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Kinetics for the reversible isomerization reaction of *trans*-astaxanthin

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Abstract

The isomerization of *cis*-astaxanthins in organic solvents, a reverse reaction of the isomerization of *trans*-astaxanthin, was investigated. HPLC analysis revealed that *cis*-astaxanthins could be also isomerized to produce *trans*-astaxanthin and the other *cis*-astaxanthin. The results showed that the isomerization of *trans*-astaxanthin to *cis*-astaxanthin was a reversible reaction and followed first-order reversible reaction kinetics. The effect of temperature on the isomerization reaction of *trans*-astaxanthin, dissolved in dimethyl sulfoxide at 20–70°C or in a mixture of dichloromethane and methanol (25:75) at 10°C, respectively, was also studied. The results indicated that increasing temperature could markedly increase the reaction rate of *trans*-astaxanthin isomerization. Temperature-dependence of the isomerization rate constants of *trans*-astaxanthins could be described by the Arrhenius equation with activation energy (E_a) of 105.8±4.2 kJ/mol.

Keywords: Astaxanthin; Isomerization; Kinetics; First-order reversible reaction

1. Introduction

Astaxanthin can be used as a pigmentation source in the poultry (Johnson, Lewis & Grau, 1980) and aquaculture industries (Johnson, Villa & Lewis, 1980; Sommer, Potts & Morrissy, 1991; Wathne, Bjerkeng, Storebakken, Vassvik & Odland, 1998) and has be permitted for use as a colour additive in salmonid feed by the Food and Drug Administration in the United States (Turujman, Wamer, Wei & Albert, 1997). Although astaxanthin does not have provitamin A activity (Jyonouchi, Hill, Tomita & Good, 1991; Jyonouchi, Sun & Gross, 1995; Jyonouchi, Sun, Mizokami & Gross, 1996; Savoure, Briand, Amory-Touz, Combre, Maudet & Nicol, 1995), recent studies have demonstrated that astaxanthin has considerable preventive activities against cancer and may be used in medicine (Gradelet, Astorg, Bon, Berges & Suschetet, 1997; Gradelet, Le Bon, Berges, Suschetet & Astorg, 1998; Mori et al., 1997; Tanaka, Morishita, Suzui, Kojima, Okumura & Mori, 1994; Tanaka, Makita, Ohnishi, Mori, Satoh & Hara, 1995). Bjerkeng and co-workers (Bjerkeng, Folling, Lagocki, Storebakken, Olli & Alsted, 1997) investigated the bioavailability of *trans*-astaxanthin and *cis*-astaxanthins (Fig. 1) in rainbow trout and found that *cis*-astaxanthins were not utilized to the same extent as *trans*-astaxanthin for flesh pigmentation. The retention of all-*trans*-astaxanthin was higher than that of *cis*-astaxanthin and the retention of 13-*cis*-astaxanthin was significantly higher than that of 9-*cis*-astaxanthin (Bjerkeng et al., 1997).

trans-Astaxanthin was readily isomerized to its *cis*-isomers, especially the 9-*cis* and 13-*cis* unhindered isomers (Johnson & An, 1991). The isomerizations of *trans*-astaxanthin dissolved in different organic solvents have been investigated in our previous work (Yuan & Chen, 1999). The result that *trans*-astaxanthin could not be isomerized completely to its *cis*-isomers indicated that the isomerization of *trans*-astaxanthin was a reversible reaction (Yuan & Chen, 1999). The thermal isomerization reaction of *trans*-lutein in a benzene solution had been showed to be a reversible reaction (Subagio, Morita & Sawada, 1998). An understanding of the isomerization of *cis*-isomers may yield important information towards the reversibility of the isomerization reaction of *trans*-astaxanthin. Although the isomeriza-

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tion reaction under mild conditions might be represented by zero-order reaction kinetics, the isomerization reaction at high temperature was fast and could not be represented by a zero-order reaction (Yuan & Chen, 1999). The major objective of the present study was to further analyze the process of isomerization of *trans*-astaxanthin at different temperatures, paying especial attention to the isomerization of *cis*-astaxanthin, and to establish a kinetics model to represent the reversible isomerization reaction of *trans*-astaxanthin.

2. Materials and methods

2.1. Chemicals and reagents

trans-Astaxanthin was obtained from Sigma Chemical Co. (St. Louis, MO, USA). Dimethyl sulfoxide, HPLC grade methanol, acetonitrile, and dichloromethane were obtained from BDH Laboratory Supplies (Poole, UK).

2.2. Isomerization of trans-astaxanthin at different temperatures

The isomerization reaction of *trans*-astaxanthin was carried out at different temperatures between 10 and 70°C in darkness. *trans*-Astaxanthin was dissolved, respectively, in dimethyl sulfoxide for the reactions at 20–70°C and in a mixture of dichloromethane and methanol (25:75) for the reaction at 10°C. A 9105 Refrig/Heat Circulating Bath (Polyscience, Niles, IL, USA) was used to maintain the reaction temperature. The reaction mixtures were sampled and analyzed over time by HPLC, for monitoring the progress of isomerization reactions.

