Food Chemistry 291 (2019) 87–93

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Effects of temperature, light, and pH on the stability of fucoxanthin in an oil-in-water emulsion

Dong Zhao[a,b,c], Daeung Yu[d], Moojoong Kim[d], Ming-Yao Gu[e], Sang-Min Kim[c,f], Cheol-Ho Pan[c,f], Gun-Hee Kim[g], Donghwa Chung[d,h,i,*]

[a] Department of Marine Food Science and Technology, Gangneung-Wonju National University, Gangneung 25457, Republic of Korea
[b] Natural Products Research Center, KIST Gangneung Institute of Natural Products, Gangneung 25451, Republic of Korea
[c] Department of Biological Chemistry, Korea University of Science and Technology (UST), Daejeon 34113, Republic of Korea
[d] Institute of Food Industrialization, Institutes of Green Bio Science and Technology, Seoul National University, Pyeongchang 25354, Republic of Korea
[e] Department of Cell Biology and Medical Genetics, School of Basic Medical Sciences, Shenzhen University Health Science Center, Shenzhen 51801, China
[f] Systems Biotechnology Research Center, KIST Gangneung Institute of Natural Products, Gangneung 25451, Republic of Korea
[g] Department of Food and Nutrition, Plant Resources Research Institute, Duksung Women's University, Seoul 01369, Republic of Korea
[h] Food Technology Major, Graduate School of International Agricultural Technology, Seoul National University, Pyeongchang 25354, Republic of Korea
[i] Center for Food and Bioconvergence, Seoul National University, Seoul 08826, Republic of Korea

ARTICLE INFO

Keywords:
Fucoxanthin
Oil-in-water emulsion
Stability
Light
pH

ABSTRACT

The effects of temperature, light, and pH on the stability of fucoxanthin in an oil-in-water emulsion were investigated with analyzing the kinetics and thermodynamics of fucoxanthin degradation. In the absence of light and air at pH 4.6, increasing the temperature from 25 to 60 °C significantly promoted fucoxanthin degradation. Total and all-*trans* fucoxanthin demonstrated an energetically unfavorable, non-spontaneous degradation with an Arrhenius temperature dependence. Increasing the light intensity up to 2000 lx at 25 °C and pH 4.6 caused a sharp degradation of total, all-*trans*, 13-*cis*, and 13′-*cis* fucoxanthin, but promoted the formation of the 9′-*cis* isomer. In the absence of light and air at 25 °C, decreasing the pH to 1.2 caused significant fucoxanthin degradation, whereas increasing the pH to 7.4 retarded the degradation. The property with the greatest influence on fucoxanthin stability was pH, followed by temperature and then light. Total and all-*trans* fucoxanthin followed first-order degradation kinetics.

1. Introduction

Fucoxanthin is one of the most abundant carotenoids in nature, and is found primarily in marine macro- and microalgae (Hosokawa, Okada, Mikami, Konishi, & Miyashita, 2009). It has many potential uses due to its medicinal and nutritional properties, such as anticancer, anti-inflammatory, antioxidant, and anti-obesity activities (Fung, Hamid, & Lu, 2013; Kumar, Hosokawa, & Miyashita, 2013; Maeda, Hosokawa, Sashima, Funayama, & Miyashita, 2005; Zhao, Kwon, Chun, Gu, & Yang, 2017). However, the use of fucoxanthin has been greatly limited because it has a highly unsaturated structure, including an allenic bond, 5,6-monoepoxide, and nine conjugated double bonds, which can be easily degraded via oxidation and isomerization in the presence of heat, air, light, strong acids and bases, metals, enzymes, and other pro-oxidant molecules (Achir, Randrianatoandro, Bohuon, Laffargue, & Avallone, 2010; Hosokawa et al., 2009). Moreover, few studies have been conducted to address the stability of fucoxanthin in food models. Hii, Choong, Woo, and Wong (2010) reported that fucoxanthin was highly susceptible to degradation in an acetone/water mixture when treated with light under acidic conditions. Our previous study revealed that, in the absence of air and light, heating (25–100 °C) caused either degradation or formation of fucoxanthin isomers (all-*trans*, 13-*cis*, 13′-*cis*, and 9′-*cis*) in canola oil (Zhao, Kim, Pan, & Chung, 2014). The thermal degradation and formation of fucoxanthin isomers were found to follow first-order kinetics, and to be energetically unfavorable, non-spontaneous reactions. Our study also demonstrated that aerial exposure promoted the oxidative degradation of fucoxanthin isomers, whereas illumination induced isomerization from 13-*cis* and 13′-*cis* to *all-trans*, isomerization from *all-trans* to 9′-*cis*, and photodegradation of the isomers (Zhao et al., 2014).

Many foods, such as milk, mayonnaise, and salad dressing, exist as oil-in-water (o/w) emulsions, and the o/w emulsion has been widely

* Corresponding author at: Food Technology Major, Graduate School of International Agricultural Technology, Seoul National University, Pyeongchang 25354, Republic of Korea.
E-mail address: dchung@snu.ac.kr (D. Chung).

https://doi.org/10.1016/j.foodchem.2019.04.002
Received 18 November 2018; Received in revised form 12 March 2019; Accepted 1 April 2019
Available online 01 April 2019
0308-8146/

used as a convenient, effective vehicle to encapsulate, protect, and deliver lipophilic functional compounds, including carotenoids, into food products with improved bioavailability (Boon, McClements, Weiss, & Decker, 2009; McClements & Li, 2010; Qian, Decker, Xiao, & McClements, 2012). Thus, the stability of carotenoids has been also examined in o/w emulsions. For examples, the stability of β-carotene in o/w emulsions was influenced by pH (3–8), ionic strength (0–500 mM NaCl), temperature (5–55 °C), and emulsifier type (nonionic and protein) (Qian et al., 2012), and the stability of lycopene in o/w emulsions was influenced by light (fluorescent), pH (3–7), iron (ferrous and ferric), antioxidant (α-tocopherol and *tert*-butylhydroquinone), metal scavenger (ethylenediaminetetraacetic acid), emulsifier type (cationic, anionic, and nonionic), and oil type (stripped or non-stripped corn oil and hexadecane) (Boon et al., 2008, 2009; Ribeiro, Ax, & Schubert, 2003). However, there are no comprehensive reports addressing the stability of fucoxanthin in o/w emulsions.

