxidants on the chemical stability of β-carotene in emulsions and food model system. Therefore, the purpose of current study was to investigate the effects of antioxidants on the stability of β-carotene in O/W emulsions formed by gum arabic as the emulsifier.

Materials and methods

Materials

β-Carotene suspension (30 % by mass β-carotene in sunflower oil) was purchased from Xinchang Pharmaceutical Co., Ltd. (Zhejiang, China). Medium chain triglyceride (MCT) oil was obtained from Lonza Inc. (NJ, USA). Gum Arabic was purchased from CNI (, France). Standard β-carotene (>95 % purity) and α-tocopherol (>97 % purity) were purchased from Sigma-Aldrich (St Louis, MO, USA). Ascorbyl palmitate (>98 % purity) was supplied by Beijing Chen Ao High-tech Development Co. Ltd (Beijing, China). Tertiary butyl hydroquinone (TBHQ, >98 % purity) was kindly offered by Crystal Quinone Pvt. Ltd (Ahmedabad, India). All other chemicals used were of analytical grade, unless otherwise stated.

Preparation of β-carotene emulsions

Gum arabic solution was prepared by dispersing gum arabic (30 wt%) in the distilled water. The solution was stirred overnight at a speed of 200 rpm to ensure complete dispersion and dissolution. Sodium azide (0.01 wt%) was added as an antimicrobial agent. β-Carotene emulsions (1.5 wt%) were prepared according to the method described by Yuan et al. with some modifications (Yuan et al. 2008b). Briefly, β-carotene was firstly dissolved in MCT oil at 140 °C for several seconds and then mixed with antioxidants and emulsifier solutions at a speed of 10,000 rpm with a blender to form coarse emulsions, which were further homogenized using a Niro-Soavi Panda two-stage valve homogenizer (Parma, Italy) for three cycles at 60 Mpa. The prepared emulsions were immediately cooled down to room temperature and sampled to measure the droplet size and stability.

Determination of droplet size and size distribution

Particle size and size distribution of β-carotene emulsions were determined by dynamic light scattering using a Zetasizer Nano-ZS90 (Malvern Instruments, Worcestershire, UK) at a fixed angle of 90°. Emulsions were diluted with Milli-Q water (0.002 wt % β-carotene in the final sample) prior to each measurement to minimize multiple scattering effects. Results

were described as cumulants mean diameter (size, nm) for droplet size, polydispersity index (PdI) for size distribution.

ζ-Potential measurements

Emulsions were diluted using Milli-Q water to avoid multiple scattering effects, the final oil concentration of diluted emulsions was 0.005 wt%. They were injected directly into the chamber of a particle electrophoresis instrument (Nano-ZS90, Malvern Instruments, Worcestershire, UK). The ζ-potential was determined by measuring the direction and velocity of droplet movement in a well-defined electric field. All measurements were performed in duplicate with freshly prepared samples and the ζ-potential measurements were reported as the mean and standard deviation of three separate injections (Chu et al. 2007; Hou et al. 2010).

Dilution of β-carotene emulsions with the reference solution

Reference solution was prepared by adding sucrose (10 wt%) and citric acid (1.0 wt%) into distilled water. β-Carotene emulsions were diluted with the reference solution to a total β-carotene concentration of 0.003 wt%, and then were further homogenized using a Niro-Soavi Panda two-stage valve homogenizer (Parma, Italy) for three cycles at 50 Mpa. Solutions from the exit of the homogenizer were boiled to remove the dissolved oxygen, and then stored in brown glass bottles at 4 °C in the dark.

Effects of storage temperature on the chemical stability of β-carotene in emulsions and diluted emulsions

In the series of experiments, the influence of antioxidants concentration on the chemical degradation of β-carotene in gum arabic stabilized emulsions was evaluated by splitting emulsion samples into four parts which were stored at 4, 25, 45 and 65 °C in the dark, respectively. Emulsion samples were diluted with 10 wt% sucrose-citric acid solution (the reference solution) to a total oil concentration of 0.01 wt% (0.003 wt% for β-carotene), or the original emulsion samples were transferred into screw-capped brown bottles flushed with nitrogen. The β-carotene concentrations were determined at different intervals of the storage time.

Effects of light exposure on the chemical stability of β-carotene in diluted emulsions

Emulsion samples were diluted with sucrose-citric acid solution (the reference solution) to a total oil concentration of 0.01 wt% (0.003 wt% for β-carotene), or the original emulsion samples were transferred into screw-capped brown bottles flushed with nitrogen in a controlled light cabinet (SUNTEST CPS+, 450 W/m^2, 45 °C). β-Carotene concentrations were determined constantly for 3 h at an interval of 30 min.

Measurements of total carotenoid content in emulsions and reference emulsions

The content of total carotenoid in the emulsion was determined following the method of Karin Ax (Karin et al. 2003) and Yuan Y (Yuan et al. 2008a). Emulsions were first diluted to an appropriate concentration with water, and then extracted with ethanol and n-hexane. Absorbance was measured at 450 nm with a SHIMADZU UV-1800 UV–Vis Spectrophotometer. The concentration of β-carotene was obtained by referring to a standard curve of β-carotene prepared under the same condition.

Thermal degradation kinetics model

β-Carotene degradation data were fitted to the following equations with regression analysis using first-order equation. Reaction rate constants (k_1-values) could be estimated according to the following equation:

$$c/c0 = \exp(-k1 \bullet t)$$

Where c=the concentration of β-carotene at any time, c_0= the initial concentration of β-carotene, t=storage time, k_1= reaction rate constant.

The Arrhenius equation is the most widely accepted method of accounting for the temperature dependence of the rate constant in food systems. The temperature and the rate constant k are related according to the Arrhenius equation:

$$d1nk_1/dT = Ea/RT^2$$

Where E_a is the activation energy of the reaction; R is the gas constant; T is the absolute temperature.

The decimal reduction time (D-value) is defined as the treatment time needed for 90 % degradation of its original value. The D-value was calculated as follows:

$$D = 2.303/k_1$$

Where k is the first-order rate constant (Takahashi et al. 1999; Zheng and Lu 2011).

