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Kinetics of Lipid Oxidation and Degradation of Flaxseed Oil Containing Crawfish (*Procambarus clarkii*) Astaxanthin

Jianing Pu · Subramaniam Sathivel

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Abstract Flaxseed oil (FO) containing crawfish (*Procambarus clarkii*) astaxanthin (FOA) was evaluated for lipid oxidation and astaxanthin degradation. The FOA was analyzed for astaxanthin content, free fatty acids (FFA), peroxide value (PV), fatty acid methyl esters (FAMEs) profile, and color. The amount of extractable astaxanthin in the crawfish byproducts was 3.02 mg/100 g of crawfish byproducts. FOA and FO had a similar alpha-linolenic acid (ALA) content (on a weight% basis). The FO was lighter and more yellow in color than FOA. The oxidation rate of FOA was lower than that of FO. When FO and FOA were heated to 30 °C, both oils exhibited minimal lipid oxidation with increasing heating time, whereas FO, when heated to 40, 50, 60 °C, had a higher lipid oxidation rate than FOA with increasing the heating time from 0 to 4 h. Astaxanthin was an effective antioxidant agent in FO when it was heated from 30 to 60 °C. The degradation of astaxanthin in FOA could be described by first order reaction kinetics. Astaxanthin was stable in flaxseed oil at 30 and 40 °C, while its stability decreased significantly at 50 and 60 °C. The rate of astaxanthin degradation in FOA was significantly influenced by temperature.

Keywords Crawfish · Flaxseed oil · Astaxanthin degradation · Lipid oxidation rate

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J. Pu · S. Sathivel (✉)
Department of Food Science, Louisiana State University Agricultural Center, Baton Rouge, LA 70803-4300, USA
e-mail: ssathivel@agcenter.lsu.edu

Introduction

On average, 85 million pounds of crawfish peeling byproducts is commercially generated in Louisiana annually. The byproducts are discarded or used as aquaculture feed so that, unfortunately, they have a low economic value. Thus, disposal of crawfish byproducts is a growing environmental problem. Methods of utilizing crawfish byproducts are needed to convert them into more marketable forms. Crawfish peeling byproducts are an excellent source of the valuable orange-red pigment, astaxanthin, and the biopolymer, chitosan [1]. Astaxanthin is the main ketocarotenoid pigment responsible for the red orange color in crustacean and salmon flesh [2] and this natural pigment serves as a powerful biological antioxidant. It has been reported that the antioxidant activity of astaxanthin is 10 times higher than other carotenoids such as zeaxanthin, lutein, canthaxanthin, and β-carotene [3]. A study has shown that supplementation of astaxanthin effectively suppresses carcinogenesis in mouse urinary bladder tissue [4]. Furthermore, the dietary administration of astaxanthin can inhibit tumor growth in breast cancer [5] and also plays a possible role(s) in preventing oxidative stress and cancer [6], cardiovascular diseases and poor eye health [7]. Astaxanthin recovered from crawfish byproducts is being used as a natural colorant in aquaculture feeds [8]. There is also a potential market for natural carotenoids, including their use in soft drinks, ice cream, desserts, candies, meat products and pet and aquaculture feeds [9].

Antioxidant-rich natural astaxanthin dispersed in alpha-linolenic acid-rich flaxseed oil will help provide healthier functional food options for consumers. Alpha-linolenic acid (ALA) in flaxseed is thought to be an essential fatty acid that promotes human health. Flaxseed oil derived

from flaxseed contains 53.3% ALA and 12.7% linoleic acid (LN), yielding the highest n-3/n-6 fatty acid ratio amongst plant sources [10]. The lipid oxidation of the oil mostly depends on the storage temperature and storage period [11]. The process of peroxidation during processing and storage of oils reduces nutritive value and affects the quality of oils. Lipid oxidation and the astaxanthin degradation rate of flaxseed oil containing astaxanthin have not been investigated. The objectives of this study were to extract astaxanthin from crawfish (*Procambarus clarkii*) byproducts using flaxseed oil and to study the oxidation rates, and astaxanthin degradation rates of flaxseed oil containing astaxanthin.

