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Influence of pH, EDTA, α-tocopherol, and WPI oxidation on the degradation of β-carotene in WPI-stabilized oil-in-water emulsions

Duoxia Xu [a], Xiaoya Wang [a], Junping Jiang [a], Fang Yuan [a], Eric Andrew Decker [b], Yanxiang Gao [a,*]

[a] College of Food Science & Nutritional Engineering, Beijing Key Laboratory of Functional Food from Plant Resources, China Agricultural University, Box 112, No. 17 Qinghua East Road, Haidian District, Beijing 100083, China
[b] Department of Food Science, University of Massachusetts, Amherst, MA, USA

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ABSTRACT

The aim of the present study was to examine the factors (pH, iron chelators, free radical scavengers) influencing the chemical stability of WPI-stabilized β-carotene emulsions and investigate the correlations between WPI oxidation and β-carotene degradation during the storage of emulsions. The pH of the emulsion had a significant influence on the stability of β-carotene, with rapid degradation in emulsions at low pH (3.0, 4.0) and relatively higher stability at high pH (6.0, 7.0). Addition of EDTA or α-tocopherol significantly increased the stability of β-carotene, exhibited a greater improvement at pH 7.0 compared to pH 4.0 and α-tocopherol was much more effective than EDTA. It revealed that both transition metals and free radicals induced β-carotene degradation, nevertheless free radicals were found to be the predominant mechanism of β-carotene degradation. WPI oxidation was measured by the loss of tryptophan fluorescence and increase in fluorescent protein oxidation products using fluorescence spectroscopy. The tryptophan fluorescence decreased in all samples during the storage and emulsions with more protein oxidation products exhibited greater β-carotene degradation rate, which confirmed that there existed a good correlation between the protein oxidation and β-carotene loss.

1. Introduction

In recent years, the benefits of carotenoids in the diet has raised great research interest in incorporating into functional food products for effects on human health including prevention of a number of serious health disorders such as cancer, heart disease and colorectal adenomas (Kiokias & Gordon, 2004). β-Carotene is one of the major carotenoids present in the diet (Grabowski, Truong, & Daubert, 2008). However, there are a number of stability issues that influence the product quality and consumer acceptance.

The conjugated polyene chain of the β-carotene makes it susceptible to degradation from a number of agents. Autoxidation of β-carotene occurs at room temperature in certain solvents, producing a number of initial oxidation products including carbon-peroxyl triplet biradicals and epoxides (Henry et al., 2000; Rascon, Beristain, Garcia, & Salgado, 2011). β-Carotene is also sensitive to low pH, oxygen, heat and light which can lead to loss of both color and bioactivity of β-carotene in foods (Marty & Berset, 1990; Ribeiro & Cruz, 2005).

Since β-carotene is lipid soluble, dispersing it in the oil phase of oil-in-water emulsions might be one of the best solutions to improve its oxidative stability and bioavailability (Neethirajan & Jayas, 2011; Yuan, Gao, Zhao, & Mao, 2008). β-Carotene is dissolved in oil, and the β-carotene emulsion is formed by emulsifying the active-compound solution with the aqueous phase containing a combination of emulsifiers (Mao, Yang, Xu, & Gao, 2010).

Factors that could influence β-carotene degradation rates in oil-in-water emulsions include droplet size, which influences surface area; emulsion droplet charge, which could cause either attraction or repulsion of transition metals; thickness of the emulsifier layer at the interface region of the emulsion droplets that can impact interactions between dispersed phase β-carotene and aqueous phase prooxidants. Prooxidants such as transition metals (iron, copper) may lead to electron transfer between metals and β-carotene through forming β-carotene radical cation, which undergoes β-carotene degradation (Cornacchia & Roos, 2011; Fomuso, Corredig, & Akoh, 2002). In addition, β-carotene may react with free radicals through hydrogen abstraction leading to β-carotene loss (Mortensen, 2002; Zanfini, Corbini, Rosa, & Dreassi, 2010). Therefore, addition of prooxidant metals chelators (EDTA) and free radical scavengers (eg. TBHQ and α-tocopherol) to emulsions have

* Corresponding author. Tel.: +86 10 62737034; fax: +86 10 62737986.
E-mail address: gyxcau@126.com (Y. Gao).

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been developed for increasing emulsion stability (Gao & Kispert, 2003).

Milk proteins, such as whey protein and casein are often utilized as emulsifying agents in foods and can inhibit the oxidation of dispersed phase by preventing the penetration of prooxidants within the emulsified droplet (Dickinson, 2008). They have been shown to undergo oxidative modification at the interface of emulsion droplet and the aqueous phase (Viljanen, Halmos, Sinclair, & Heinonen, 2005). Tryptophan, cysteine and methionine were determined to have the highest antioxidant activity. Tryptophan plays an important role in antioxidation by serving as a hydrogen donor, thus allowing oxygen radicals to quench its indolic hydrogen (Hernandez-Ledesma, Davalos, Bartolome, & Amigo, 2005). In the oil-in-water emulsions, amino acids residues located in the interface of the oil droplets, such as tryptophan, might be preferentially affected by oxidizing unsaturated dispersed phase (Katsuda, McClements, Miglioranza, & Decker, 2008).