Statistical analysis

All experiment were conducted in duplicate. Data were subjected to analysis of variance (ANOVA) using the software

package SPSS 18.0 for Windows (SPSS Inc., Chicago, USA). Means of treatments were separated at the 5 % significance level using the LSD method.

Results and discussion

Carotenoids were degraded through free radical mediated autoxidation reactions (Alekseev et al. 1967; Kasaikina et al. 1976, 1998). Therefore, antioxidants which might act as free radical scavengers should be effective in oil-in-water emulsions. Dissolved sucrose also contributed to the reduction of oxidative degradation, because it diminished the solubility of oxygen, increased the viscosity of the aqueous phase, which led to decreased mobility of reactive oxygen species, and it acted as a free radical scavenger (Hu et al. 2003). β-Carotene containing gum arabic stabilized emulsions were prepared with MCT as the oil phase (with or without the addition of antioxidants). Various antioxidants were evaluated for the protection of the emulsified β-carotene.

Effects of antioxidants on the droplet characteristics of β-carotene emulsions

Gum arabic is a hybrid polyelectrolyte containing both protein and polysaccharide subunits. It consists mainly of a mixture of arabinogalactan (AG) (80–90 % of the total gum in weight), glycoprotein (GP) (2–4 % of the total gum in weight) and arabinogalactan protein (AGP) (10–20 % of the total gum in weight) fractions (Bouyer et al. 2011). Previous studies showed that gum arabic was widely used in preparation of emulsions, and the droplet characteristics were then evaluated (Jayme et al. 1999; Djordjevic et al. 2007, 2008; Karaiskou et al. 2008; Padala et al. 2009; Bouyer et al. 2011; Charoen et al. 2011; Zhang and Liu 2011). However, no information is available concerning the effect of antioxidants on the stability of β-carotene in the emulsions stabilized by gum arabic. Table 1 showed the particle size, polydispersity index (PdI) and particle electrical charge (ζ-potential) of β-carotene emulsions prepared with different antioxidants (1.5 wt% β-carotene, 3.5 wt% MCT oil and 0.1 wt% antioxidants). It was found that only slight differences were observed on the mean particle diameters of the emulsions with α-tocopherol, TBHQ and ascorbyl palmitate. Polydispersity index (PdI) of the emulsions remained lower than 0.2, which indicated that the emulsions with different antioxidants were all in identical size distributions. In addition, the net charge on the gum arabic stabilized emulsions was ranged from −23.8 to −26.6 mV. The droplets in emulsions with α-tocopherol exhibited a little less negative net charge. Fig. 1 showed the effect of different antioxidants on the size distributions of gum arabic stabilized emulsions, and these results verified that the general size distributions of gum arabic stabilized emulsions had no distinct discrepancy.

Table 1 Dependence of particle size, polydispersity index (PdI) and particle electrical charge (ζ-potential) of β-carotene emulsions prepared by different antioxidants

Antioxidants	α-Tocopherol	TBHQ	Ascorbyl Palmitate
Size (nm)	644.3±7.0[a]	625.0±6.9[b]	614.0±11.1[c]
PdI	0.16±0.05	0.17±0.05	0.15±0.07
ζ-potential (mV)	−26.6±0.2	−24.2±0.4	−23.8±0.2

Values are means±SD (n=3). Means with different superscript letters in the same row are expressed as a significant difference at $p<0.05$

Effects of antioxidants on the stability of β-carotene in O/W emulsions

In the actual storage or transportation, β-carotene emulsions were mainly stored in the seal in prevent of light conservancy at room temperature or in the cold condition. To identify the effects of storage temperatures and types of antioxidants on the degradation of β-carotene in original oil-in-water emulsions, α-tocopherol, TBHQ and ascorbyl palmitate were added into the oil phase. The degradation of β-carotene was determined by splitting emulsion samples into two groups. One group was stored at 4 °C and the other was stored at 25 °C. Results were shown in Fig. 2. The loss of β-carotene in oil-in-water emulsions without the presence of antioxidants was about 2.8 % at 4 °C and 4.7 % at 25 °C, after a 4 weeks storage. More significant carotenoids content drop of 10 % was reported for the cold-pressed linseed oil emulsion after 4 weeks of storage, 5 % for refined palm olein as well (Szterk et al. 2013). In

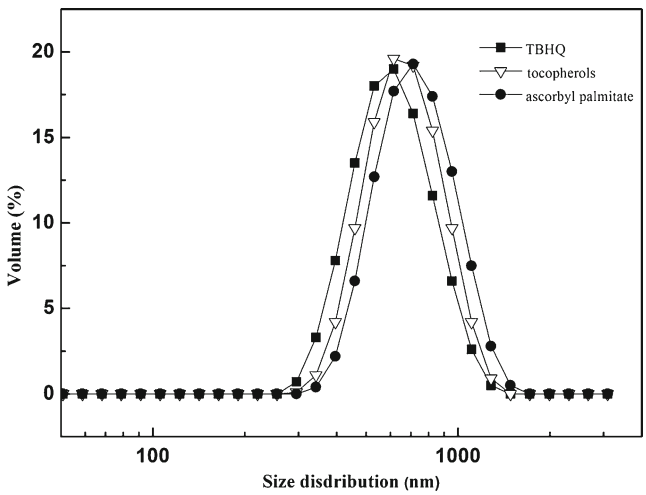


Fig. 1 Size distribution of β-carotene emulsions prepared by gum arabic (30 wt%) with ascorbyl palmitate, α-tocopherol and TBHQ (1.5 wt% β-carotene, 3.5 wt% MCT and 0.1 wt% antioxidants in the final emulsion)