The objective of this study was to investigate the effects of temperature, light, and pH on the stability of fucoxanthin, purified from a brown algae, in an o/w emulsion of canola oil. First, the effect of temperature on fucoxanthin stability was examined within a range of 25–60 °C in the absence of light, air, and pH treatment. Second, the effect of light (at intensities of 300 and 2000 lx) on fucoxanthin stability was examined at 25 °C and pH 4.6 in the absence of air. Last, the effect of pH (1.2, 4.6, or 7.5) on fucoxanthin stability was examined at 25 °C in the absence of light and air. The kinetics and thermodynamics of the degradation of fucoxanthin isomers in the o/w emulsion were also analyzed.

2. Materials and methods

2.1. Materials

Fucoxanthin ($C_{42}H_{58}O_6$, 95% purity) was extracted from *Costaria costata*, a brown algae, using centrifugal partition chromatography according to the method of Kim, Shang, and Um (2011). Canola oil was obtained from CJ Co., Ltd. (Seoul, Korea). HPLC analytical grade solvents, including water, methyl *tert*-butyl ether (MTBE), acetonitrile (ACN), and methanol (MeOH), were obtained from Daejung Co., Ltd (Busan, Korea). Sodium hydroxide (NaOH), hydrochloric acid (HCl), Tween 80, and butylated hydroxytoluene (BHT) were purchased from Sigma-Aldrich (St. Louis, Mo, USA).

2.2. Preparation of emulsion

The purified fucoxanthin was dissolved in degassed canola oil at a concentration of 200 mg/L with nitrogen flushing and six repetitions of sonication (3 min sonication followed by 1 min vortex, at 20 min intervals) at room temperature. Deionized water containing 0.2% (w/w) Tween 80 was prepared separately. Batches of o/w emulsion were prepared by adding 2 g of the fucoxanthin-containing canola oil into vials filled with 38 g of the Tween 80-containing water in an ice bath, followed by homogenization using a high-intensity ultrasonic processor (VCX 750, Sonics & Materials Inc., Newtown, CT, USA) with 4200 J strength and 30% amplitude for 5 min. The sample vials, which contained 40 g of the fucoxanthin-loaded o/w emulsion, were flushed with nitrogen and sealed gas-tight. The pH of the fucoxanthin-loaded o/w emulsion was 4.6.

2.3. Thermal treatment

Sample vials containing 40 g of the fucoxanthin-loaded o/w emulsion (pH 4.6) were prepared as described above, wrapped with aluminum foil, and incubated at three different temperatures (25, 37, or 60 °C) in a water bath in the dark. The incubated vials were periodically sampled by transferring 1 mL of the emulsion from each vial to a 1.5 mL centrifugal tube, flushing with nitrogen, sealing gas-tight, and storing at −20 °C until further analysis.

2.4. Light treatment

Sample vials containing 40 g of the fucoxanthin-loaded o/w emulsion (pH 4.6) were prepared as described above and incubated in a chamber at 25 °C under fluorescent light (18 W). The sample vials were separately exposed to two light intensities, 300 or 2000 lx, to simulate indoor and outdoor illumination conditions, respectively. The light intensity was controlled by adjusting the distance between the sample vials and light source. The light intensity was monitored using a luxmeter (JT813, Bluebird Hi-tech. Co., Ltd., Shenzhen, China). The incubated vials were periodically sampled by transferring 1 mL of the emulsion from each vial to a 1.5 mL centrifugal tube, flushing with nitrogen, sealing gas-tight, and storing at −20 °C until further analysis.

2.5. pH treatment

The pH of the fucoxanthin-loaded o/w emulsion prepared as described above was initially 4.6, and further adjusted to 1.2 or 7.5 in the dark at 25 °C using 1 M HCl and NaOH, respectively. The sample vials were wrapped with aluminum foil and incubated at 25 °C in the dark. The incubated vials were periodically sampled by transferring 1 mL of the emulsion from each vial into a 1.5 mL centrifugal tube, flushing with nitrogen, sealing gas-tight, and storing at −20 °C until further analysis. In the experiment at pH 1.2, 10 μL of 1 M NaOH was added into each sampled emulsion in the centrifuge tube immediately after sampling.

2.6. Droplet size measurement

The volume-weighted mean diameter ($d_{4,3}$; nm) of the oil droplets in the emulsions was determined immediately after the preparation and treatments of the emulsions using a laser diffraction particle size analyzer (Mastersizer 2000, Malvern Instruments, Worcestershire, UK). The span of droplet size distribution was calculated as follows:

$$\text{Span} = \frac{D_{0.9} - D_{0.1}}{D_{0.5}} \quad (1)$$

where $D_{0.1}$, $D_{0.5}$, and $D_{0.9}$ are the diameters of oil droplets at 10%, 50%, and 90% of the cumulative volume in droplet size distribution, respectively. An average of triplicates was calculated.

2.7. Fucoxanthin analysis

The fucoxanthin contained in the sampled emulsions was extracted as described below and analyzed immediately after extraction. Each sampled emulsion was centrifuged for 10 min at 11000 × *g*, and the water phase was transferred to a 5 mL vial. The oil phase was mixed with 1 mL of an MeOH-ACN (60:40, v/v) mixture containing 50 mg/L BHT, vortexed for 15 s, and incubated for 30 min at room temperature in the dark. This extraction was repeated two times. The solvent phase was collected, mixed with the water phase in a 5 mL capped tube, and stored at −20 °C overnight to crystallize the extracted fatty acids, followed by filtration with a 0.2 μm polytetrafluoroethylene membrane filter (Advantec, Tokyo, Japan). The concentrations of all-*trans* and three *cis* fucoxanthin (13-*cis*, 13′-*cis*, and 9′-*cis*) in the oil phase were determined as described in our previous study (Zhao et al., 2014). Briefly, the filtrate (10 μL) was injected into a high performance liquid chromatography-mass spectrometry (HPLC-MS) system (model 1100; Agilent Technologies, St. Clara, CA, USA) equipped with a diode array detector (DAD) and C_{30} reversed-phase column (4.6 × 250 mm, 3 μm; YMC Co. Ltd., Tokyo, Japan). The following gradient program was applied for the fucoxanthin analysis: mobile phase A (91% (v/v) MeOH in water) from 100 to 90% in 10 min, and to 50% in the next 10 min with MTBE as mobile phase B at a flow rate of 1 mL/min and a column

temperature of 30 °C. The *trans* and *cis* isomers in the purified fucoxanthin were identified and quantified using the DAD chromatograms at 450 nm in combination with ultraviolet-visible and mass spectra, as described in our previous study (Zhao et al., 2014). The standard fucoxanthin-loaded o/w emulsions were prepared by dissolving known amounts (10–200 mg/L) of purified fucoxanthin in canola oil, as described above. These standard emulsions were treated according to the same procedure described for the sampled emulsions.

