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Clove Oil Protects β-Carotene in Oil-in-Water Emulsion against Photodegradation

Yi-Ming Zhou [1], Hui-Ting Chang [1], Jian-Ping Zhang [1,*] and Leif H. Skibsted [2,*]

[1] Department of Chemistry, Renmin University of China, Beijing 100872, China; zym1990@ruc.edu.cn (Y.-M.Z.); htchang1991@163.com (H.-T.C.)
[2] Department of Food Science, University of Copenhagen, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark
* Correspondence: jpzhang@ruc.edu.cn (J.-P.Z.); ls@food.ku.dk (L.H.S.); Tel.: +86-10-62516604 (J.-P.Z.); +45-3533-3221 (L.H.S.); Fax: +86-10-62516444 (J.-P.Z.)

Abstract: β-Carotene degrades rapidly in a 2% oil-in-water emulsion, made from food-grade soy oil with 7.4 mg β-carotene/mL oil, during storage and when exposed to light. Added clove oil (2.0, 4.0, or 8.0 μL/mL of emulsion) protects against the photodegradation of β-carotene, regardless of the ratio between clove oil and β-carotene in the concentration range studied, suggesting that the regeneration of β-carotene is caused by eugenol, the principal plant phenol of clove oil to occur in the oil-water interface. Therefore, clove oil in low concentrations may find use as a natural protectant of provitamin A in enriched foods during retail display.

Keywords: eugenol; photoprotection; provitamin A

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1. Introduction

While β-Carotene (β-Car) is an important provitamin A, it is sensitive to light and degrades rapidly in plant oils or in plant oil emulsions during storage under ambient conditions [1–4]. Vitamin A deficiency is a major challenge worldwide, especially for children's nutrition, and urgently calls for practical solutions [5,6].

Recently, it was found that plant phenols regenerate β-Car and other carotenoids (Car) from their initial photooxidation product, the carotenoid radical cations ($Car^{\bullet+}$), through electron transfer from the reducing phenol group φ-OH [7]:

$$Car^{\bullet+} + \varphi\text{-OH} \rightarrow Car + \varphi\text{-}O^{\bullet} + H^{+} \quad (1)$$

The regeneration of β-Car corresponding to the reaction of Equation (1) was surprisingly found to be the most efficient for moderately reducing plant phenols, such as eugenol, while strongly reducing plant phenols, like tea catechins, showed no regeneration of β-Car, but displayed enhanced photobleaching [7–9].

Eugenol and isoeugenol, the main constituents of clove oil [10], are moderately reducing plant phenols that have been found to regenerate β-Car efficiently from the radical cation formed by photolysis of β-Car. This reduction occurs in alkaline chloroform/methanol as an electron-withdrawing solvent [11]. The ordering of the anions of the plant phenols according to the rate of regeneration of carotenoids could further be accounted for by the Marcus theory of electron transfer [12]. According to this theory, the maximal rate of electron transfer corresponds to a driving force matching the reorganization energy in the transition state for electron transfer. Notably, for a larger driving force, the rate of electron transfer enters the so-called inverted region with a higher activation barrier, and accordingly, lower rates are seen for quercetin and tea catechins [7,12].

The more practical aspects of the Marcus theory for electron transfer have not yet been exploited in relation to food preservation. However, the protection of β-Car, as a provitamin A in an oil-in-water emulsion in a functional food, could provide a proof of

concept, where the use of Marcus theory could be moved from model systems involving chlorinated solvents of high pH into a real food system. Accordingly, clove oil, with high content of moderately reducing plant phenols and its worldwide use in food and beverages, was combined with β-Car in an oil-in-water emulsion and stored under illumination in ambient conditions with the objective of protecting provitamin A against degradation during retail display. The present study aimed to explore whether the Marcus theory for electron transfer could be used to design optimal protection of a light-sensitive vitamin.