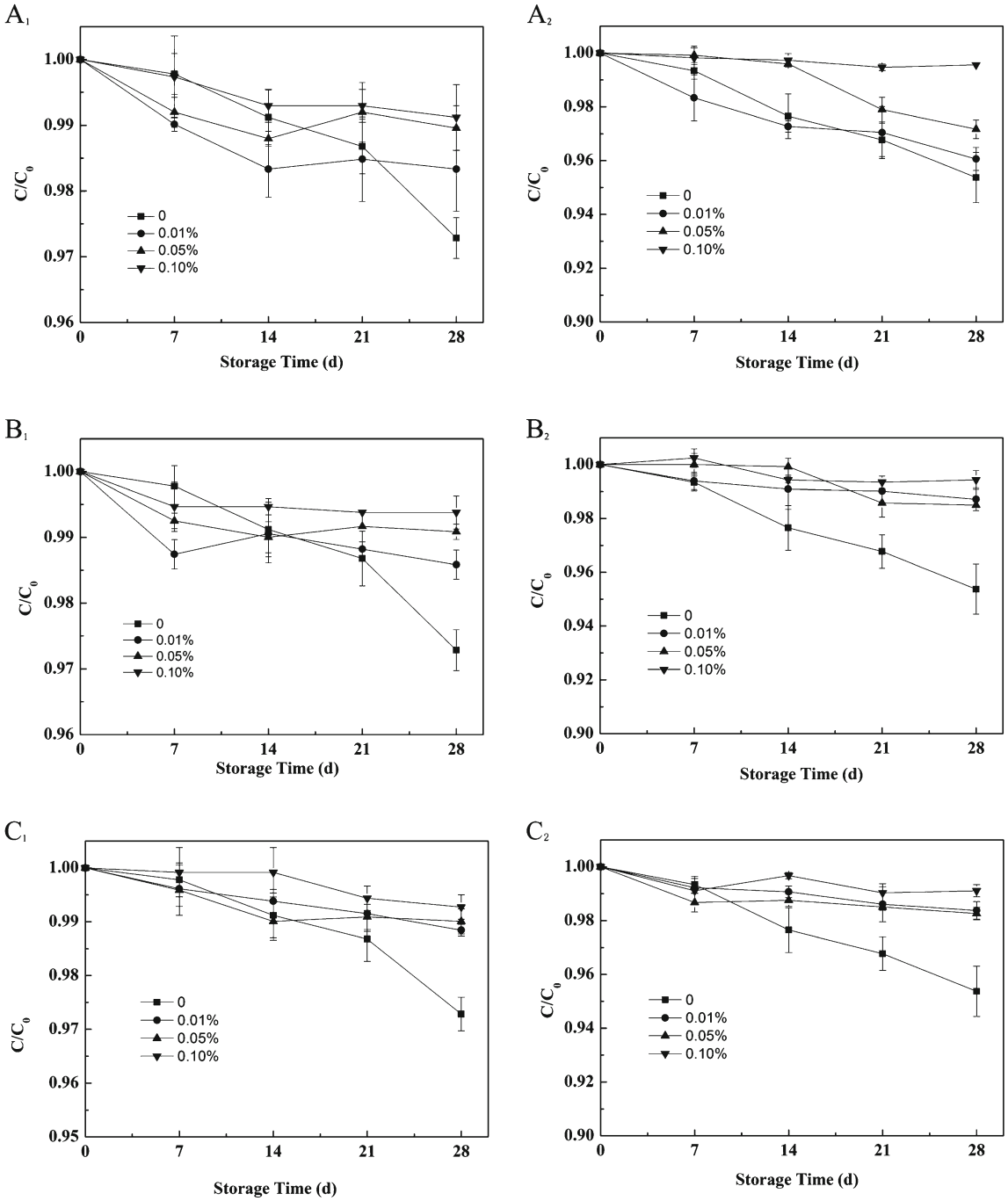


Fig. 2 Stability of β-carotene emulsions prepared by gum arabic (30 wt%) with different antioxidants (1.5 wt% β-carotene, 3.5 wt% MCT and 0.1 wt% antioxidants in the final emulsion) at 4 °C (marked with the subscript 1) and 25 °C (marked with the subscript 2). **a**: ascorbyl palmitate; **b**: α-tocopherol; **c**: TBHQ

addition, the presence of antioxidants was effective in retarding the oxidation of β-carotene in oil-in-water emulsions. However, there was no significant difference among different antioxidants.

In this study, the degradation of β-carotene was relatively slow under both low temperature and room temperature, where only about 0.7~2.8 % and 0.5~4.7 % of the original β-carotene in the emulsion were lost at 4 and 25 °C respectively. This was attributed to the high viscosity of the gum arabic emulsion and the strong surface layer of oil phase which could prevent the creaming of β-carotene and reduce the exposure of β-carotene to the external environment. Therefore, β-carotene had identical chemical stability in gum arabic stabilized emulsion. Moreover, slower degradation of β-carotene was observed in emulsions with higher concentration of antioxidants.

Effects of antioxidants on the photo stability of β-carotene in the reference solution

The previous experiments indicated that antioxidants, such as α-tocopherol, TBHQ and ascorbyl palmitate, could protect β-carotene from degradation in oil-in-water emulsions. Food systems are more complex because of the presence of numerous other compounds. Studies showed that food systems had a great influence on the stability of β-carotene within the oil droplets. Sucrose is the most common compound present in many food formulations, such as orange juice and milk. Therefore, in the present study, sucrose-citric acid solution was used as the reference solution. The effects of various antioxidants on the stability of β-carotene emulsion in the reference solution were investigated.

Light exposure degrades carotenoids. Photo-oxidation produces carotenoid radical cautions (Mortensen and Skibsted 1996; Konovalova et al. 2001). Rapid degradation of β-carotene was observed in the diluted emulsion samples without antioxidants (Fig. 3a, b and c). The presence of the antioxidants had more significant effects on the degradation of β-carotene in the reference solution ($p<0.05$). About 75.5 % of β-carotene was lost in the diluted emulsions without antioxidants after the light exposure for 1 h, while those with ascorbyl palmitate concentrations of 0.01, 0.05 and 0.10 wt% exhibited the losses of 73.2, 69.1 and 62.3 %, respectively. Treated with the light for 3 h, over 85 % of β-carotene was lost in the diluted emulsions (Fig. 3a). The addition of ascorbyl palmitate was found to have little impact on β-carotene stability in gum arabic stabilized emulsions under light exposure. This might be due to the fact that the effectiveness of free radical scavenging antioxidants was decreased as their polarity increased and ascorbyl palmitate has the strongest polarity among the three antioxidants (Huang et al. 1997). However, slower degradation rates of β-carotene were observed in the diluted emulsions with TBHQ. Higher levels of TBHQ were more effective in inhibiting β-carotene degradation, which was illustrated in Fig. 3c. These results revealed that the degradation of β-carotene was inhibited by the presence of chain-breaking antioxidants. As shown in Fig. 3b, after light exposure for 3 h at 45 °C, the degradation of β-carotene was differed among the diluted emulsions with different α-tocopherol levels. The degradation of β-carotene was the slowest in diluted emulsions with α-tocopherol concentration of 0.10 wt%, which indicated that the degradation of β-carotene in the reference solution was significantly influenced by α-tocopherol concentration ($p<0.05$).

