

Degradation rate of astaxanthin from *Haematococcus pluvialis*

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Abstract

Astaxanthin is an antioxidant that is sensitive to environmental conditions. This study aimed to determine the effect of temperature and lighting on the concentration of astaxanthin, then derive the kinetic equation for its degradation. The half-life was calculated to determine the astaxanthin degradation time at which its concentration drops to half of its initial concentration. Standard astaxanthin was dissolved in acetone and left to stand under three different conditions. The results indicated that temperature and lighting can both cause degradation of astaxanthin. Degradation appeared to follow the second-order kinetics. The calculation showed that lower storage temperatures and less intense light exposure extended the astaxanthin half-life.

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1. Introduction

Astaxanthin is a carotenoid with stronger antioxidant properties than vitamins C and E (Krichnavaruk *et al.*, 2008; Wang *et al.*, 2012; Lee *et al.*, 2020). In nature, astaxanthin can be found in the microalga *Haematococcus pluvialis*, which can account for 0.5%–5% of the algal dry weight (Haque *et al.*, 2016; Shang *et al.*, 2016; Sun *et al.*, 2016; Dewati *et al.*, 2021). It has been reported that astaxanthin is sensitive to heat, light, and other oxidative conditions (Lim *et al.*, 2017). Having many double bonds, it easily undergoes oxidation (Takeungwongtrakul and Benjakul, 2016). Free astaxanthin is more easily degraded than esterified astaxanthin (Breithaupt, 2004). The main products of astaxanthin degradation are 13-apoastaxanthinone, 11-apoastaxanthinal, 9-apoastaxanthinone, and 10-apoastaxanthinal (Etoh *et al.*, 2012). The degradation is characterized by a fading orange-red colour from the original solution.

Storage and distribution of astaxanthin compounds require knowledge of the astaxanthin degradation rate equation and its half-life. Several previous studies have

reported that astaxanthin obtained from several marine animals follows first-order degradation kinetics (Niamnuy *et al.*, 2008; Song *et al.*, 2017). Due to limited information about the degradation kinetics of astaxanthin, it is necessary to study its degradation under different conditions to determine the reaction order for each circumstance. A total of three reaction orders were used. The aim of this research is to find the best reaction order which represent astaxanthin degradation reaction. Degradation reaction rate constant and degradation half life calculated based on the best reaction order.

2. Materials and methods

2.1 Materials

Standard astaxanthin (98% purity, CAS 472-61-7) was purchased from Sigma Aldrich (Singapore) as the standard reference for high-performance liquid chromatography (HPLC). EMSURE-grade acetone was purchased from Merck (Darmstadt, Germany) and used as the solvent for astaxanthin. Trifluoroacetic acid and methanol suitable for HPLC from Merck (Darmstadt,

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Germany).

2.2 Instrumentation

HPLC was used to determine astaxanthin concentration. HPLC was conducted with a Shimadzu (Kyoto, Japan) LC-2010CHT with a photodiode array detector SPD-M10A_{VP} on a C18 column, with 0.05% trifluoroacetic acid/methanol (3:97 (v/v)) as the mobile phase. The retention time was 4.2 mins.

2.3 Method

Standard astaxanthin (in acetone) at 15.26 mg/L was left under three conditions: 1. Under light at room temperature (28-30°C) and analyzed daily. 2. In the dark at room temperature and analyzed daily. 3. In the dark at 4°C (in the refrigerator) and analyzed weekly. The concentrations of all samples were then analyzed by HPLC.

2.4 Calculations

2.4.1 Astaxanthin degradation kinetics

The rate equation for astaxanthin degradation was modelled using the degradation equations for zero-, first-, and second-order reactions. The equations for these reactions were derived from the equations for the reactions are written by Mon *et al.* (2012).

For a zero-order reaction, the degradation rate is not affected by the concentration of astaxanthin, as shown in Equation 1 below.

$$\frac{dC_A}{dt} = -kC_A^0 \quad (1)$$

Where C_A = concentration of astaxanthin (mg/L), t = time (min), k = degradation rate constant (1/d or 1/week)

The concentration of astaxanthin at $t = 0$ is C_{A0} , and at $t = t$ is C_A . Therefore, Equation 1 can be rewritten as Equation 2.

$$\int_{C_A=C_{A0}}^{C_A=C_A} dC_A = -k \int_{t=0}^{t=t} dt \quad (2)$$

$$C_A - C_{A0} = -kt \quad (3)$$

$$C_A = -kt + C_{A0} \quad (4)$$

Equation 4 is a linear equation in the form of $y = ax + b$, where $y = C_A$, $x = t$, $a = k$, and $b = C_{A0}$.