Materials and Methods

Extraction of Astaxanthin

Fresh crawfish peeling byproducts consisting of the cephalothorax, abdominal exoskeleton, and viscera were obtained from a local Louisiana crawfish processor and stored at −20 °C until astaxanthin extraction. Flaxseed oil (FO) containing astaxanthin (FOA) was prepared using a modified method of Sachindra and Mahendrakar [12] as described in Fig. 1. The thawed crawfish byproducts were finely ground in a Hobart grinder (K5SS, Hobart Corporation, Troy, OH) through a 12-mm diameter plate. A 100-g sample of ground crawfish byproducts was mixed with an equal volume of FO (iHerb, Irwindale, CA), stirred for 60 min at 60 °C, and then the mixture was centrifuged at 8,630×*g* (Beckman J2-HC, GMI, Inc., Ramsey, MN) for 15 min at 4 °C and recovered FOA and water phase from the solid phase. FOA was separated from the water phase using a separatory funnel.

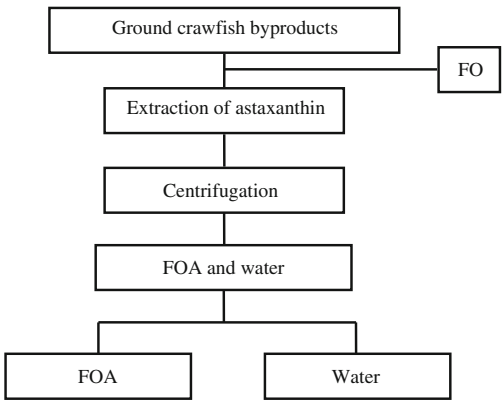

Fig. 1 Extracting astaxanthin from crawfish byproducts using flaxseed oil. *FO* flaxseed oil, *FOA* flaxseed oil containing astaxanthin

Astaxanthin, Color, Peroxide Value, and Free Fatty Acids of FOA and FO

The amount of extractable astaxanthin in the crawfish byproducts was determined spectrophotometrically at a wavelength of 500 nm as described by Chen and Meyers [13]. The astaxanthin content was reported as mg astaxanthin/100 g byproducts using Eq. 1:

$$\text{Astaxanthin}(\text{mg}/100\,\text{g byproducts}) = \frac{A \times V \times D \times 10^6}{1{,}000 \times W \times E^{\%}_{1\,\text{cm}}} \quad (1)$$

where A is the absorbance at 500 nm; V is the volume of FOA recovered (mL); D is the dilution factor; W is the weight of byproducts (g); $E^{\%}_{1\,\text{cm}}$ is the extinction coefficient (1827); FOA is flaxseed oil containing astaxanthin.

The amount of astaxanthin in 100 g of oil was calculated using Eq. 2:

$$\text{Astaxanthin}(\text{mg}/100\,\text{g FOA}) = \frac{A \times D \times 10^5}{100 \times d \times S \times E^{\%}_{1\,\text{cm}}} \quad (2)$$

where A is the absorbance at 500 nm; D is the dilution factor, d is the cell width (1 cm); S is the specific gravity of FOA; $E^{\%}_{1\,\text{cm}}$ is the extinction coefficient (1827); FOA is flaxseed oil containing astaxanthin.

Color of FOA and FO was measured in triplicate using a LabScan® XE spectrophotometer (Hunter Associates Laboratory, Inc., Resbon, VA) and was reported in CIELAB color scales (L^*, a^*, and b^* values). The peroxide value (PV) of the FOA and FO samples was measured according to AOAC method 965.33 [14]. Free fatty acids (FFA) content was determined according to AOAC procedure 940.28 [14]. The percentage of FFA was expressed as oleic acid equivalents.

Fatty Acid Methyl Esters Profile of FOA and FO

The fatty acid methyl esters composition of samples was determined at the USDA-ARS Laboratory, University of Alaska Fairbanks, AK, USA. Fatty acid methyl esters (FAMEs) were prepared using a modified method of Maxwell and Marmer [15]. A 20-mg sample of FO or FOA was dissolved in 4.5 mL isooctane and 500 µL internal standard (10 mg methyl tricosanoate (23:0)/mL isooctane and 500 µL 2 N KOH (1.12 g/10 mL MeOH) was added to convert the fatty acids into their methyl esters. Saturated ammonium acetate was used to neutralize the KOH and distilled water was used to wash the ammonium acetate from the isooctane layer. The isooctane layer containing methyl ester was analyzed by Gas Chromatographic (GC

model 7890A, Agilent) fitted with a HP-88 (100 m × 0.25 mm ID × 0.25 μm film) column.