2. Materials and Methods

2.1. Materials

All-*trans*-β-carotene (β-Car) was from Sigma-Aldrich (St. Louis, MO, USA). Clove oil, containing 85% eugenol and isoeugenol, was from O'plants (Shanghai, China). Soybean oil was from Yihai Kerry Food Co., Ltd. (Beijing, China). Whey protein isolates (WPI) were from HIRMAR (Los Angeles, CA, USA). Lecithin (95%) was from Arbor Star Biological Technology Co., Ltd. (Beijing, China).

2.2. Preparation of Emulsion

β-Car (40 mg, accurately weighted) and lecithin (2400 mg) were mixed in 5.4 mL soybean oil and stirred at 1000 rpm for 3 h in the dark to fully dissolve the mixture in the oil phase. The water phase contained 12 g WPI in 280 mL deionized water with the pH of the aqueous solution adjusted to 7.0. This solution was adjusted by dropwise addition of dilute HCl and NaOH, while pH was monitored electrochemically. The emulsion was prepared by mixing the oil phase with the aqueous solution and homogenizing the mixture at 13,000 rpm for 5 min using an FA25 homogenizer (Shanghai, China). Subsequently, 10 mL of emulsion samples were added to glass jars before adding the clove oil (0.02 mL, 0.04 mL, or 0.08 mL) to different samples, which were homogenized at 13,000 rpm for 2 min. In this study, 10 mL emulsion without clove oil served as the control sample. The emulsion had a fat content of approximately 2%, which is comparable to milk and other nutritive beverages. The final concentration of eugenol and isoeugenol from clove oil was 1.7, 3.4, or 6.8 μL/mL emulsion. All samples were stored under light (spectral distribution in the 300−800 nm range, 22,000 Lx warm white similar to light used for illumination during retail display) at 25 °C. Control emulsion samples were stored in the dark at 25 °C. The main experiment, as described in Figures 1 and 2 as well as in Table 1, was in storage for three weeks. The standard deviation of each color measurement was less than 1%.

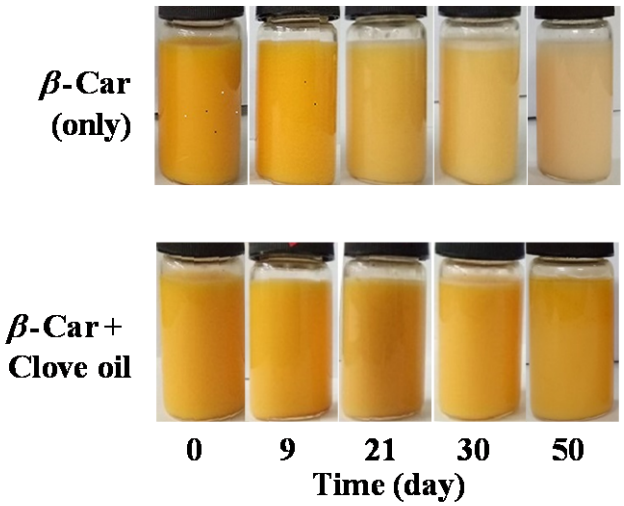

Figure 1. Appearance of oil-in-water with β-Car during storage under ambient conditions and on exposure to light in glass jars with and without addition of 4 μL/mL emulsion of clove oil.