For a first-order reaction, the degradation rate is influenced by the concentration of astaxanthin, as shown in Equation 5 below.

$$\frac{dC_A}{dt} = -kC_A \quad (5)$$

The concentration of astaxanthin at $t = 0$ is C_{A0} , and at $t = t$ is C_A . Therefore Equation 5 can be rewritten as Equation 6.

$$\int_{C_A=C_{A0}}^{C_A=C_A} \frac{dC_A}{C_A} = -k \int_{t=0}^{t=t} dt \quad (6)$$

$$\ln \frac{C_A}{C_{A0}} = -kt \quad (7)$$

Equation 8 is a linear equation in the form of $y = ax + b$, where $y = \ln C_A$, $x = t$, $a = k$, and $b = \ln C_{A0}$.

$$\ln C_A = -kt + \ln C_{A0} \quad (8)$$

For a second-order reaction, the degradation rate is influenced by the square of the astaxanthin concentrations, as shown in Equation 9 below.

$$\frac{dC_A}{dt} = -kC_A^2 \quad (9)$$

The concentration of astaxanthin at $t = 0$ is C_{A0} , and at $t = t$ is C_A . Therefore, Equation 9 can be rewritten as Equation 10.

$$\int_{C_A=C_{A0}}^{C_A=C_A} \frac{dC_A}{C_A^2} = -k \int_{t=0}^{t=t} dt \quad (10)$$

$$\frac{1}{C_{A0}} - \frac{1}{C_A} = -kt \quad (11)$$

$$\frac{1}{C_A} = kt + \frac{1}{C_{A0}} \quad (12)$$

Equation 12 is a linear equation in the form of $y = ax + b$, where $y = \frac{1}{C_A}$, $x = t$, $a = k$, and $b = \frac{1}{C_{A0}}$.

2.4.2 Astaxanthin degradation half-life

The half-life of astaxanthin is the time it takes to degrade to one-half its initial concentration. It is calculated using the order of degradation kinetics.

If the degradation kinetics are zero-order, the half-life equation becomes:

$$C_A = -kt + C_{A0} \quad (13)$$

$$0.5C_{A0} = -kt + C_{A0} \quad (14)$$

$$t = \frac{0.5C_{A0}}{k} \quad (15)$$

If the degradation kinetics are first-order, the half-life equation becomes:

$$\ln C_A = -kt + \ln C_{A0} \quad (16)$$

$$\ln(0.5C_{A0}) = -kt + \ln C_{A0} \quad (17)$$

$$kt = \ln\left(\frac{C_{A0}}{0.5C_{A0}}\right) \quad (18)$$

$$t = \frac{\ln 2}{k} \quad (19)$$

If the degradation kinetics are second-order, the half-life equation is:

$$\frac{1}{C_A} = kt + \frac{1}{C_{A0}} \quad (20)$$

$$\frac{1}{0.5C_{A0}} = kt + \frac{1}{C_{A0}} \tag{21}$$

$$t = \frac{1}{kC_{A0}} \tag{22}$$

3. Results and discussion

Table 1 shows the degradation of astaxanthin as measured by the decreasing concentration of astaxanthin under three different conditions, which are at room temperature in the presence of light, at room temperature in the absence of light, and the temperature of 4°C in the absence of light. After 5 days at room temperature under illumination, astaxanthin has been degraded by 23.59%. The concentration decreased from 15.26 mg/L to 11.66 mg/L. At room temperature in the dark (no illumination), the concentration of astaxanthin has decreased 20.77% from 15.26 mg/L to 12.09 mg/L, indicating that astaxanthin is photosensitive. In the dark refrigerated conditions, astaxanthin concentration decreased from 15.26 mg/L to 12.27 mg/L, a loss of 19.59%. These results indicate that, though astaxanthin is temperature-sensitive, photodegradation has a more significant effect.

Takeungwongtrakul *et al.* (2015) also reported that light, air, and temperature caused the degradation of astaxanthin and the oxidation of lipid compounds in shrimp oil.