Oxidation Study of FOA and FO

Three batches of the opened amber bottles containing 20 g of FOA and/or FO samples were placed in a water bath and heated at 30, 40, 50, and/or 60 °C. An oil sample was drawn from the amber bottles using a pipette every hour from 0 to 4 h for PV and astaxanthin concentration analysis. A plot of PV versus time was constructed for 30, 40, 50, and/or 60 °C. The resulting straight line yielded the magnitude of the oxidation rate (mequiv kg oil^{-1} h^{-1}) for the corresponding temperature. The effect of temperature on the oxidation rate was described using the Arrhenius relationship as shown in Eq. 3:

$$k = k_{\infty} \exp(-E_a/RT). \quad (3)$$

The logarithms were taken on both sides of Eq. 3:

$$\ln k = \frac{-E_a}{\mathrm{RT}} + \ln k_{\infty} \quad (4)$$

where k is the reaction rate constant; k_{∞} is the frequency factor; R is the universal gas constant (8.3145 J/mol K); T is the absolute temperature (K); E_a is the activation energy (J/mol). A plot of ln oxidation rate constant versus $1/T$ (i.e., 1/absolute temperature) was constructed for FO and FOA oil. The slope of the straight line was obtained from the trend line of the plot. The magnitude of E_a was calculated as the slope of the trend line multiplied by the universal gas constant.

Kinetic Parameters of Astaxanthin Degradation for FOA

First order model was used to describe the astaxanthin degradation kinetics as described by Eq. 5 [16]:

$$\ln\left(\frac{C}{C'}\right) = -kt \quad (5)$$

where C' is the initial concentration (mg 100 g FOA^{-1}) of astaxanthin; C is the concentration (mg 100 g FOA^{-1}) of astaxanthin at time t; k is the degradation rate constant (h^{-1}); t is the oxidation time (h).

A plot of $\ln\left(\frac{C}{C'}\right)$ versus t was constructed to determine the k values and the correlation coefficients. The resulting k values from first order model (ln k) versus $1/T$ were plotted and the activation energy was obtained for astaxanthin degradation using the Arrhenius model (Eq. 4). The magnitude of E_a was calculated as the slope of the plot multiplied by the gas constant.

Statistical Analysis

Mean values and standard deviations of triplicate determinations were reported. Analysis of variance (ANOVA) was carried out to determine the difference among treatments means (SAS Version 8.2, SAS Institute Inc., Cary, NC) using the post hoc Tukey's studentized range test.

Results and Discussion

Astaxanthin Concentration, Color, PV, and FFA of FOA and FO

The amount of extractable astaxanthin in the crawfish byproducts and the amount of astaxanthin in 100 g of FOA oil were 3.02 mg/100 g of crawfish byproducts and 3.9 mg/100 g of FOA, respectively (Table 1), which was lower than previously reported by Meyers and Bligh [17]. In their study, a petroleum ether-acetone–water mixture was used to extract the astaxanthin from the crawfish byproducts giving a concentration of 15.3 mg of astaxanthin/100 g of crawfish peeling byproducts. A number of studies have demonstrated that the yield of astaxanthin depends upon the extraction methods, solvents or media, and other factors used for extracting astaxanthin from seafood processing byproducts. For example, De Holanda and Netto [18] enzymatically hydrolyzed dried shrimp byproducts and extracted 4.90 mg astaxanthin/100 g shrimp byproducts, while Saito and Regier [19] and Shahidi and Synowiecki [2] obtained concentrations of 14.8 and 11.1 mg of astaxanthin/100 dry shrimp processing byproducts, respectively, using cod liver oil for the extraction. Shahidi and Synowiecki [2] have also reported that factors such as the amount of carotenoid available in the byproducts, particle size of the byproducts, extraction temperature, and byproducts/oil ratio can influence the extraction yield of astaxanthin. In our study, however, neither proteolytic enzymes and/nor solvents were used to extract the astaxanthin, which might explain why FOA had