Results mentioned above interpreted that higher level of antioxidants was directly correlated with the decrease of β-carotene loss in all samples. The types and concentrations of antioxidants exhibited a notable impact on the stability of β-carotene. Severe degradation of β-carotene was detected in the diluted emulsions without presence of the antioxidants,

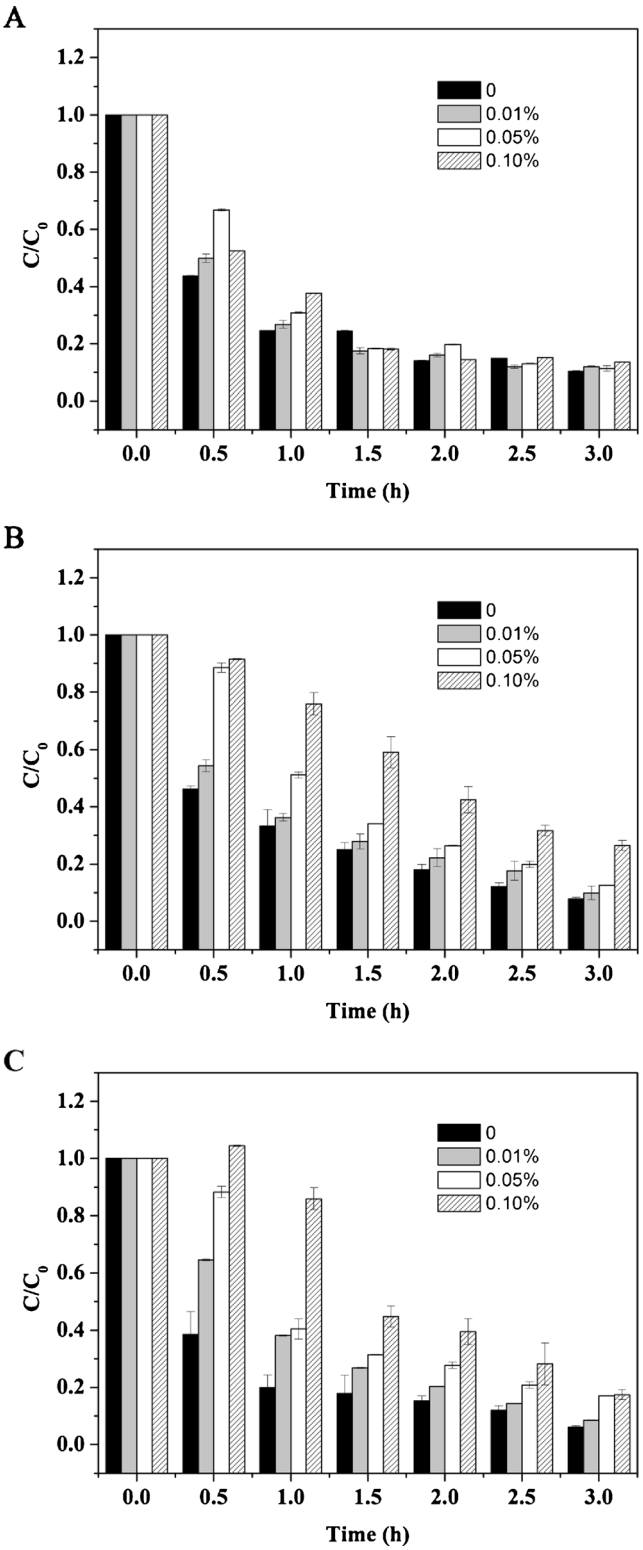
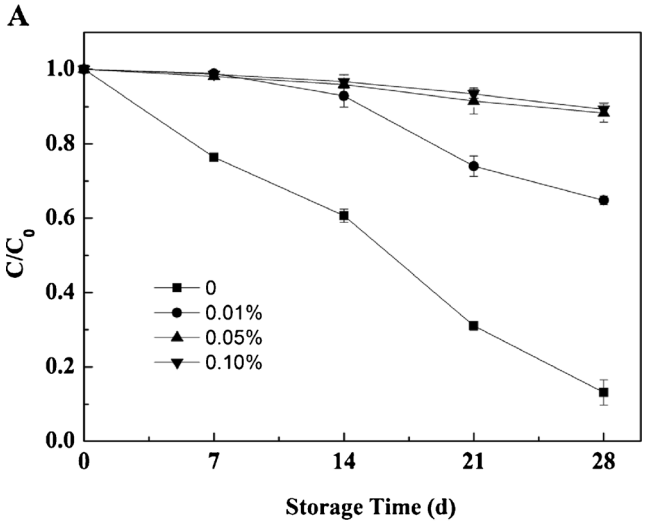
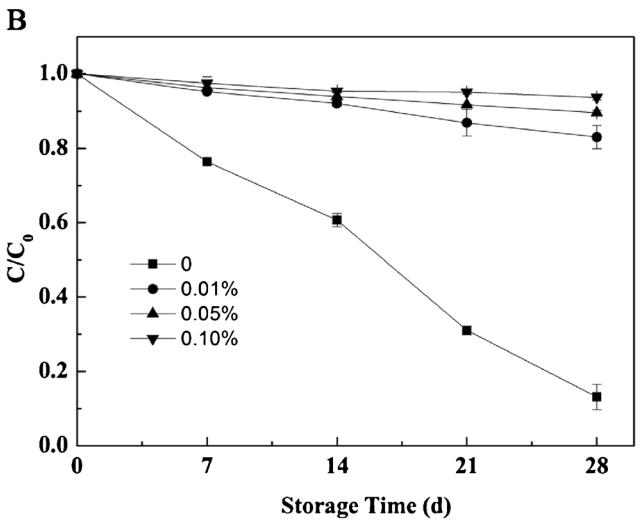