2.8. Kinetic analysis

The degradation of total and all-*trans* fucoxanthin in the emulsions was analyzed using the following first-order kinetic model:

$$\frac{dC_i}{dt} = -kC_i \tag{2}$$

where C_i is the concentration of fucoxanthin at any time t (mg fucoxanthin/L of oil, i = AT, C, or T for all-*trans*, *cis*, or total (all-*trans* plus *cis*) fucoxanthin, respectively) and k is the reaction rate constant (h^{-1}). The integration of Eq. (2) yields:

$$C_i = C_{i,0} \cdot e^{-kt} \tag{3}$$

where $C_{i,0}$ is the initial concentration of fucoxanthin at time zero (mg fucoxanthin/L of oil). The k values were determined by nonlinear regression of the experimental concentration-time profiles. The coefficient of determination (R^2) and the mean relative percentage deviation modulus (E) were calculated to evaluate the goodness of the fit of Eq. (3):

$$E(\%) = \frac{100}{N}\sum_{i=1}^{N}\frac{|C_{i,exp} - C_{i,pred}|}{C_{i,exp}} \tag{4}$$

where $C_{i,exp}$ is the experimentally obtained fucoxanthin concentration, $C_{i,pred}$ is the predicted fucoxanthin concentration, and N is the population of experimental data. Generally, an E value below 10% indicates a good fit (Zhao et al., 2014).

2.9. Thermodynamic analysis

The thermodynamic nature of the degradation of total and all-*trans* fucoxanthin in the emulsions was examined by analyzing the temperature dependence of k. Assuming its temperature-independence, the activation energy (E_a) was determined using the following Arrhenius equation:

$$\ln k = \ln k_0 - \frac{E_a}{RT} \tag{5}$$

where k_0 is the pre-exponential factor, R is the universal gas constant (8.314 J/mol K), and T is the absolute temperature (K). The E_a values were obtained from the slopes of the regression lines in the plots of ln k versus $1/T$. Assuming their temperature-independence, the enthalpy ($\Delta H^{\ddagger}$) and entropy ($\Delta S^{\ddagger}$) of activation were determined using the following equation derived from the Eyring equation in transition state theory (Atkins & de Paula, 2006; Zhao et al., 2014):

$$\ln\left(\frac{k}{T}\right) = \ln\frac{k_B}{h} + \frac{\Delta S^{\ddagger}}{R} - \frac{\Delta H^{\ddagger}}{RT} \tag{6}$$

where k_B is the Boltzmann constant (1.381×10^{-23} J/K) and h is the Planck's constant (6.626×10^{-34} J s). The values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ were estimated from the slopes and y-intercepts of the regression lines in the plots of ln (k/T) versus $1/T$, respectively. The Gibbs energy of activation ($\Delta G^{\ddagger}$) at each incubation temperature examined was estimated using the following relationship:

$$\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger} \tag{7}$$

2.10. Statistical analysis

All of the experiments were conducted at least in triplicate. Data are reported as means ± standard deviation. Regression analyses were carried out using SigmaPlot software (ver. 10.0; SPSS Inc., Chicago, IL, USA). Statistical comparisons were performed by analysis of variance (ANOVA) with Duncan's multiple range tests, at a 0.05 confidence level using SPSS software (ver. 15.0; SPSS Inc.).

3. Results and discussion

3.1. Emulsion stability

The volume-weighted mean diameter ($d_{4,3}$) of the oil droplets in the emulsions did not significantly change during all of the treatments performed in the present study. Fig. S1 shows an example droplet size distribution, measured at pH 4.6 and 25 °C, where the size distribution maintained almost the same during a 100-day storage period. The $d_{4,3}$ and span values were 2.12–2.17 μm and 1.24–1.30, respectively. The results indicate that the fucoxanthin-containing o/w emulsion remained stable during the treatments.

3.2. Effects of temperature

Fig. 1 shows the changes in concentrations of the all-*trans* isomer (C_{AT}), three *cis* isomers (C_C for 13-*cis*, 13′-*cis*, and 9′-*cis*), and total fucoxanthin (C_T) in the o/w emulsion (pH 4.6) incubated at 25, 37, or 60 °C in the absence of light and air. The initial concentrations of fucoxanthin isomers in the oil phase were as follows: all-*trans* (162.7 mg/L), 13-*cis* (17.5 mg/L), 13′-*cis* (10.1 mg/L), and 9′-*cis* (5.3 mg/L). The C_{AT}, C_C for 13-*cis* and 13′-*cis*, and C_T demonstrated rapidly decreasing trends during the treatments, whereas 9′-*cis* demonstrated a relatively stable concentration profile.

The C_T and C_{AT} continuously decreased with time at all temperatures tested, indicating the degradation of total and all-*trans* fucoxanthin due to oxidation and isomerization reactions. The fucoxanthin dissolved in canola oil also showed decreasing trends of C_T and C_{AT} when treated at the same temperatures (Zhao et al., 2014). The decreases in C_T and C_{AT} were much faster and greater in the o/w emulsion prepared in the present study than those reported previously for fucoxanthin in canola oil (Zhao et al., 2014) (see the kinetic analysis in the next section for a quantitative comparison). This is likely due to the availability of a higher amount of oxygen in water than in canola oil, resulting in more significant oxidative degradation of fucoxanthin in the emulsion than in canola oil (Chen, Shi, Xue, & Ma, 2009).