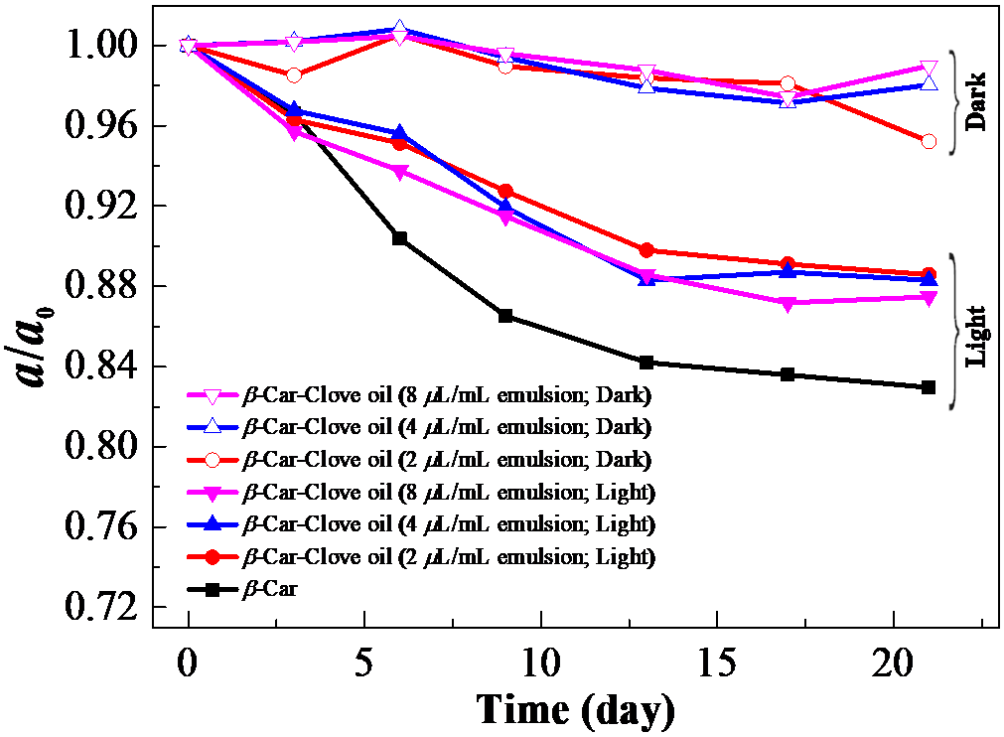

Figure 2. Red bleaching (relative redness a/a_0) of oil-in-water emulsion with β-Car with or without the addition of clove oil during storage in the dark or exposed to light of 22,000 Lx at 25 °C.

Table 1. The redness parameter a of β-Car and β-Car-clove oil on light exposure compared to dark storage for different days. The redness parameter of 0 day is defined as a_0.

Sample \ Day	0	3	6	9	13	17	21
β-Car	24.12	23.31	21.80	20.87	20.31	20.16	20.01
β-Car-clove oil (2 μL/mL emulsion; Light)	24.69	23.78	23.49	22.90	22.17	22.00	21.87
β-Car-clove oil (4 μL/mL emulsion; Light)	24.45	23.66	23.38	22.48	21.59	21.69	21.59
β-Car-clove oil (8 μL/mL emulsion; Light)	23.39	22.39	21.93	22.48	20.72	20.39	20.46
β-Car-clove oil (2 μL/mL emulsion; Dark)	24.27	23.91	24.40	24.02	23.88	23.81	23.11
β-Car-clove oil (4 μL/mL emulsion; Dark)	24.07	24.12	24.27	23.93	23.56	23.38	23.60
β-Car-clove oil (8 μL/mL emulsion; Dark)	22.93	22.97	23.04	22.84	22.65	22.34	22.70

2.3. UV-Visible Absorption Spectroscopy

UV-visible absorption spectra were measured on a Cary50 spectrophotometer (Varian Inc., Palo Alto, CA, USA), using 1.0 cm quartz cells. According to Lambert-Beer's law, the soybean oil acted as a mixed low-polarity solvent for the concentration of β-Car as it relates to the absorbance:

$$c = \frac{A_\lambda}{\varepsilon_\lambda \cdot b} \quad (2)$$

Equation (2) was used for quantification in this study [13]. In Equation (2), c is the molar concentration (mol·L^{-1}) of β-Car. A_λ is the measured absorbance, and ε_λ is the molar extinction coefficient of β-Car in pure soybean oil (1.43×10^5 L·mol^{-1}·cm^{-1}) at the maximal absorption wavelength (λ) of 462 nm. b is the optical pathlength of the cuvette (1 cm).