Fig. 3 Dependence of photo stability of β-carotene in the reference solution (0.01 wt% total oil, 0.003 wt% β-carotene) with different antioxidants at concentrations of 0.01, 0.05 and 0.10 wt% during storage under light exposure (450 W/m^2, 45 °C). (**a**: ascorbyl palmitate; **b**: α-tocopherol; **c**: TBHQ)





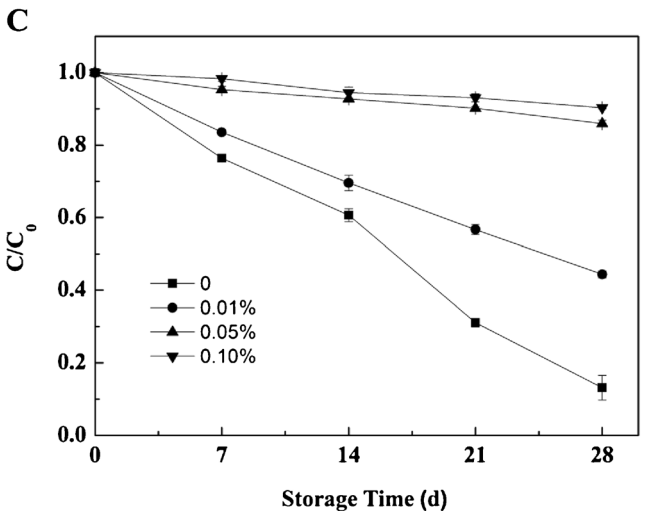
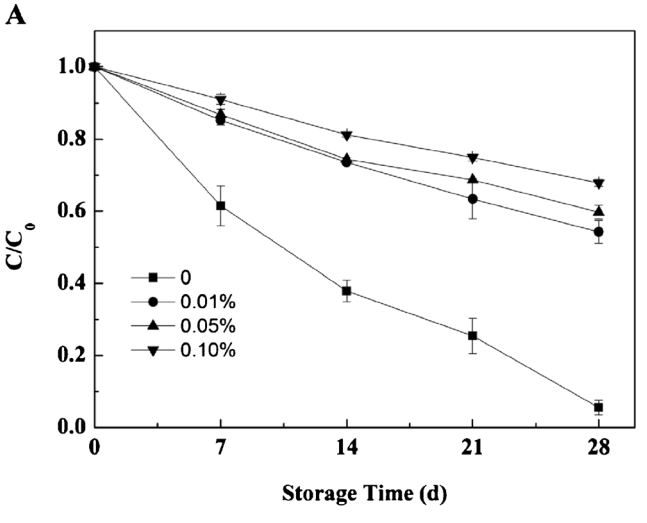
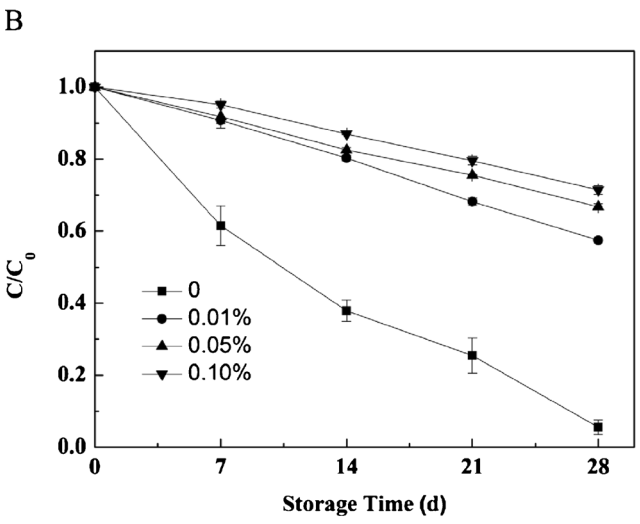


Fig. 4 Thermal stability of β-carotene in the reference solution (0.01 wt% total oil, 0.003 wt% β-carotene) with different antioxidants at concentrations of 0.01, 0.05 and 0.10 wt% at 4 °C.(**a**: ascorbyl Palmitate; **b**: α-tocopherol; **c**: TBHQ)





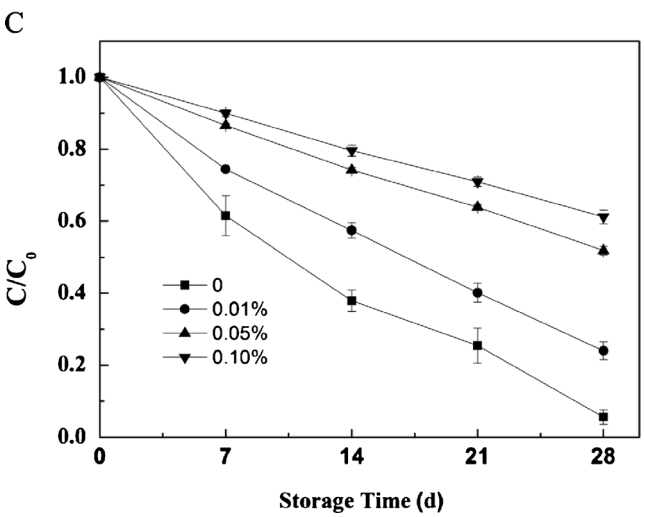


Fig. 5 Thermal stability of β-carotene in the reference solution (0.01 wt% total oil, 0.003 wt% β-carotene) with different antioxidants at concentrations of 0.01, 0.05 and 0.10 wt% at 25 °C.(**a**: ascorbyl palmitate; **b**: α-tocopherol; **c**: TBHQ)