Zero-, first- and second-order were used to determine the kinetic reaction order for astaxanthin degradation. The zero-order kinetics equation in Figure 1 shows the rate of reduction in the concentration of astaxanthin, which can be modelled with a linear fit. The value of the reaction rate constant can be observed from the slope of the linear line. The decrease in the ln value of astaxanthin concentration is modelled by the first-order kinetics equation in Figure 2. The reaction rate constant was obtained from the modelled linear slope. The increase in the value of 1/concentration of astaxanthin modelled by the second-order kinetics equation is shown in Figure 3. The degradation reaction rate constant was obtained from the slope of the linear line.

Based on Table 2, the coefficients of determination of the equations for zero-, first-, and second-order, it can

Table 1. Astaxanthin degradation results

Day	Astaxanthin concentration, mg/L		Week	Astaxanthin concentration, mg/L
	Room temperature, light	Room temperature, dark		Refrigerator temperature, dark
0	15.26	15.26	0	15.26
1	14.07	13.99	1	14.45
2	13.12	13.24	2	14.06
3	12.14	12.96	3	12.96
4	11.95	12.28	4	12.45
5	11.66	12.09	5	12.27

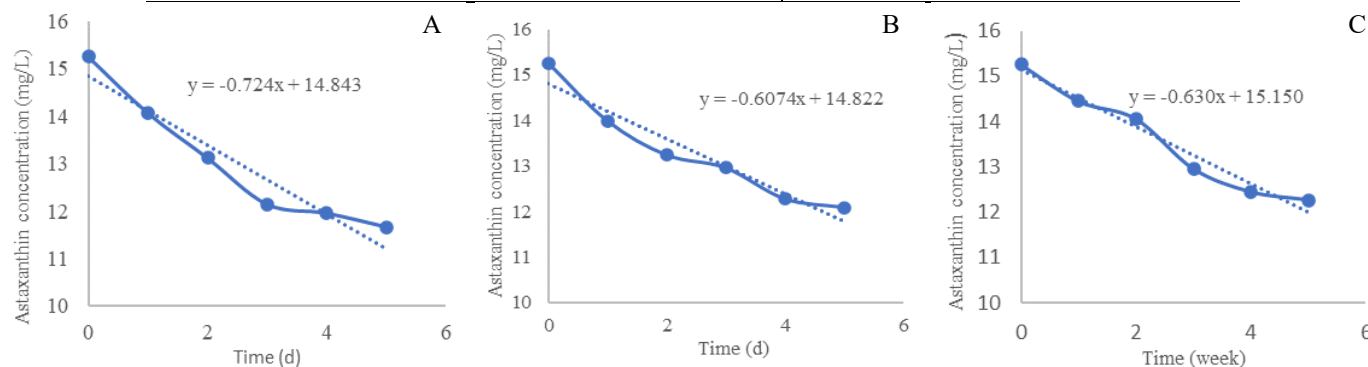


Figure 1. Astaxanthin degradation modeled for zero-order reaction kinetics: (a) room temperature, light; (b) room temperature, dark; (c) refrigerator temperature, dark

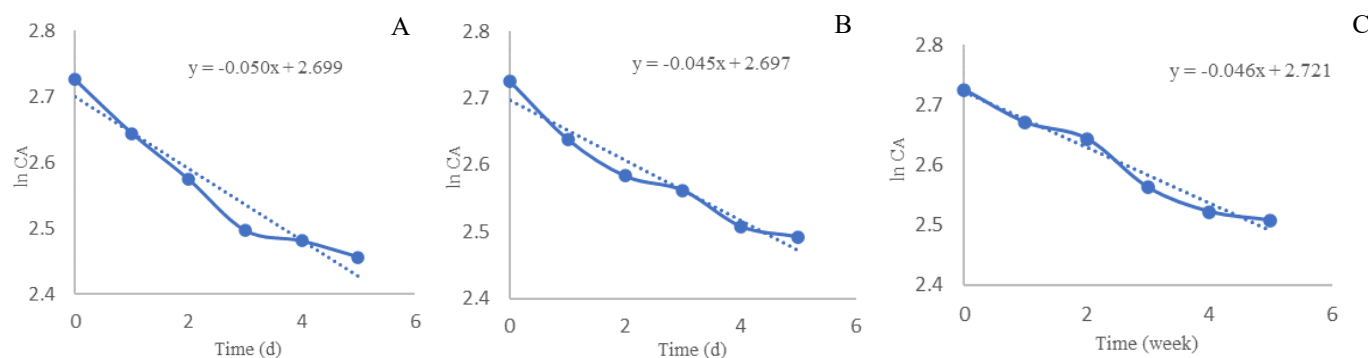


Figure 2. Astaxanthin degradation modeled for first-order reaction kinetics: (a) room temperature, light; (b) room temperature, dark; (c) refrigerator temperature, dark