The C_C for 13-*cis* and 13′-*cis* also demonstrated a sharply decreasing trend at all temperatures tested (Fig. 1); the two *cis* isomers were completely degraded after 5 and 4 days, respectively, at 60 °C (Fig. 1C). This indicates that the two *cis* isomers underwent oxidative degradation rather than the formation by isomerization. Zhao et al. (2014) reported that, for fucoxanthin in canola oil, the C_C for 13-*cis* and 13′-*cis* also decreased with time at 25 °C, but increased at 37 °C and showed a faster and greater increase at 60 °C. This is because the isomerization of all-*trans* to 13-*cis* and 13′-*cis* was enhanced at increased temperatures, resulting in the increased formation of the two *cis* isomers in the heated canola oil. In the o/w emulsion prepared in the present study, the formation of 13-*cis* and 13′-*cis* may have been also enhanced as the temperature increased, but this formation was not apparent due to the greater effects of oxidative degradation.

The C_C for 9′-*cis* remained relatively stable during the treatment periods (Fig. 1), indicating that the 9′-*cis* is more resistant to oxidative degradation than the other two *cis* isomers. More stable concentration profiles were reported for the 9′-*cis* in canola oil due to the greatly reduced amount of oxygen in the oil (Zhao et al., 2014).

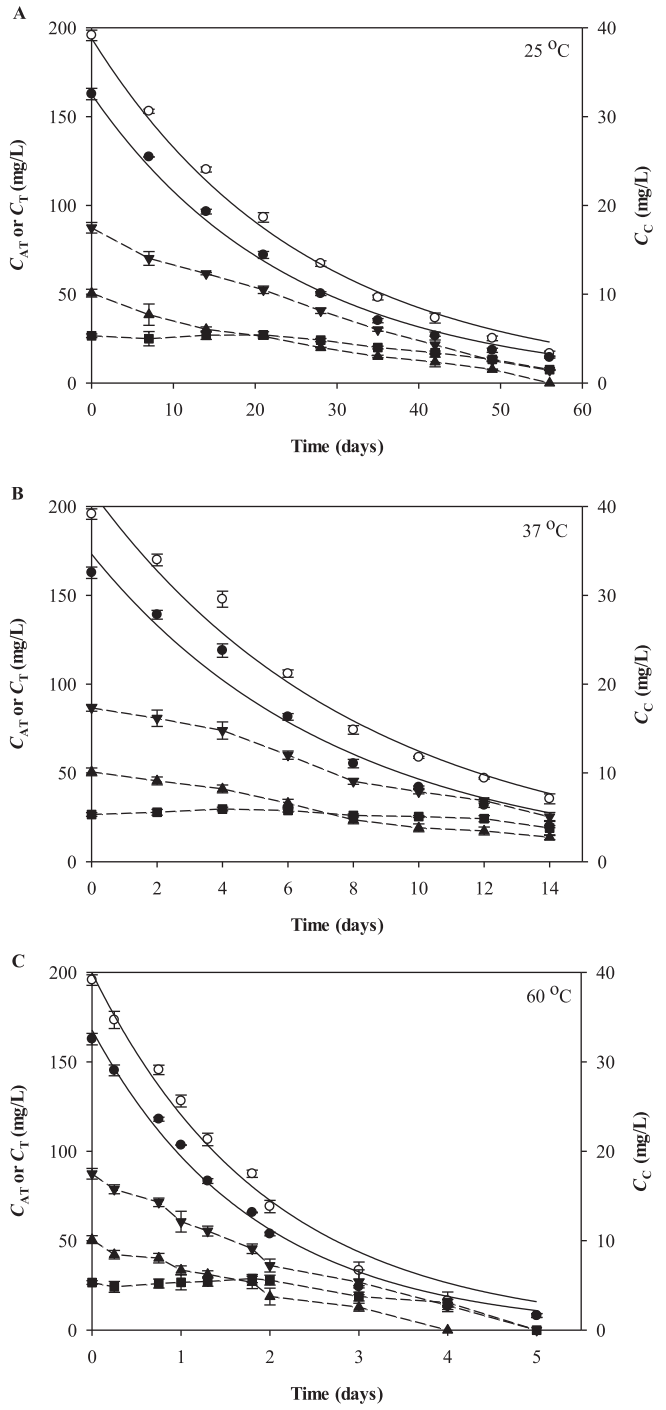

Fig. 1. Changes in the concentrations of all-*trans* isomer (C_{AT}), three *cis* isomers (C_C), and total fucoxanthin (C_T) in o/w emulsions (no pH treatment; pH 4.6) at (A) 25 °C, (B) 37 °C, and (C) 60 °C in the absence of light and air: all-*trans* (●), 13-*cis* (▼), 13′-*cis* (▲), 9′-*cis* (■), and total fucoxanthin (○). Solid lines are simulated results obtained from Eq. (3).

3.3. Kinetics for thermal degradation of total and all-trans fucoxanthin

The fit of Eq. (3) to the C_T and C_{AT} profiles in Fig. 1 exhibited high R^2 values ($R^2 \geq 0.977$) and acceptably low E values ($E \leq 11.05$) at all tested temperatures (Table S1). This indicates that the degradation of total and all-*trans* fucoxanthin in the emulsion (pH 4.6) followed first-order kinetics in the absence of light and air. However, the changes in the C_C revealed for the three *cis* isomers were not adequately described by first-order kinetics.

As the temperature increased from 25 to 60 °C, the degradation rate constant (k) sharply increased from 0.0017 to 0.0210 h^{-1} (a 12-fold increase) for total fucoxanthin, and from 0.0017 to 0.0228 h^{-1} (a 13-fold increase) for the all-*trans* isomer. This indicates that the degradation of total and all-*trans* fucoxanthin in the emulsion was greatly accelerated with an increase in temperature in the absence of light and air. The k values reported for total and all-*trans* fucoxanthin dissolved in canola oil also dramatically increased as the temperature increased 25 to 60 °C in the absence of light and air, from 0.00029 to 0.0035 h^{-1} and from 0.00014 to 0.0073 h^{-1}, respectively (Zhao et al., 2014). The k values were much larger in the emulsion than in canola oil (3–12 times larger depending on the temperature), indicating that oxidative degradation was much stronger in the emulsion due to the presence of a higher amount of available oxygen in water than in canola oil, as discussed above.

3.4. Thermodynamics for thermal degradation of total and all-trans fucoxanthin

A clear negative linear relationship ($R^2 \geq 0.989$) was found between ln k and $1/T$ for the degradation of total and all-*trans* fucoxanthin in the emulsion (pH 4.6) in the absence of light and air (Fig. S2), and thus the activation energy (E_a) for the degradation was determined using Eq. (5) (Table 1). The relationship between ln (k/T) and $1/T$ was also found to be linear ($R^2 \geq 0.989$), and therefore the enthalpy ($\Delta H^{\ddagger}$) and entropy ($\Delta S^{\ddagger}$) of activation of the degradation were estimated using Eq. (6) (Table 1).