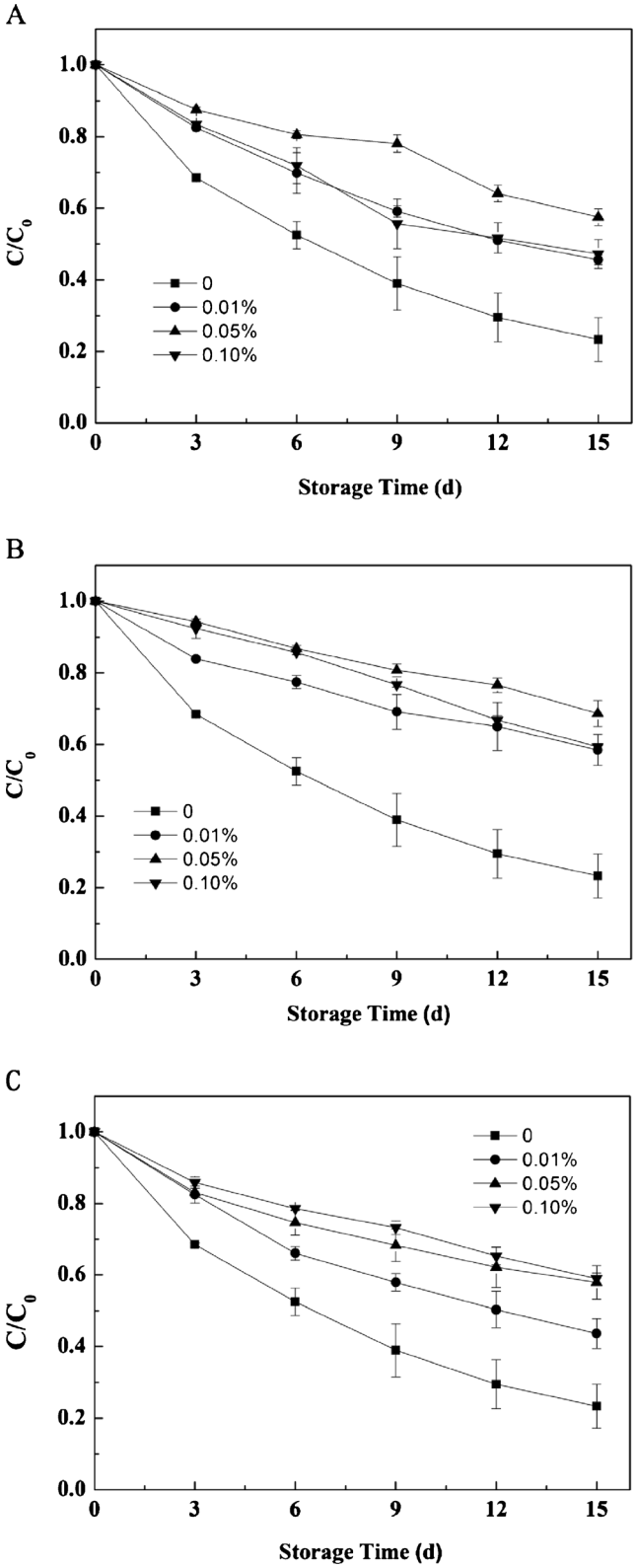


Fig. 6 Thermal stability of β-carotene in the reference solution (0.01 wt% total oil, 0.003 wt% β-carotene) with different antioxidants at concentrations of 0.01, 0.05 and 0.10 wt% at 45 °C.(**a**: ascorbyl palmitate; **b**: α-tocopherol; **c**: TBHQ)

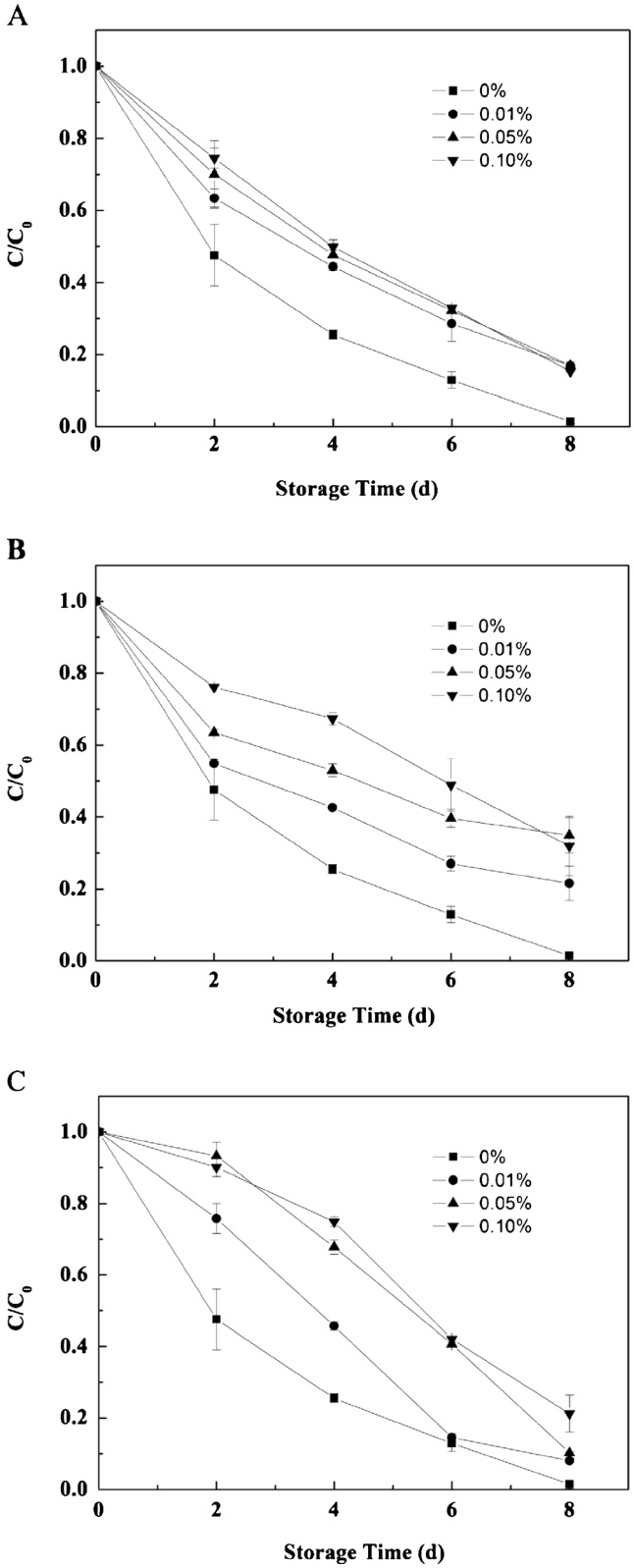


Fig. 7 Thermal stability of β-carotene in the reference solution (0.01 wt% total oil, 0.003 wt% β-carotene) with different antioxidants at concentrations of 0.01, 0.05 and 0.10 wt% at 65 °C.(**a**: ascorbyl palmitate; **b**: α-tocopherol; **c**: TBHQ)