2.4. Color Measurement

The LAB Hunter values of the emulsion samples were measured multiple times by a PR-780 Spectrophotometer (Photo Research, Los Angeles, CA, USA) during storage for up to 21 days. In the main experiment, photographs of the samples were taken regularly during storage using a digital camera. The light source for color measurement was a tungsten lamp (40 W), and a standard, white tile served as a background.

3. Results and Discussion

β-Car is lipophilic (log$P$ = 12.2) and dissolves in the soy oil of the oil-in-water emulsion. Clove oil consists mainly of eugenol with log$P$ = 2.49 [14,15] which distributes between the oil and the aqueous phase. As evident from Figure 1, the emulsion appeared homogeneously red. The concentration of β-Car in the emulsion oil phase was 7.4 mg/mL soy oil, while the phenols from clove oil were distributed between the two phases. Soy oil was selected for the oil phase of the emulsion as it is edible, with good nutritive value, and is available worldwide. Lecithin with 8.4 mg/mL emulsion was also added because it is commonly used as an emulsifier in foods.

When stored in the dark, the color remained constant, as depicted in Figure 2. In Table 1, a, i.e., the redness parameter of the LAB color system, is shown for 21 days of storage, while a_0 is the redness parameter of day 0. The presence of clove oil did not affect the color during dark storage at any of the three concentrations. This finding was in agreement with the robustness toward uncatalyzed degradation of β-Car which was previously observed [16].

Upon exposure to light, the redness faded, as was evident from visual inspection; see Figure 1. Carotenoids are generally sensitive to radiation, including light and γ-irradiation [17]. The redness parameter a also showed a significant decrease during storage when exposed to light (Table 1 and Figure 2). The presence of clove oil clearly provided protection, as bleaching was reduced to approximately half of that in the emulsion without clove oil. Notably, the protection of color, and accordingly, of β-Car, was not dependent on either the amount of clove oil added or the concentration of the plant phenols in the concentration range studied (clove oil between 2.0 µL/mL and 8.0 µL/mL), due to the saturation of plant phenols at the emulsion interface. The decrease of the redness parameter a could be described by a mono-exponential model function for each of the three independent experiments for which the rate constant was 0.089 days^{-1}, and was not dependent on the clove oil concentration. This type of protection was similar to that of plant phenols toward the carotenoids involved in the visual function [18].

The light source used for the storage experiment had an intensity of 22,000 Lx, was mainly in the visible region, and had a minor UV-component. The glass of the jars further served as a UV-filter. As seen from the absorption spectra of Figure 3, the light was absorbed by β-Car rather than by the clove oil phenols. Excitation of β-Car to the singlet or triplet states generated radical cations, leading to bleaching:

$$\mathrm{Car} + \mathrm{h}\nu \rightarrow {}^1\mathrm{Car}* \quad (3)$$

$${}^1\mathrm{Car}* \rightarrow {}^3\mathrm{Car}* \quad (4)$$

$${}^3\mathrm{Car}*/{}^1\mathrm{Car}* \rightarrow \mathrm{Car}^{\bullet+} + \mathrm{e}^- \text{ (solv.)} \quad (5)$$

