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Effects of heating, aerial exposure and illumination on stability of fucoxanthin in canola oil

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ABSTRACT

The effects of heating, aerial exposure and illumination on the stability of fucoxanthin was investigated in canola oil. In the absence of air and light, the heating caused the degradation of total and all-*trans* fucoxanthin at all tested temperatures between 25 and 100 °C. The increase of heating temperature promoted the formation of 13-*cis* and 13′-*cis* and the degradation of 9′-*cis*. The degradation and formation reactions were found to follow simple first-order kinetics and to be energetically unfavorable, non-spontaneous processes. Arrhenius-type temperature dependence was observed for the degradation of total and all-*trans* fucoxanthin but not for the reactions of *cis* isomers. The aerial exposure promoted the oxidative fucoxanthin degradation at 25 °C, whilst illumination caused the initial formation of all-*trans*, with concurrent sudden degradation of 13-*cis* and 13′-*cis*, and the considerable formation of 9′-*cis*. The fucoxanthin degradation was synergistically promoted when exposed to both air and light.

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1. Introduction

Fucoxanthin, a marine carotenoid belonging to the xanthophyll class, is abundantly found not only in macroalgae but also in microalgae, and is known to possess diverse health-promoting properties, such as antioxidant, anticancer, anti-inflammatory, antiobesity and antidiabetic activities (Heo et al., 2010; Hosokawa, Okada, Mikami, Konishi, & Miyashita, 2009; Yan, Chuda, Suzuki, & Nagata, 1999). These remarkable biological activities may be attributed to its unique molecular structure, in which an unusual allenic bond, a 5,6-monoepoxide and 9 conjugated double bounds are present (Hosokawa et al., 2009; Yan et al., 1999). Since such functional groups are highly susceptible to oxidation and isomerization, fucoxanthin, like other carotenoids, would be prone to degradation during processing and storage as a result of exposure to heat, light, oxygen, metals, enzymes, unsaturated lipids and other pro-oxidant molecules (Achir, Randrianatoandro, Bohuon, Laffargue, & Avallone, 2010). A spectrophotometric analysis by Hii, Choong, Woo and Wong (2010) showed that fucoxanthin was highly unstable when exposed to light and acidic pH, however, to the best of our knowledge, this is the only published study regarding the stability of fucoxanthin.

Thermal processing is one of the most common and effective food preservation techniques and temperature is a key factor influencing the quality of food during storage. Therefore, the thermal stability of carotenoids has been extensively investigated in different types of medium in the presence or absence of air or light, mostly along with the analysis of degradation kinetics, in order to predict their loss during processing and storage. Examples include, β-carotene and lutein in palm olein and Vegetaline® (Achir et al., 2010), lycopene, lutein and β-carotene in safflower seed oil (Henry, Catignani, & Schwartz, 1998), lutein, β-carotene and β-cryptoxanthin in virgin olive oils (Aparicio-Ruiz, Mínguez-Mosquera, & Gandul-Rojas, 2011), lycopene in an olive oil/tomato emulsion (Colle et al., 2010), lycopene in oil-in-water emulsions (Ax, Mayer-Miebach, Link, Schuchmann, & Schubert, 2003), β-carotene in organic solvents (Chen & Huang, 1998), lycopene in hexane (Lee & Chen, 2002), all-*trans* retinol mixed with microcrystalline cellulose (Manan, Baines, Stone, & Ryley, 1995), lycopene in tomato pulp (Sharma & Le Maguer, 1996), bixin in a water/ethanol solution (Rios, Borsarelli, & Mercadante, 2005) and β-carotene and β-cryptoxanthin in an apple juice (Zepka, Borsarelli, Azevedo, Da Silva, & Mercadante, 2009). The first-order kinetic model and the Arrhenius relationship have been widely adopted to describe the thermal degradation of carotenoids and their temperature dependence, respectively, regardless of the presence of air or light. Heating in the presence or absence of air or light is known to induce not only the degradation of all-*trans*, *cis* and total (all-*trans* plus *cis*) carotenoids via oxidation or photodegradation, but also the formation of some *cis* isomers by isomerization, such as 9-*cis*, 13-*cis*, or 13, 15-di-*cis* forms, depending on treatment conditions, medium and type of carotenoids (Aman, Schieber, & Carle, 2005; Ax et al., 2003; Chen & Huang, 1998; Henry et al., 1998; Lee & Chen,

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2002; Manan et al., 1995; Pesek & Warthesen, 1990; Rios et al., 2005; Sharma & Le Maguer, 1996; Shi, Le Maguer, Bryan, & Kakuda, 2003). However, the information on the stability of individual fucoxanthin isomers, influenced by heat, air and light, and on the corresponding degradation kinetics is rarely available.

The objective of this study was to investigate the effects of heating, aerial exposure and illumination on the stability of fucoxanthin, purified from a brown algae, in canola oil. The all-*trans* isomer and also three *cis* isomers of fucoxanthin were identified and monitored. Firstly, the kinetics and thermodynamics of the degradation or formation of fucoxanthin isomers were analysed in a temperature range of 25–100 °C in the absence of air and light. Then, the influence of aerial exposure and illumination (300 and 2000 lux) on the kinetics and the concentration-time profiles of fucoxanthin isomers was examined at 25 °C. Canola oil was used as a model solvent phase for fucoxanthin, because the food oil was found to improve the human bioavailability of carotenoids, such as α-carotene, β-carotene and lycopene (Brown et al., 2004).