Table 2 Thermal reaction rate constants (k_1) of β-carotene degradation in the reference solution

T (°C)	k_1 [h^{-1}]									
	Control	Ascorbyl Palmitate			α-Tocopherol			TBHQ		
	0	0.01 %	0.05 %	0.10 %	0.01 %	0.05 %	0.10 %	0.01 %	0.05 %	0.10 %
	0	0.01 %	0.05 %	0.10 %	0.01 %	0.05 %	0.10 %	0.01 %	0.05 %	0.10 %
4	$2.95\cdot10^{-3i}$	$6.88\cdot10^{-4abcde}$	$2.08\cdot10^{-4ab}$	$1.67\cdot10^{-4a}$	$2.75\cdot10^{-4abc}$	$1.58\cdot10^{-4a}$	$9.17\cdot10^{-5a}$	$1.20\cdot10^{-3efg}$	$2.13\cdot10^{-4ab}$	$1.54\cdot10^{-4a}$
25	$3.98\cdot10^{-3j}$	$9.04\cdot10^{-4def}$	$7.54\cdot10^{-4cde}$	$5.79\cdot10^{-4abcd}$	$8.29\cdot10^{-4cde}$	$5.96\cdot10^{-4\ abcd}$	$5.08\cdot10^{-4\ abcd}$	$2.07\cdot10^{-3h}$	$9.63\cdot10^{-4defg}$	$7.25\cdot10^{-4bcde}$
45	$4.01\cdot10^{-3j}$	$2.20\cdot10^{-3h}$	$1.48\cdot10^{-3g}$	$2.16\cdot10^{-3h}$	$1.41\cdot10^{-3fg}$	$1.02\cdot10^{-3defg}$	$1.47\cdot10^{-3g}$	$2.29\cdot10^{-3h}$	$1.47\cdot10^{-3g}$	$1.40\cdot10^{-3fg}$
65	$2.05\cdot10^{-2p}$	$9.32\cdot10^{-3m}$	$9.14\cdot10^{-3m}$	$9.54\cdot10^{-3m}$	$7.94\cdot10^{-3l}$	$5.44\cdot10^{-3k}$	$5.72\cdot10^{-3k}$	$1.40\cdot10^{-2o}$	$1.12\cdot10^{-2n}$	$8.01\cdot10^{-3l}$

Values are means±SD (n=3). Means with different superscript letters are expressed as a significant difference at $p<0.05$

and the protecting capacity of different antioxidants in reference solution under light exposure was in the following order: α-tocopherol>TBHQ>ascorbyl palmitate.

Effects of antioxidants on the thermal stability of β-carotene in the reference solution

Thermal processing was regarded as another important factor that influenced the β-carotene degradation. Emulsion samples prepared with various concentrations of antioxidants (0.01, 0.05 and 0.10 wt% in the emulsion) were diluted in the reference solutions to a total β-carotene concentration of 0.003 wt%, and then the solutions were stored at 4, 25 and 45, 65 °C in dark, respectively. The results of β-carotene degradation could be found in Figs. 4, 5, 6 and 7. The retarded loss of β-carotene suggested that antioxidants could effectively protect β-carotene from degradation in the reference solutions. Accordingly, within the temperature range investigated, β-carotene degradation could be reduced significantly by increasing the concentration of antioxidants ($p<0.05$).

Figure 4 showed the stability of β-carotene in the diluted reference solution at 4 °C, the degradation of β-carotene in solutions was slower with the presence of α-tocopherol, the main reason might be that the preferential oxidation of α-tocopherol could inhibit the oxidation of β-carotene since α-tocopherol was oxidized prior to β-carotene. Similar phenomena were found in the study on lycopene emulsions (Bou et al. 2011). Compared with α-tocopherol, the degradation of β-carotene in solutions was faster with ascorbyl palmitate and TBHQ, especially at low concentration. Ascorbyl palmitate and TBHQ were more effective in protecting β-carotene in solutions at concentrations above 0.05 %. The samples with ascorbyl palmitate and TBHQ at the level of 0.01 wt% exhibited 35.2 and 55.6 % β-carotene losses, while those with higher concentration (0.05 wt%) showed only 13.2 and 14 % losses, respectively.

The protecting capacity of different antioxidants for β-carotene in the reference solutions stored at 25 °C was in the order of α-tocopherol>ascorbyl palmitate>TBHQ (Fig. 5). Reference solutions containing β-carotene containing antioxidants exhibited a greater stability than those without antioxidants.

Degradation of β-carotene in the reference solution stored at 45 °C was much faster than that at 25 and 4 °C (Fig. 6). These results implied that β-carotene degradation could be reduced by increasing the concentration of TBHQ; however, α-tocopherol and ascorbyl palmitate of a lower concentration (0.05 wt%) were more effective than those with higher concentration (0.10 wt%), which might be attributed to the prooxidant effects related to metal ions (Let et al. 2007).

As illustrated in Fig. 7, over 85 % of β-carotene had lost in the emulsions stabilized by gum arabic without antioxidants in only 1 week at 65 °C. Antioxidants of lower concentrations were found to have little impact on β-carotene stability in the reference solution at a relatively high temperature. After the storage of 8 days, it was found that severe degradation of β-carotene was detected in the solutions with all concentrations of ascorbyl palmitate, and the least degradation occurred in the reference solution with α-tocopherol concentration of 0.05 wt%.