The E_a was determined to be 188.13 kJ/mol for total fucoxanthin and 190.84 kJ/mol for the all-*trans* isomer (Table 1). These E_a values were about two times larger than those reported for the total fucoxanthin (83.35 kJ/mol) and all-*trans* isomer (98.93 kJ/mol) in canola oil at a temperature range of 25–100 °C (Zhao et al., 2014). These values were also much larger than the E_a values reported for other carotenoids dissolved in food oils; the value reported for all-*trans* β-carotene in safflower seed oil at 75–95 °C in the dark with air is 109.5 kJ/mol (Henry, Catignani, & Schwartz, 1998) and that for all-*trans* lutein in commercial virgin olive oils at 60–120 °C in the absence of light and air is about 71 kJ/mol (Aparicio-Ruiz, Mínguez-Mosquera, & Gandul-Rojas, 2011). The results indicate that the total and all-*trans* fucoxanthin in the emulsion degraded with a greater temperature dependence than those in canola oil and other reported carotenoids in food oils.

The $\Delta H^{\ddagger}$ was estimated to be 56.07 kJ/mol for total fucoxanthin and 57.75 kJ/mol for all-*trans* isomer (Table 1). The positive $\Delta H^{\ddagger}$ value implies that the transition from the initial to the activated molecular states is endothermic and energetically unfavorable. The $\Delta H^{\ddagger}$ values obtained in the present study were about 60–70% of those reported for total and all-*trans* fucoxanthin in canola oil (80.59 and 96.19 kJ/mol, respectively) (Zhao et al., 2014). This indicates that the molecular transition to the activated states for fucoxanthin degradation was more favored in the emulsion than in canola oil.

Table 1
Thermodynamic functions estimated for the degradation of total and all-*trans* fucoxanthin in o/w emulsions (pH 4.6) at three temperatures in the absence of light and air.

	T (°C)	E_a (kJ/mol)	$\Delta G^{\ddagger}$(kJ/mol)	$\Delta H^{\ddagger}$(kJ/mol)	$\Delta S^{\ddagger}$(kJ/mol K)
Total fucoxanthin (C_T)	25	188.13 (0.991)	108.97	56.07 (0.995)	−0.177
	37		111.10		
	60		115.18		
All-*trans* (C_{AT})	25	190.84 (0.989)	108.90	57.75 (0.989)	−0.172
	37		110.96		
	60		114.91		

Values in parentheses represent R^2 of regression curves in the plot of ln k versus $1/T$.

The $\Delta S^{\ddagger}$ was determined to be −0.177 kJ/mol K for total fucoxanthin and −0.172 kJ/mol K for the all-*trans* isomer (Table 1). The negative $\Delta S^{\ddagger}$ value indicates that the molecular transition to the activated states is an entropically unfavorable process, where overall molecular disorder decreased during the transition, likely due to the formation of some activated complexes (Atkins & de Paula, 2006; Zhao et al., 2014). The $\Delta S^{\ddagger}$ values in the present study were more negative than those reported for total and all-*trans* fucoxanthin in canola oil (−0.044 and 0.007 kJ/mol K, respectively) (Zhao et al., 2014). This indicates that total and all-*trans* fucoxanthin in the emulsion, compared to those in canola oil, formed more structured activated complexes for faster thermal degradation reactions.

The Gibbs energy of activation ($\Delta G^{\ddagger}$) for the degradation, obtain from Eq. (7), was in the range of 108.90–115.18 kJ/mol, demonstrating that there was no considerable temperature dependence (Table 1). The positive $\Delta G^{\ddagger}$value indicates a non-spontaneous degradation of total and all-*trans* fucoxanthin in the emulsion. The $\Delta G^{\ddagger}$values in the emulsion were slightly larger than those reported for total and all-*trans* fucoxanthin in canola oil (93.68–96.97 kJ/mol), although relatively large differences were found in the $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values (Zhao et al., 2014). This indicates that the activated states of the molecules in the emulsion, compared to those in canola oil, had more free energy that resulted in faster and greater degradation.

3.5. Effects of light

Fig. 2 shows that all-*trans*, 13-*cis*, 13′-*cis*, and total fucoxanthin also degraded with time in the emulsion (pH 4.6) exposed to a light intensity of 300 or 2000 lx in the absence of air at 25 °C, as was observed in the absence of light (Fig. 1A). However, the profile of the C_C for 9′-*cis* was distinctly different when under illumination; the concentration sharply increased during the early stage of treatment, indicating the formation of 9′-*cis* (Fig. 2), which was not observed under dark conditions (Fig. 1A).

The decreasing profiles exhibited in the C_T and C_{AT} in Fig. 2 were well-fitted by Eq. (3), with high R^2 values ($R^2 \geq 0.982$) and acceptably low E values ($E \leq 13.39$), indicating that the degradation of total and all-*trans* fucoxanthin in the light-exposed emulsion was also consistent with first-order kinetics. The k values for total and all-*trans* fucoxanthin increased from 0.0017 to 0.0028 h^{-1} and from 0.0017 to 0.0029 h^{-1} as the light intensity increased from 0 to 2000 lx (Table 2). The results indicate that the illumination accelerated the degradation of total and all-*trans* fucoxanthin in the emulsion via photodegradation and photoisomerization. A similar illumination effect was also reported for fucoxanthin in canola oil (Zhao et al., 2014). However, the degradation of total and all-*trans* fucoxanthin in canola oil did not follow first-order kinetics, and was much weaker than the degradation observed in the present study. It is notable that the k values determined at 25 °C under a light intensity of 2000 lx were only about 53–56% of those determined at 37 °C in the dark (k = 0.0050 and 0.0055 h^{-1} for total and all-*trans* fucoxanthin, respectively; Table S1). This indicates that the degradation of total and all-*trans* fucoxanthin was more influenced by temperature than light.