Table 1 Astaxanthin, color, PV, and FFA of FO and FOA

	FO	FOA
Astaxanthin extracted (mg/100 g byproducts)	–	3.02 ± 0.02
Astaxanthin extracted (mg/100 g FOA)	–	3.90 ± 0.34
Color L^*	50.42 ± 0.22^{a}	35.63 ± 0.26^{b}
Color a^*	4.04 ± 0.07^{b}	35.27 ± 0.12^{a}
Color b^*	81.30 ± 0.35^{a}	61.78 ± 0.25^{b}
PV (mequiv/kg)	3.97 ± 0.08^{b}	4.59 ± 0.16^{a}
FFA (%)	0.31 ± 0.00^{a}	0.30 ± 0.00^{a}

Values are means and SD of three determinations

FO flaxseed oil, *FOA* flaxseed oil containing astaxanthin

[ab] Means with different letters in each row are significantly different ($p < 0.05$)

a lower concentration of astaxanthin. Although, our astaxanthin concentration in FOA was lower than previously reported astaxanthin extraction concentrations, the process presently used could significantly reduce the processing time and lower the chemical waste generation during extraction of astaxanthin from crawfish byproducts. Thus, our process might reduce the overall processing cost.

The FO was lighter ($L^* = 50.42$) and more yellow ($b^* = 81.30$) in color than FOA, while FOA had higher redness ($a^* = 35.27$) than the FO, which indicated that carotenoids pigments were extracted from crawfish byproducts into flaxseed oil (Table 1). The PV of FO was 3.97 mequiv/kg, lower than the PV of 5.2 mequiv/kg for FO extraction reported by previous study [20]. Both FO and FOA had similar FFA values, which indicated that extraction had not affected the FFA levels in oil.

Fatty Acid Methyl Esters Profile of FOA and FO

The fatty acid compositions of FOA and FO are given in Table 2. Both oil samples had similar fatty acid compositions, which demonstrated that the procedure of astaxanthin extraction that we used had no effect on the fatty acid composition of FO. Alpha-linolenic acid (ALA) was the predominant fatty acid accounting for 56.3 and 56.5% for FOA and FO, respectively, which is a similar ALA in FO profile to that reported by previous researchers [10]. Our ALA values in FO and FOA were slightly higher than those reported for FO (50.4%) by Bera, Lahiri, and Nag [20]. FO contained 88% unsaturated fatty acids; therefore, the oil probably reacted with the oxygen in the air. The present study showed that FO sharply oxidized at 30, 40, 50, and 60 °C with increasing time without astaxanthin. The high content of unsaturated fatty acids in FO has a greater tendency to be oxidized [20].

Table 2 Fatty acid methyl esters profile of FO and FOA

Weight (%)	FO	FOA
C16:0 palmitic	6.07 ± 0.05[a]	5.81 ± 0.72[a]
C18:0 stearic	3.35 ± 0.03[a]	3.03 ± 0.38[a]
C18:1n9c oleic	16.61 ± 0.14[a]	15.08 ± 1.88[a]
C18:2n6c linoleic	15.06 ± 0.12[a]	13.02 ± 1.73[a]
C18:3n3 alpha-linolenic	56.50 ± 0.51[a]	56.3 ± 1.61[a]
Total omega 3	56.50 ± 0.51[a]	56.33 ± 1.11[a]
Total omega 6	15.17 ± 0.12[b]	14.11 ± 0.26[a]
Saturates	9.57 ± 0.08[a]	9.67 ± 0.21[a]
Monounsaturates	17.85 ± 0.15[a]	17.52 ± 0.36[a]
Polyunsaturates	71.71 ± 0.63[a]	70.33 ± 1.41[a]
Omega 3/omega 6	3.73 ± 0.00[a]	4.00 ± 0.01[b]
Polyunsaturates/saturates	7.49 ± 0.00[b]	7.29 ± 0.02[a]
Total fatty acids	99.13 ± 0.86[a]	97.71 ± 1.96[a]