2. Materials and methods

2.1. Materials

Fucoxanthin ($C_{42}H_{58}O_6$, purity = 95%) was purified from a brown algae, *Costaria costata*, by a centrifugal partition chromatography according to Kim, Shang and Um (2011). Canola oil and butylated hydroxytoluene (BHT) were produced from CJ Co. Ltd. (Seoul, Korea) and Sigma Chemical Co. (St. Louis, MO), respectively. All the solvents used, including water, methyl *tert*-butyl ether (MTBE), acetonitrile and methanol, were of analytical grade from Daejung Co. Ltd. (Busan, Korea).

2.2. Thermal treatment

The fucoxanthin purified (4.4 mg) was added separately into each of the 50-ml glass vials containing 20 g of degassed canola oil at room temperature (fucoxanthin concentration = 200 mg/l, due to the specific oil density = 0.91). The vials were flushed with nitrogen, gas-tightly sealed and then sonicated 6 times (3 min sonication followed by 1 min vortex with a 20 min interval) in a bath sonicator (UC-02, Jeio Tech Co. Ltd., Seoul, Korea; 70 W, 40 kHz) at room temperature in the dark. The vials were wrapped with aluminium foil and incubated at five different temperatures (25, 37, 60, 80 and 100 °C) in the dark. Incubation at 100 °C was conducted in an oil bath, whereas the other incubations were performed in a water bath. The time required to raise the temperature from ambient to 60, 80 or 100 °C was minimised by heating the vials in an oil bath at 150 °C before the isothermal incubation. The incubated vials were sampled periodically, and 0.5 g of the oil mixture in each vial was transferred to 5 ml glass vials in an ice bath. The vials were then flushed with nitrogen, gas-tightly sealed, wrapped with aluminium foil and kept at −20 °C until further analysis.

2.3. Aerial exposure treatment

The sample vials, in which 20 g of canola oil containing 200 mg/l of purified fucoxanthin was sealed, were prepared as described in Section 2.2, wrapped or not wrapped with aluminium foil, cap-opened and incubated in an oven in open air at 25 °C. The incubated vials were sampled periodically, and the oil mixture in each vial was treated as described in Section 2.2.

2.4. Illumination treatment

The sample vials, in which 20 g of canola oil containing 200 mg/l of purified fucoxanthin was sealed, were prepared according to Section 2.2, cap-opened or not opened without any wrapping around the vials, and incubated in the oven in open air at 25 °C under 18 W fluorescent light. Two different illumination intensities, 300 and 2000 lux, were achieved by varying the distance between the sample vials and light source in order to mimic indoor and outdoor illumination conditions, respectively. The illumination intensity was measured with a luxmeter (JT813, Bluebird Hi-tech. Co. Ltd., Shenzhen, China). The incubated vials were sampled periodically and the oil mixture in each vial was treated as described in Section 2.2.

2.5. Fucoxanthin analysis

The fucoxanthin contained in the sampled oil mixtures was extracted as follows. Each of the sampled oil mixtures (0.5 g) was mixed with 2 ml of a methanol–acetonitrile (60:40, v/v) mixture containing 50 mg/l of BHT, vortexed for 15 s and left for 30 min at room temperature in the dark for phase separation. The extraction was repeated three times. The solvent phase was collected in a 15 ml cap tube, placed at −20 °C overnight to crystallize extracted fatty acids, filtered with a 0.2 μm polytetrafluoroethylene membrane filter (Toyo Roshi Kaisha, Ltd., Tokyo, Japan) and injected (10 μl) into an HPLC system (model 1100, Agilent Technologies, Inc., St. Clara, CA) equipped with an autosampler, column oven, diode array detector (DAD) and C_{30} reversed-phase column (4.6 × 250 mm, 3 μm; YMC Co. Ltd., Tokyo, Japan), which was coupled to a mass spectrometer (MS). Two mobile phases were used: 91% (v/v) methanol in water as mobile phase A and MTBE as mobile phase B. The following gradient program was applied: mobile phase A from 100% to 90% in 10 min and to 50% in the next 10 min at a flow rate of 1 ml/min with a column temperature of 30 °C. Fucoxanthin isomers were identified and quantified using the DAD chromatograms obtained at 450 nm in combination with the UV–VIS and MS spectra. The standard mixtures of fucoxanthin, prepared by dispersing known amounts of purified fucoxanthin in canola oil as described above, were treated in the same manner described for the sampled oil mixtures.

2.6. Kinetic analysis

The first-order kinetic model has been widely used to describe the thermal degradation of carotenoids regardless of the presence of air or light (Ax et al., 2003; Chen & Huang, 1998; Henry et al., 1998; Lee & Chen, 2002; Manan et al., 1995; Pesek & Warthesen, 1990; Sharma & Le Maguer, 1996). In addition, in the present study, the concentrations of fucoxanthin isomers were found to reach non-zero equilibrium levels during the thermal treatments in the absence of air and light. Therefore, the following kinetic model was employed to describe the thermal degradation or formation of fucoxanthin isomers in canola oil in the absence of air and light:

$$\frac{dC_i}{dt} = -k(C_i - C_{i,\infty}) \quad (1)$$

where C_i is the concentration of fucoxanthin at any time t (mg/l, i = AT, C, or T for all-*trans*, *cis*, or total (all-*trans* plus *cis*) fucoxanthin, respectively), $C_{i,\infty}$ is the concentration of fucoxanthin at thermodynamic equilibrium (mg/l), k is the reaction rate constant (h^{-1}), and t is the reaction time (h). The integrated form of Eq. (1) is:

$$\frac{C_i - C_{i,\infty}}{C_{i,0} - C_{i,\infty}} = e^{-kt} \quad (2)$$

where $C_{i,0}$ is the concentration of fucoxanthin at $t = 0$ (mg/l). Eq. (2) was fitted to the experimental concentration profiles by

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