There were several possible explanations why different antioxidants influenced the thermal degradation of β-carotene significantly. With certain antioxidants in the emulsions, the reaction between β-carotene and singlet oxygen, or other free radicals, might be inhibited by the antioxidants presented in the emulsion systems. Previous study revealed

Table 3 Activation energy (E_a) of β-carotene degradation in the reference solution

Concentration	E_a (10^3 J/mol)			
	Control	Ascorbyl Palmitate	α-Tocopherol	TBHQ
0.01 %	5.6^{a}	20.4^{c}	29.5^{d}	11.8^{b}
0.05 %		35.3^{f}	33.7^{e}	35.0^{f}
0.10 %		45.7^{i}	49.8^{h}	39.8^{g}

Values are means±SD (n=3). Means with different superscript letters are expressed as a significant difference at $p<0.05$

Table 4 The decimal reduction time (*D*-value) of β-carotene degradation in the reference solution

T (°C)	*D*-value (days)									
	Control	Ascorbyl Palmitate			α-Tocopherol			TBHQ		
	0	0.01 %	0.05 %	0.10 %	0.01 %	0.05 %	0.10 %	0.01 %	0.05 %	0.10 %
	0	0.01 %	0.05 %	0.10 %	0.01 %	0.05 %	0.10 %	0.01 %	0.05 %	0.10 %
4	$7.81\cdot10^{2h}$	$3.35\cdot10^{3lmnop}$	$1.11\cdot10^{4op}$	$1.38\cdot10^{4p}$	$8.37\cdot10^{3nop}$	$1.46\cdot10^{4p}$	$2.51\cdot10^{4p}$	$1.92\cdot10^{3jkl}$	$1.08\cdot10^{4op}$	$1.50\cdot10^{4p}$
25	$5.79\cdot10^{2g}$	$2.55\cdot10^{3klm}$	$3.05\cdot10^{3lmn}$	$3.98\cdot10^{3mnop}$	$2.78\cdot10^{3lmn}$	$3.86\cdot10^{3mnop}$	$4.53\cdot10^{3mnop}$	$1.11\cdot10^{3i}$	$2.39\cdot10^{3jklm}$	$3.18\cdot10^{3lmno}$
45	$5.74\cdot10^{2g}$	$1.05\cdot10^{3i}$	$1.56\cdot10^{3j}$	$1.07\cdot10^{3i}$	$1.63\cdot10^{3jk}$	$2.26\cdot10^{3jklm}$	$1.57\cdot10^{3j}$	$1.01\cdot10^{3i}$	$1.57\cdot10^{3j}$	$1.65\cdot10^{3jk}$
65	$1.12\cdot10^{2a}$	$2.47\cdot10^{2d}$	$2.52\cdot10^{2d}$	$2.41\cdot10^{2d}$	$2.90\cdot10^{2e}$	$4.23\cdot10^{2f}$	$4.03\cdot10^{2f}$	$1.65\cdot10^{2b}$	$2.06\cdot10^{2c}$	$2.88\cdot10^{2e}$

Values are means±SD (n=3). Means with different superscript letters are expressed as significant difference at $p<0.05$

that tocopherol protected β-carotene from deterioration (Palozza and Krinsky 1991; Haila et al. 1996). Moreover, studies interpreted that TBHQ presented the greater effect on enhancing the sensory stability of MCT than β-carotene (De Azeredo et al. 2003). However, it was reported that ascorbyl palmitate at a low concentration acted as a radical-type inhibitor of autoxidation, while the prooxidant effects were observed as the concentration was increased (Let et al. 2007).

Another considered reason might be that the free radical scavenging activity was consistently related to the inhibition of lipid oxidation in the diluted emulsions. Once the oxidation was initiated by lipid, β-carotene might further react with itself or other chemical species produced by the initial reactions. Therefore, the effects of antioxidants on the oxidation of lipids influenced the stability of β-carotene.

The activity of an antioxidant to capture free radicals was also depended on its physical location in the systems. It was suggested that the activity of chain-breaking antioxidants in retarding lipid oxidation in the O/W emulsions would increase because of their decreased polarity or the increased surface activity, which made them more likely to be localized at the oil–water interface where the oxidation would occur. All what mentioned above indicated that the polarity difference among α-tocopherol, TBHQ and ascorbyl palmitate affected their protection capacity (McClements and Decker 2000).

Degradation kinetics of β-carotene in the reference solution

To get a better understanding of the effects of antioxidant concentration and storage temperature on the degradation of β-carotene in gum arabic stabilized emulsions, the thermal degradation kinetics of β-carotene was developed. k_1-values were listed in Table 2. It was found that the degradation of β-carotene in the diluted emulsions stabilized by gum arabic followed the first-order kinetics. R^2 values were ranged from 0.8124 to 0.9998, the first-order kinetics model had a well description for the degradation. These phenomena were similar to those reported in organic solutions (Takahashi et al. 1999) and oil in water emulsions (Karin et al. 2003). Results mentioned above illustrated that higher temperatures were directly related to distinct increase of β-carotene loss in all samples. . It could be also found from k_1-values that relative high concentration of antioxidants offered stronger protection for β-carotene from thermal degradation. α-Tocopherol was more effective than ascorbyl palmitate and TBHQ in the reference solution.

Activation energy (E_a) was estimated by using Arrhenius plots of β-carotene loss given in Table 3. It was shown that higher activation energies were required in the reactions at higher temperatures and those in presence of α-tocopherol in the reference solution. Table 4 showed the *D*-values of β-carotene degradation in the diluted emulsions with different antioxidants. These results verified that significant decreases of β-carotene were observed during the storage at all temperatures without antioxidants and the relative high concentration of antioxidants significantly inhibited the loss of β-carotene in the reference solution ($p<0.05$).

Conclusion

In conclusion, β-carotene stability in O/W emulsions could be significantly improved by the presence of antioxidants. The thermal degradation of β-carotene in the diluted emulsions at different temperatures was greatly affected by the types and dosages of antioxidants. Taking the results from photo exposure into account, the least degradation occurred in the reference solution with α-tocopherol concentration of 0.10 wt%. Overall, the gum arabic emulsion with antioxidants could be a potential β-carotene delivery system for food industry and the results were of more significance for increasing the shelf life of food products containing β-carotene. Further research will be carried out to clarify the reaction mechanism of the antioxidants in β-carotene emulsions and their in vitro digestive properties.

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