The C_C for 13-*cis* and 13′-*cis* demonstrated larger decreases with increasing light intensity (Fig. 2), indicating that illumination induced the photodegradation of 13-*cis* and 13′-*cis*. Zhao et al. (2014) also reported a decrease in the C_C for13-*cis* and 13′-*cis* in canola oil during illumination. The authors suggested that the decrease was more likely due to the photoisomerization of the two *cis* isomers to the all-*trans* isomer rather than to their photodegradation, because the decrease in the C_C for 13-*cis* and 13′-*cis* coincided with the formation of the all-*trans* isomer, and the decrease was not significantly dependent on the light intensity. This photoisomerization from the two *cis* isomers to the all-*trans* isomer may have also occurred in the emulsion prepared in the present study, although it was not clearly observed due to the strong photodegradation of the all-*trans* isomer.

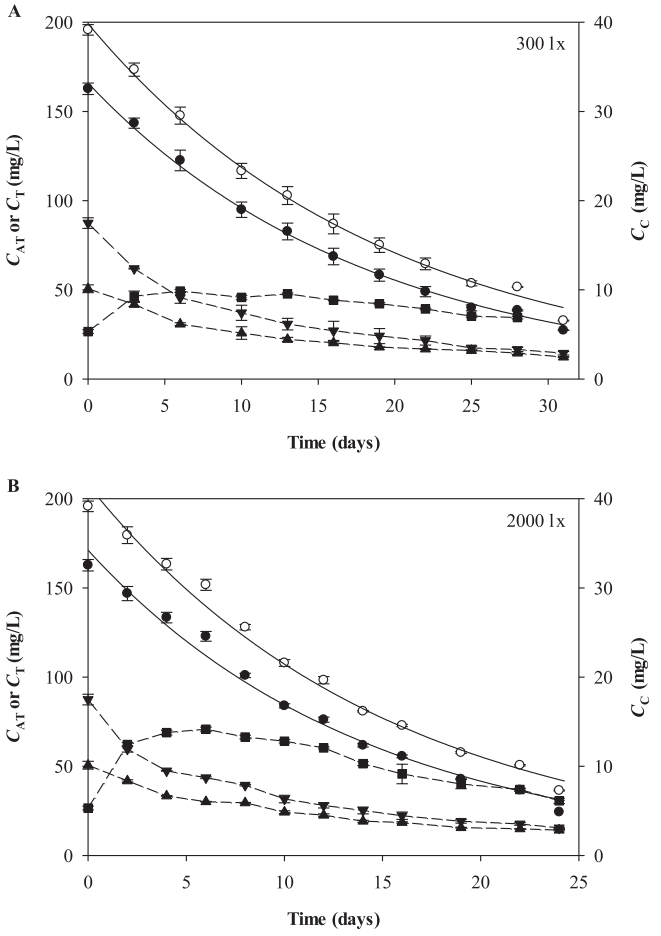

Fig. 2. Changes in the concentrations of all-*trans* isomer (C_{AT}), three *cis* isomers (C_C), and total fucoxanthin (C_T) in o/w emulsions (no pH treatment; pH 4.6) exposed to (A) 300 lx or (B) 2000 lx in absence of air at 25 °C: all-*trans* (●), 13-*cis* (▼), 13′-*cis* (▲), 9′-*cis* (■), and total fucoxanthin (○). Solid lines are simulated results obtained from Eq. (3).

The initial increase in the C_C for 9′-*cis* increased further with light intensity (Fig. 2). This implies that the 9′-*cis* isomer was formed by the photoisomerization from the all-*trans* to the 9′-*cis* isomer, due to the reduction in the rotational barrier for isomerization under illumination (Zhao et al., 2014). It is notable that a decrease in the C_C for 9′-*cis* was observed during the later stage of treatment at 2000 lx. This indicates that the 9′-*cis* isomer was more heat-resistant than the other two *cis* isomers, but that it also underwent photodegradation when exposed to a light of high intensity. The photoisomerization-induced formation and photodegradation of 9′-*cis* were also reported in canola oil (Zhao et al., 2014). The formation of 9′-*cis* by illumination has been also reported for β-carotene in vegetables, fruits, and organic solvents (Aman, Schieber, & Carle, 2005; Pesek & Warthesen, 1990; Pott, Marx, Neidhart, Mühlbauer, & Carle, 2003).

3.6. Effects of pH

Fig. 3A shows that the C_{AT}, C_C (for the three *cis* isomers), and C_T rapidly decreased with time in the emulsion adjusted to a low pH of 1.2 in the absence of light and air at 25 °C. About 94% of total fucoxanthin disappeared after 48 h treatments, and complete degradation was observed after 22 h for the 13-*cis* and 13′-*cis* isomers, and after only 12 h for the 9′-*cis* isomer. Acids are known to promote the destruction of carotenoids by protonation-induced isomerization and degradation reactions (Mortensen & Skibsted, 2000). At a higher pH of 7.5, the decreases in the concentrations became much smaller, where total

Table 2
Rate constants (k) estimated for the degradation of total and all-*trans* fucoxanthin in o/w emulsions (pH 4.6) exposed to three light intensities in the absence of air at 25 °C.

Light (lx)	Total fucoxanthin (C_T)			All-*trans* (C_{AT})		
	k (h^{-1})	R^2	E (%)	k (h^{-1})	R^2	E (%)
0	0.0017 ± 0.0001[a]	0.991	10.22	0.0017 ± 0.0005[a]	0.998	8.14
300	0.0022 ± 0.0000[b]	0.998	4.25	0.0023 ± 0.0000[b]	0.995	3.35
2000	0.0028 ± 0.0000[c]	0.982	13.39	0.0029 ± 0.0000[c]	0.998	9.68

Values with different letters in the same column are significantly different at $p < 0.05$ according to one-way ANOVA followed by Duncan's multiple range test.