In order to better understand the predominant mechanisms of β-carotene degradation in WPI-stabilized oil-in-water emulsions, this study will examine the impact of factors (pH, iron chelators and free radical scavengers) known to play a role in β-carotene degradation and the relationship between WPI oxidation and β-carotene degradation using Spectrofluorometry. In addition, the physical stability was determined by changes of droplet size and zeta-potential of the β-carotene emulsions after storage. The ultimate goal of this study is to develop a delivery system of oxidatively stable WPI-coated β-carotene emulsion.

2. Materials and methods

2.1. Materials

β-Carotene suspension (30 g/100 g by mass β-carotene in sunflower oil) was purchased from Xinchang Pharmaceutical Co., Ltd. (Shaoxing, Zhejiang, China). Medium-chain triglyceride (MCT) oil was obtained from Lonza Inc. (Allendale, NJ, USA). Whey protein isolate (WPI) was obtained from Davisco Foods International Inc. (Le Sueur, MN, USA). The product contains 97.6 g/100 g protein (dry basis), as determined by the supplier's standard proximate analysis procedures. Standard β-carotene (>95 g/100 g purity), disodium EDTA and α-tocopherol were purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade.

2.2. Preparation of β-carotene emulsion

WPI was dispersed in the 10 mmol/L phosphate buffer at pH 7.0. The pH was then adjusted back to pH 7.0 using 1.0 mol/L NaOH if necessary. The solutions were kept overnight to ensure complete dispersion and dissolution, while sodium azide (0.01 g/100 g) was added to prevent microbial growth. Our previous study showed that 0.5 g/100 g WPI was able to stabilize 5 g/100 g MCT incorporating 0.075 g/100 g β-carotene emulsions at pH 7.0 which had homogenous droplet size distributions. Therefore, β-carotene emulsion (0.5 g/100 g WPI) was prepared with 5 g/100 g MCT oil containing β-carotene (0.075 g/100 g in the final emulsion) as the dispersed phase and 95 g/100 g aqueous phase solution at room temperature. The mixture was then pre-homogenized using an Ultra-Turrax at a speed of 10,000 rpm for 3 min to form coarse emulsions, which were subsequently homogenized using a M110-EH Microfluidizer processor (Microfluidics international Corp., Newton, MA, USA) at the operational pressure of 50 MPa for three times. After preparation, the pH was adjusted to appropriate pH using 1.0 mol/L HCl or NaOH. Because the original emulsions were too chemically stable, emulsion samples were diluted with buffer solution (10 mmol/L phosphate buffer) to a total oil concentration of 1 g/100 g (0.015 g/100 g β-carotene) and then transferred into screw-capped brown bottles flushed with nitrogen for the chemical analysis.

To evaluate the effect of transition metals and free radical scavengers on the stability of β-carotene in emulsions, EDTA and α-tocopherol were added directly to the diluted emulsions, respectively. EDTA was added to emulsion samples at a final concentration of 200 μmol/L. α-Tocopherol was added at a final concentration of 0.02 and 0.05 g/100 g.

2.3. Determination of droplet size

Droplet size of β-carotene emulsions was determined by dynamic light scattering using a Zetasizer Nano-ZS90 (Malvern Instruments, Worcestershire, UK) at a fixed detector angle of 90°. Emulsions were diluted to a final oil droplet concentration of 0.005 g/100 g with phosphate buffer solution prior to each measurement to minimize multiple scattering effects. Results were described as cumulants mean diameter (size, nm) for droplet size.

2.4. Determination of zeta potential

Zeta potential of β-carotene emulsions was determined using a Zetasizer Nano-ZS90 (Malvern Instruments, Worcestershire, UK). Zeta-potential is determined by measuring the direction and velocity of droplet movement in a well-defined electric field. Emulsions were diluted to a final oil droplet concentration of 0.005 g/100 g with buffer solution prior to each measurement to minimize multiple scattering effects. After loading the samples into the instrument they were equilibrated for about 120 s before droplet charge data was collected over 20 continuous readings.

2.5. Analysis of β-carotene content

Samples were stored at 55 °C to accelerate β-carotene degradation in dark. The β-carotene content was measured during the storage. The content of β-carotene in the emulsion was determined following the method of Yuan et al. (2008). Samples (1 mL) were first extracted with a mixture of 2 mL ethanol and 3 mL of n-hexane. After the mixture was shaken, the hexane phase was removed. The extraction was repeated twice more and the hexane phases were combined. After appropriate dilutions with hexane, the absorbance at 450 nm was measured with a Shimadzu UV-1800 UV–vis spectrophotometer (Shimadzu, Japan). The concentration of β-carotene was obtained by referring to a standard curve of β-carotene prepared under the same condition. The β-carotene content was expressed as relative β-carotene C in percent: $C(t)/C_0$, where $C(t)$ is the β-carotene content after storage for a period t and C_0 is the β-carotene content at the time of preparation.

2.6. WPI oxidation

The oxidation of WPI was evaluated by assessing both the loss of tryptophan fluorescence and the emission of fluorescence by protein oxidation products in emulsions using fluorescence spectroscopy (LS 55 Perkin Elmer luminescence spectrometer, Shelton, USA) (Heinonen et al., 1998).

β-Carotene emulsion samples (0.1 g/100 g WPI) after different storage was dispensed in a quartz spectrofluorometer cell. Emission spectra of tryptophan were recorded from 300 to 400 nm with the excitation wavelength established at 283 nm. Emission spectra of WPI oxidation products were recorded from 490 to 600 nm with the excitation wavelength set at 390 nm.