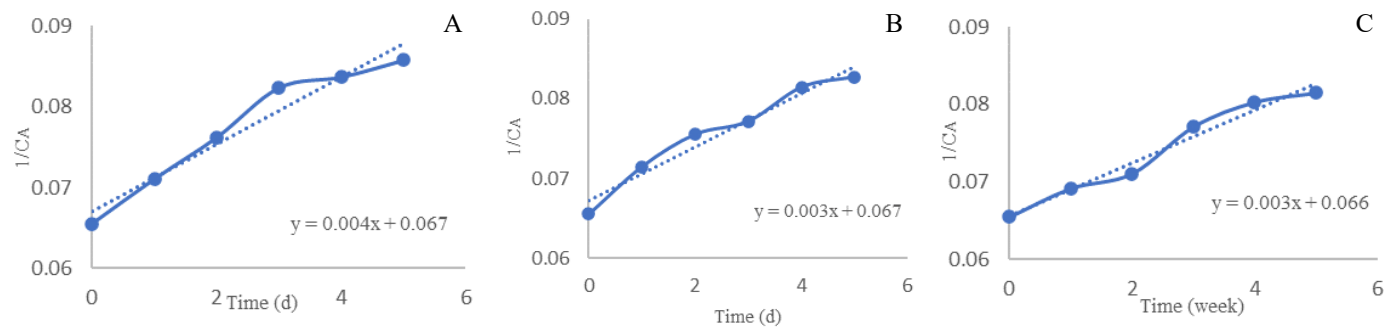


Figure 3. Astaxanthin degradation modeled for second-order reaction kinetics: (a) room temperature, light; (b) room temperature, dark; (c) refrigerator temperature, dark

be concluded that all orders are suitable for modelling the degradation process. However, a second-order equation is most suitable as it has the highest determination value under all conditions.

The rate constant of degradation reaction can be summarized in Table 3. This constant closed to Takeungwongtrakul *et al.* (2016) which mentioned that with first order kinetics equation, astaxanthin degradation process in 30°C has rate constant, $k = 0.0029 \pm 0.2 \text{ hour}^{-1}$ or equivalent to 0.0696 day^{-1} .

Astaxanthin has a longer half-life when kept dark and under illumination, even at the same temperature. This indicates that light can degrade astaxanthin. Based on Table 4, astaxanthin stored in the dark at 4°C has a much longer half-life than that stored at room temperature. The difference in half-lives for the degradation of astaxanthin stored at room temperature and refrigerator temperature in dark conditions for zero-, first-, and second-order reactions was one week.

4. Conclusion

The degradation rate of astaxanthin follows the kinetics of zero-, first-, and second-order reactions. As

the second-order reaction has the best coefficient of determination, it is the most suitable equation for modelling astaxanthin degradation. Astaxanthin stored at room temperature under light conditions had a higher degradation rate and shorter half-life than when stored at the same temperature without light. And at the condition without light, astaxanthin had higher degradation and shorter half-life when stored at room temperature than in a refrigerator (4°C). Thus, it can be concluded that temperature and light are factors that cause astaxanthin degradation.

Conflict of interest

The authors declare no conflict of interest.

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Table 2. Coefficients of determination

	Coefficient of Determination, R^2		
	Zero-order	First-order	Second-order
Room temperature, light	0.9268	0.9399	0.9511
Room temperature, dark	0.9302	0.9456	0.9586
4 °C, dark	0.9664	0.9695	0.9714

Table 3. Degradation reaction rate constants

	Degradation reaction rate constant (k)		
	Zero-order	First-order	Second-order
Room temperature, light (1/d)	0.724	0.050	0.004
Room temperature, dark (1/d)	0.607	0.045	0.003
4 °C, dark (1/week)	0.630	0.046	0.003

Table 4. Degradation half-life

	Half-time, $t(1/2)$		
	Zero-order	First-order	Second-order
Room temperature, light (day)	10.54	13.86	16.38
Room temperature, dark (day)	12.57	15.40	21.84
Refrigerator temperature, dark (week)	12.11	15.07	21.84

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