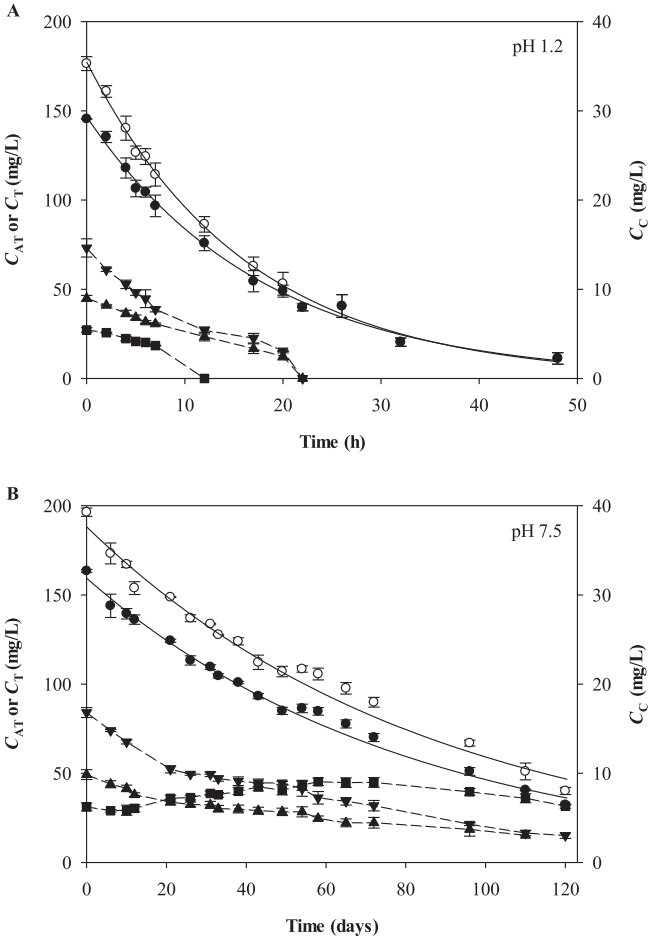

Fig. 3. Changes in the concentrations of all-*trans* isomer (C_{AT}), three *cis* isomers (C_C), and total fucoxanthin (C_T) in o/w emulsions at (A) pH 1.2 and (B) pH 7.5 in the absence of light and air at 25 °C: all-*trans* (●), 13-*cis* (▼), 13′-*cis* (▲), 9′-*cis* (■), and total fucoxanthin (○). Solid lines are simulated results obtained from Eq. (3).

fuxocanthin still remained at about 50% of the initial value after 60 days, and at 20% after 120 days (Fig. 3B). Even the C_C for 9′-*cis* exhibited a notable increase up to 72 days, which was not observed at pH 1.2 or pH 4.6 (Fig. 1A). The higher fucoxanthin stability at pH 7.5 may be attributed to reduced protonation and increased reduction potential at the neutral pH, compared to the acidic pH, resulting in less destruction of fucoxanthin.

The decreases in C_T and C_{AT} at pH 1.2 and 7.5 (Fig. 3) were well-fitted by Eq. (3), with high R^2 values ($R^2 \geq 0.996$) and low E values ($E \leq 6.61$), indicating that the degradation of total and all-*trans* fucoxanthin at these pH values also followed first-order kinetics, as was observed at pH 4.6. The k values determined at pH 7.5 ($k = 0.0005\ h^{-1}$ for both total and all-*trans* fucoxanthin) was only 29% of the values obtained at pH 4.6 ($0.0017\ h^{-1}$ for both total and all-*trans*

Table 3
Rate constants (k) estimated for the degradation of total and all-*trans* fucoxanthin in o/w emulsions at three pH values in the absence of light and air at 25 °C.

pH	Total fucoxanthin (C_T)			All-*trans* (C_{AT})		
	k (h^{-1})	R^2	E (%)	k (h^{-1})	R^2	E (%)
1.2	0.061 ± 0.006[a]	0.996	6.61	0.056 ± 0.005[a]	0.998	5.51
4.6	0.0017 ± 0.0001[b]	0.991	10.22	0.0017 ± 0.0005[b]	0.998	8.14
7.5	0.0005 ± 0.0000[c]	0.998	6.35	0.0005 ± 0.0001[c]	0.996	4.64

Values with different letters in the same column are significantly different at $p < 0.05$ according to one-way ANOVA followed by Duncan's multiple range test.

fucoxanthin), whereas the k values determined at pH 1.2 ($k = 0.061$ and $0.056\ h^{-1}$ for total and all-*trans* fucoxanthin, respectively) were more than 30 times larger than the values at pH 4.6 (Table 3). In addition, the k values determined at pH 1.2 and 25 °C were about 3-fold larger than those obtained at 60 °C and pH 4.5 ($k = 0.0210$ and $0.0228\ h^{-1}$ for total and all-*trans* fucoxanthin, respectively; Table S1). The results indicate that acidification greatly accelerated the degradation of fucoxanthin in the emulsion, even more so than heating under the conditions investigated, whereas neutralization significantly enhanced the stability of fucoxanthin.

The 13-*cis* and 13′-*cis* isomers in the emulsion also exhibited much greater degradation at pH 1.2 than at pH 7.5 (Fig. 3) due to the isomerization and degradation induced by protonation (Mortensen & Skibsted, 2000). At pH 1.2, complete degradation was observed after 22 h for both *cis* isomers, whereas at pH 7.5, the 13-*cis* and 13′-*cis* were present at 18 and 31% of their initial values after 120 days, respectively.

The 9′-*cis* isomer exhibited a fast and strong degradation in the emulsion of pH 1.2, and complete degradation was observed only after 12 h (Fig. 3A). At pH 7.5, however, the 9′-*cis* exhibited a gradual formation from 6.2 to 9.0 mg/L during the first 72 days, followed by a degradation to 6.3 mg/L during the subsequent 48 days (Fig. 3B). The formation of 9′-*cis* was not even observed at pH 4.6 (Fig. 1A). The neutralization could not only weaken the degradation of 9′-*cis* isomer by oxidation and protonation, but could also reduce the rotational barrier for the isomerization from all-*trans* to 9′-*cis* isomer. This may result in the predominance of formation over degradation for the 9′-*cis* isomer during the early treatment period, when the C_{AT} was sufficiently high.

4. Conclusion

The present study demonstrated that total, all-*trans*, 13-*cis*, and 13′-*cis* fucoxanthin underwent significant degradation in the o/w emulsion in response to heating, illumination, and acidification. The 9′-*cis* isomer was relatively resistant to the treatments except at pH 1.2, and even increased considerably in concentration under illumination or at a neutral pH of 7.5 due to the formation reaction by isomerization being favored over the degradation reactions by oxidation, illumination, and

protonation. This degradation of total, all-*trans*, 13-*cis*, and 13′-*cis* fucoxanthin in the emulsion was much greater than that reported for fucoxanthin dissolved in canola oil, and the formation of 9′-*cis* isomer was weaker than that observed in canola oil. This may be primarily attributed to the presence of a high amount of available oxygen in the water phase of the emulsion, which can promote oxidative degradation during all of the treatments. The thermodynamic analysis indicated that the molecular transition to the activated states for the thermal degradation of total and all-*trans* fucoxanthin was more favored in the emulsion than in canola oil, and more structured activated complexes that could undergo faster degradation were formed in the emulsion. Under the current treatment conditions, the factors most significantly affecting the stability of fucoxanthin were, in the order of greatest to least influence, pH, temperature, and light. The pH was able to greatly alter the magnitude of protonation and oxidation-reduction potential, which determine the degree of degradation, resulting in the strongly pH-dependent stability of fucoxanthin in the emulsion. This study provides essential findings to enable the use of o/w emulsion as a convenient, effective system to deliver fucoxanthin into food products with improved stability and bioavailability.