Values are means and SD of three determinations. Only major Fatty acid methyl esters were listed in Table 2

FO flaxseed oil, *FOA* flaxseed oil containing astaxanthin

[ab] Means with different letters in each row are significantly different ($p < 0.05$)

Kinetics of Lipid Oxidation of FO and FOA

The peroxide value is a useful indicator of lipid oxidation, especially at the beginning of lipid oxidation [21]. Figures 2 and 3 show the changes in PV of FO and FOA as a function of time at different temperatures. Lipid oxidation, as indicated by the PV, increased with increasing time and temperature. The FO and FOA heated at 30 °C exhibited minimal lipid oxidation with increasing time, whereas FOA heated at 40–60 °C showed a lower lipid oxidation rate than FO with increasing time from 0 to 4 h (Table 3). Lipid

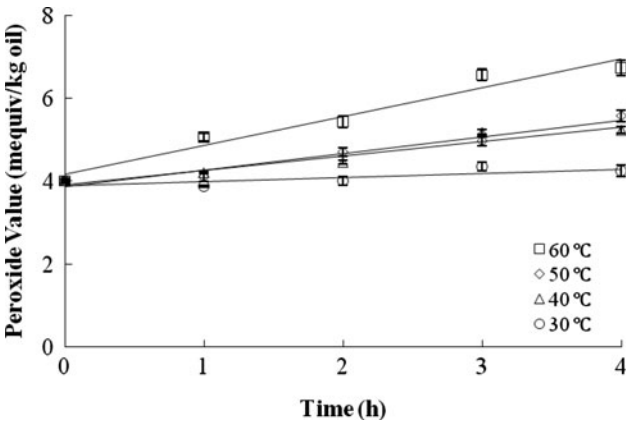

Fig. 2 Effect of time on peroxide formation in the FO at different temperatures. *FO* flaxseed oil

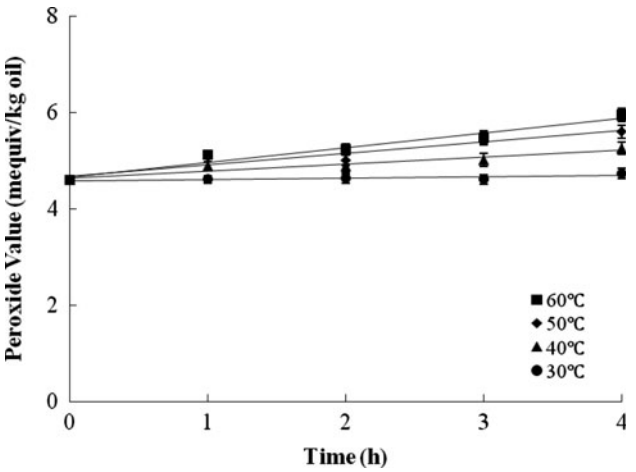

Fig. 3 Effect of time on peroxide formation in the FOA at different temperatures. *FOA* flaxseed oil containing astaxanthin

Table 3 Oxidation rate of FO and FOA

Temperature (°C)	Oxidation rate (mequiv kg oil^{-1} h^{-1})	
	FO	FOA
30	0.098 ± 0.01^{aD}	0.027 ± 0.02^{bD}
40	0.348 ± 0.01^{aC}	0.144 ± 0.01^{bC}
50	0.404 ± 0.04^{aB}	0.24 ± 0.03^{bB}
60	0.698 ± 0.05^{aA}	0.305 ± 0.04^{bA}
E_a (kJ/mol)	51.07 ± 1.62^{b}	65.84 ± 0.89^{a}

Values are means and SD of three determinations

E_a activation energy, *FO* flaxseed oil, *FOA* flaxseed oil containing astaxanthin

^{ab} Means with different letters in each row are significantly different ($p < 0.05$)

^{ABCD} Means with different letters in each column are significantly different ($p < 0.05$)

oxidation in edible oils occurs very slowly but it is accelerated when subjected to heat, air and light [22].