$$\mathrm{Car}^{\bullet+} \rightarrow \text{Degradation products} \quad (6)$$

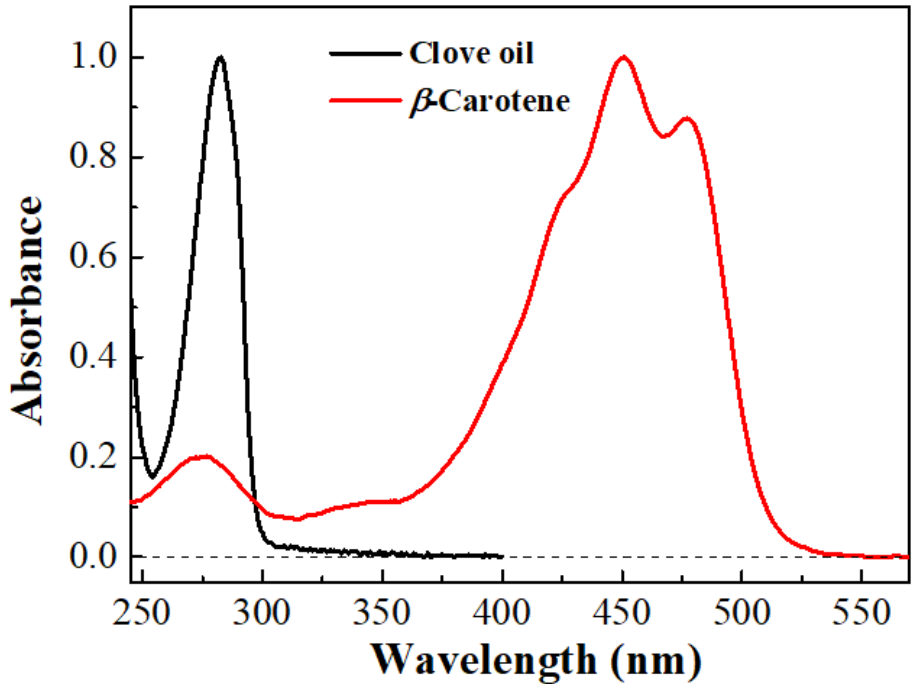

Figure 3. Absorption spectra of β-Car in methanol normalized at 450 nm and of clove oil in methanol normalized at 283 nm.

According to Equations (1) and (6), the regeneration of the carotenoid from radical cations will compete with its degradation. As seen in Figure 2, the bleaching was independent of the concentration of eugenol in the concentration range studied.

The regeneration rate previously found for homogeneous solution [7]:

$$\frac{d[\text{Car}]}{dt} = k_2[Car^+][\varphi\text{–OH}] \quad (7)$$

seems for the present conditions independent of the total phenol concentration in the emulsion. This apparent zero-order dependence on the plant phenol for the emulsion probably indicated: (i) a rapid electron transfer, and (ii) an apparent similar excess of phenol available for reduction under all conditions investigated. These observations pointed toward a mechanism occurring in the emulsion interface that was saturated with the plant phenols. The rate expression of Equation (7) was based on a series of more systematic kinetic studies in homogenous solutions [7–9,11]. The observed kinetics for the photodegradation of β-Car in the oil-in-water emulsion can be accommodated within this theory, including the partition of eugenol between the homogeneous aqueous phase and the heterogeneous oil phase. The distribution between water and oil may be adjusted as eugenol is consumed.

In the oil-in-water emulsion, the protection of β-Car by clove oil is an important finding, since regeneration occurs at neutral pH as compared to the conditions of high pH used in model studies [7–9,11]. The phenols of clove oil and not only their anions are sufficiently reducing for the donation of an electron, and have matching reduction potential according to Marcus' theory to reduce the carotenoid radical cation [12]. Isoeugenol and especially eugenol may be unique in this respect; nevertheless, other plant oils and plant phenols with similar, moderate reduction potentials are now being investigated for their ability to protect carotenoids against light degradation in food.

Designing functional foods with better shelf life is encouraging, as there is a serious problem with vitamin A deficiency worldwide [19]. Moreover, the use of plant oils will provide such products with a natural image of sustainability. The practical application of these findings still needs further development, but in light of the simple procedures required, the perspective seems encouraging, especially for developing countries.

4. Conclusions

Our results show that clove oil protects β-carotene in an oil-in-water emulsion from photodegradation due to the content of moderately reducing plant phenols. It serves as a proof of concept for the use of the Marcus theory for electron transfer as a strategy for the protection of vitamin A and provitamin A compounds, thus addressing a global problem.

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