Conflict of interest statement

The authors have declared that there is no conflict of interest.

Acknowledgements

This research was part of a project titled “Development of Global Senior-friendly Healthy Functional Food Materials from Marine Resources” funded by the Ministry of Oceans and Fisheries, Korea.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.04.002.

References

Achir, N., Randrianatoandro, V. A., Bohuon, P., Laffargue, A., & Avallone, S. (2010). Kinetic study of β-carotene and lutein degradation in oils during heat treatment. *European Journal of Lipid Science and Technology, 112*, 349–361.

Aman, R., Schieber, A., & Carle, R. (2005). Effects of heating and illumination on *trans-cis* isomerization and degradation of β-carotene and lutein in isolated spinach chloroplasts. *Journal of Agricultural and Food Chemistry, 53*, 9512–9518.

Aparicio-Ruiz, R., Mínguez-Mosquera, M. I., & Gandul-Rojas, B. (2011). Thermal degradation kinetics of lutein, β-carotene and β-cryptoxanthin in virgin olive oils. *Journal of Food Composition and Analysis, 24*, 811–820.

Atkins, P., & de Paula, J. (2006). *Physical chemistry* (8th ed.). New York: Oxford University Press.

Boon, C. S., McClements, D. J., Weiss, J., & Decker, E. A. (2009). Role of iron and hydroperoxides in the degradation of lycopene in oil-in-water emulsions. *Journal of Agricultural and Food Chemistry, 57*, 2993–2998.

Boon, C. S., Xu, Z., Yue, X., McClements, D. J., Weiss, J., & Decker, E. A. (2008). Factors affecting lycopene oxidation in oil-in-water emulsions. *Journal of Agricultural and Food Chemistry, 56*, 1408–1414.

Chen, J., Shi, J., Xue, S. J., & Ma, Y. (2009). Comparison of lycopene stability in water- and oil-based food model systems under thermal- and light-irradiation treatments. *LWT-Food Science and Technology, 42*, 740–747.

Fung, A., Hamid, N., & Lu, J. (2013). Fucoxanthin content and antioxidant properties of *Undaria pinnatifida*. *Food Chemistry, 136*, 1055–1062.

Henry, L. K., Catignani, G. L., & Schwartz, S. J. (1998). Oxidative degradation kinetics of lycopene, lutein, and 9-*cis* and all-*trans* β-carotene. *Journal of the American Oil Chemists' Society, 75*, 823–829.

Hii, S. L., Choong, P. Y., Woo, K. K., & Wong, C. L. (2010). Stability studies of fucoxanthin from *Sargassum binderi*. *Australian Journal of Basic and Applied Sciences, 4*, 4580–4584.

Hosokawa, M., Okada, T., Mikami, N., Konishi, I., & Miyashita, K. (2009). Bio-functions of marine carotenoids. *Food Science and Biotechnology, 18*, 1–11.

Kim, S. M., Shang, Y. F., & Um, B.-H. (2011). A preparative method for isolation of fucoxanthin from *Eisenia bicyclis* by centrifugal partition chromatography. *Phytochemical Analysis, 22*, 322–329.

Kumar, S., Hosokawa, M., & Miyashita, K. (2013). Fucoxanthin: A marine carotenoid exerting anti-cancer effects by affecting multiple mechanisms. *Marine Drugs, 11*, 5130–5147.

Maeda, H., Hosokawa, M., Sashima, T., Funayama, K., & Miyashita, K. (2005). Fucoxanthin from edible seaweed, *Undaria pinnatifida*, shows antiobesity effect through UCP1 expression in white adipose tissues. *Biochemical and Biophysical Research Communications, 332*, 392–397.

McClements, D. J., & Li, Y. (2010). Structured emulsion-based delivery systems: Controlling the digestion and release of lipophilic food components. *Advances in Colloid and Interface Science, 159*, 213–228.

Mortensen, A., & Skibsted, L. H. (2000). Kinetics and mechanism of the primary steps of degradation of carotenoids by acid in homogeneous solution. *Journal of Agricultural and Food Chemistry, 48*, 279–286.

Pesek, C. A., & Warthesen, J. J. (1990). Kinetic model for photoisomerization and concomitant photodegradation of β-carotenes. *Journal of Agricultural and Food Chemistry, 38*, 1313–1315.

Pott, I., Marx, M., Neidhart, S., Mühlbauer, W., & Carle, R. (2003). Quantitative determination of β-carotene stereoisomers in fresh, dried, and solar-dried mangoes (*Mangifera indica* L.). *Journal of Agricultural and Food Chemistry, 51*, 4527–4531.

Qian, C., Decker, E. A., Xiao, H., & McClements, D. J. (2012). Physical and chemical stability of β-carotene-enriched nanoemulsions: Influence of pH, ionic strength, temperature, and emulsifier type. *Food Chemistry, 132*, 1221–1229.

Ribeiro, H. S., Ax, K., & Schubert, H. (2003). Stability of lycopene emulsions in food systems. *Journal of Food Science, 68*, 2730–2734.

Zhao, D., Kim, S. M., Pan, C. H., & Chung, D. (2014). Effects of heating, aerial exposure and illumination on stability of fucoxanthin in canola oil. *Food Chemistry, 145*, 505–513.

Zhao, D., Kwon, S. H., Chun, Y. S., Gu, M. Y., & Yang, H. O. (2017). Anti-neuroinflammatory effects of fucoxanthin via inhibition of Akt/NF-κB and MAPKs/AP-1 pathways and activation of PKA/CREB pathway in lipopolysaccharide-activated BV-2 microglial cells. *Neurochemical Research, 42*, 667–677.