Lipid oxidation can be delayed by adding antioxidants. One previous study reported that sunflower oil containing leafy vegetable extracts minimized peroxide formation during heating and storage processes [23]. Another study showed that the PV of FO decreased when ajowan extract was added to FO during heating [20]. Our study was in accordance with previous reports of delayed lipid oxidation of FO by adding antioxidants [20], which indicates that the presence of the astaxanthin in the FO minimized the oxidation of the FO. Oxidation was significantly higher for FO than FOA regardless of different heating temperatures used. The oxidation rate (mequiv kg oil^{-1} h^{-1}) increased from 0.098 to 0.698 for FO while it increased from 0.027 to 0.305 for FOA when the oils were heated from 30 to 60 °C for 4 h. The oxidation rates for the FO and FOA oils could be described by the Arrhenius equation (Fig. 4) and the R^2 values for FO and FOA were 0.89 and 0.87, respectively, indicating that the peroxide formation rates of these oils could be modeled by the Arrhenius equation. The activation energies for lipid oxidation of FO and FOA were 51.07 and 65.84 kJ/mol. The lower activation energy and higher oxidation rate indicated that FO had a higher heat sensitive than did FOA during heating. Antioxidants such as tocopherols and propyl-gallate either delay or inhibit the initiation and propagation stages of oxidation by reacting with lipid-free radicals and peroxy or alkoxy radicals, respectively [24]. The presence of astaxanthin in the FO might function as a free radical acceptor, terminating free radicals at the initiation stage of oxidation during heating. Astaxanthin has a phenolic hydroxyl group in its structure, which may have actively prevented oxidation of the glycerides in the FO during heating. Our results show that astaxanthin

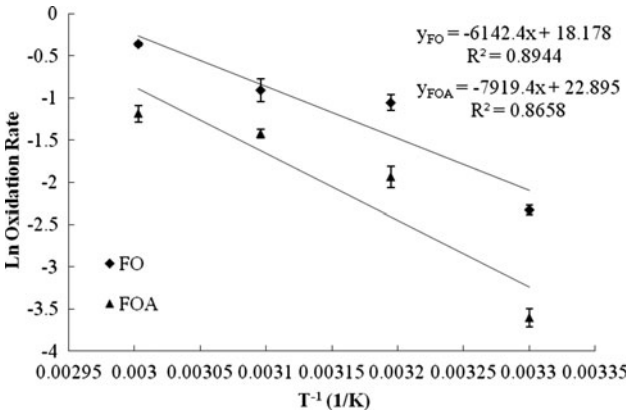

Fig. 4 The Arrhenius plot for the peroxide values of the FO and FOA. *FO* flaxseed oil, *FOA* flaxseed oil containing astaxanthin

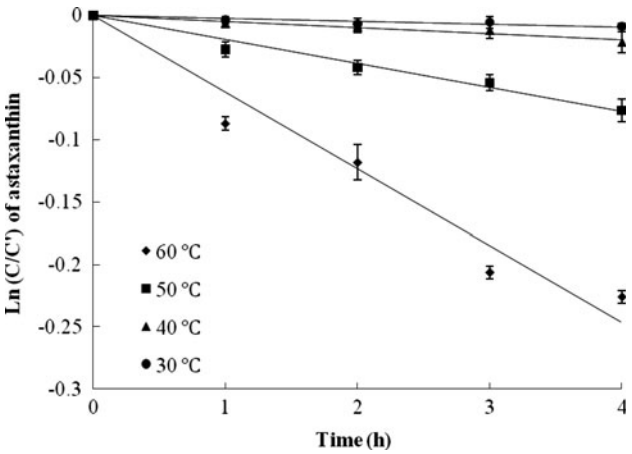

Fig. 5 First–order kinetic plots of astaxanthin degradation in FOA at various temperatures. *FOA* flaxseed oil containing astaxanthin, C' is the initial concentration of astaxanthin, C is the concentration of astaxanthin at time t

exhibited an effective antioxidant activity in FO when FOA was heated from 30 to 60 °C.

Kinetics Parameters of Astaxanthin Degradation

Astaxanthin is sensitive to heat, oxidation, and light because of its unsaturated structure [16]. The results of astaxanthin degradation in the FOA during heating are given in Fig. 5. The rate of degradation of astaxanthin was determined by linear regression of ln (C/C') against heating time. The astaxanthin degradation fitted with the first order (Fig. 5) kinetics model. The first order kinetics showed that the rate constant (k) for astaxanthin degradation increased with increasing temperature. The R^2 for the first order kinetics model was 0.85, 0.95, 0.97 and 0.95 for 30, 40, 50, and 60 °C, respectively. This indicated that first order kinetics could be used to describe the degradation of astaxanthin in FOA between 30 and 60 °C. Astaxanthin was stable in FO at

Table 4 Kinetics of astaxanthin degradation in FOA

Temperature (°C)	$k \times 10^2$ (h^{-1})	R^2	*Ea* (kJ/mol)
30	0.22 ± 0.02^{c}	0.85	
40	0.50 ± 0.11^{c}	0.95	96.79 ± 1.37
50	1.93 ± 0.21^{bc}	0.97	
60	6.49 ± 0.30^{a}	0.95	

Values are means and SD of three determinations

k degradation rate constant, E_a activation energy, *FOA* flaxseed oil containing astaxanthin

[abc] Means with different letters in each column are significantly different ($p < 0.05$)

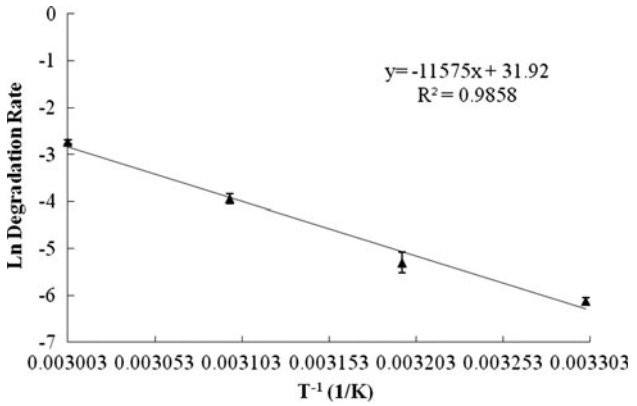

Fig. 6 The Arrhenius plot for the astaxanthin degradation of FOA. *FOA* flaxseed oil containing astaxanthin

30 and 40 °C, while astaxanthin concentrations decreased at 50 and 60 °C by 7.53 and 20.63%, respectively. The rate of constant (*k)* for astaxanthin degradation increased with increased temperature (Table 4). The *k* was 0.22×10^{-2} h^{-1}at 30 °C and it increased to 6.49×10^{-2} h^{-1} when the FOA was heated to 60 °C for 4 h. Levenspiel [25] and Niamnuy, Devahastin, Soponronnarit, and Raghavan [16] have reported a higher *k* for a higher rate of reaction, which indicated astaxanthin degraded faster at a higher temperature than that at a lower temperature. Rao, Sarada, and Ravishankar [26] have also reported drastic reductions in astaxanthin concentrations at elevated heat processing temperatures. Astaxanthin degradation was well described by the Arrhenius equation (Fig. 6) and the R^2 value of 0.99 was determined using the rate of constants obtained from first order kinetics model. The E_a value for astaxanthin degradation was 96.79 kJ/mol, which was slightly lower than previously reported E_a values (110 kJ/mol) for carotenoid degradation during heat treatment [27].

Conclusions

Astaxanthin extracted from crawfish byproducts using FO (i.e., FOA) showed a lower lipid oxidation rate than that was found for FO. Both FO and FOA showed minimal lipid oxidation at 30 °C with increasing time, whereas FO had a higher oxidation at 40–60 °C than FOA on increasing the heating time from 0 to 4 h. Collectively, the latter results demonstrate that astaxanthin can effectively reduce lipid oxidation in FO when it is heated from 30 to 60 °C. The degradation of astaxanthin during heating could be described by first order reaction kinetics. Astaxanthin was stable in FO at 30 and 40 °C, while astaxanthin was unstable at 50 and 60 °C. The rate of astaxanthin degradation in FOA was significantly influenced by temperature. Byproducts generated from crawfish peeling operations are a good source of high quality astaxanthin, which can be used as a natural colorant and antioxidant ingredient in human food and industrial applications.

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