2.3. Preparation of cis-astaxanthin solution and isomerization of cis-astaxanthin

trans-Astaxanthin was dissolved in dichloromethane and isomerized to produce *cis*-astaxanthin at 35°C for 24–48 h. *cis*-Astaxanthins in *cis*-*trans* mixture were obtained by using the semipreparative HPLC method (Yuan & Chen, 2000). A Beckman Ultrasphere C_{18} (5 μm; 250×10 mm) semipreparative column was used. The mobile phase consisted of methanol (90%), water (8%) and dichloromethane (2%). The 9-*cis*-astaxanthin and 13-*cis*-astaxanthin fractions were collected together. Five millilitres of dichloromethane and then 20 ml of distilled water were added to the *cis*-astaxanthin fraction collected. The solution separated into two layers after the addition of distilled water. The *cis*-astaxanthin solution, the red dichloromethane layer containing 9-*cis*- and 13-*cis*-astaxanthins, was obtained and used to observe the isomerization reaction of *cis*-astaxanthins to *trans*-astaxanthin.

2.4. HPLC analysis

The isomers of astaxanthin were separated and analyzed (20-ml aliquots) by using a Waters Symmetry C_{18} column (5 μm; 150×3.9 mm) at ambient temperature. The mobile phase consisted of methanol (82.5%), dichloromethane (5.0%), acetonitrile (5.5%), and water (7.0%). The flow rate was set at 1.0 ml/min. The tridimensional chromatogram was recorded from 250 to 700 nm. The isomers of astaxanthin were identified according to their retention times and spectra by photodiode array detection (Yuan & Chen, 1997, 1999). Peaks were measured at a wavelength of 480 nm. The concentrations of *trans*-astaxanthin, 9-*cis*-astaxanthin, and 13-*cis*-astaxanthin were calculated by dividing their peak areas at 480 nm by their respective coefficients 306323, 247660, and 190512. The coefficient of *trans*-astaxanthin was calculated by the response of *trans*-astaxanthin standard at 480 nm. The coefficients of 9-*cis*-astaxanthin and 13-*cis*-astaxanthin were estimated according to the extinction coefficients at 470 nm (Bjerkeng et al., 1997), the spectra of *trans*-astaxanthin, 9-*cis*-astaxanthin, and 13-*cis*-astaxanthin, and the coefficient of *trans*-astaxanthin.

2.5. First order reversible isomerization reaction

Under a mild condition for the reversible isomerization reaction, the reaction from *trans*-astaxanthin to *cis*-astaxanthin was slow and the change in concentration of *trans*-astaxanthin was small, and then the reverse reaction from *cis*-astaxanthin to *trans*-astaxanthin was negligible, the isomerization reaction might be represented approximately by a zero-order reaction kinetics. But zero-order kinetics were not applicable to the isomerization reaction at high temperature (Yuan & Chen, 1999). According to Drenth and Kwart (1980), the rate for first-order with a reverse reaction was proportional to the distance removed from equilibrium. That is, at the beginning of reaction, the rate (dc/dt) of isomerization reaction was high, and then the isomerization reaction rate gradually decreased along with the progress of the reaction and the decrease of *trans*-astaxanthin concentration (Yuan & Chen, 1999). Therefore, for the reversible isomerization reaction, first-order reaction kinetics were suggested

For the reversible isomerization reaction of *trans*-astaxanthin to *cis*-astaxanthins:

$$\textit{trans}\text{-astaxanthin} \underset{k_2}{\overset{k_1}{\leftrightarrow}} \textit{cis}\text{-astaxanthins}$$

Suppose that at the beginning of the isomerization reaction and time t, the concentrations of *trans*-astaxanthin were C_0 and c, respectively. After an appropriate time interval, equilibrium will practically be reached.

Designating the concentration (%) of *trans*-astaxanthin as C_{trans} and that of *cis*-astaxanthins as C_{cis} at equilibrium:

$$k_1\ C_{trans} = k_2\ C_{cis} \quad (1)$$

The equilibrium constant expression is:

$$K_{iso} = k_1/k_2 = C_{cis}/C_{trans} \quad (2)$$

At any moment:

$$dc/dt = -k_1\ c + k_2\ (C_0 - c) \quad (3)$$

Integration gives (Drenth & Kwart, 1980):

$$\ln\ \{[(k_1 + k_2)\ c - k_2\ C_0]/\ k_1\ C_0\ \} = -(k_1\ +\ k_2)\ t \quad (4)$$

Substitution of Eq. (2) into Eq. (4) leads to:

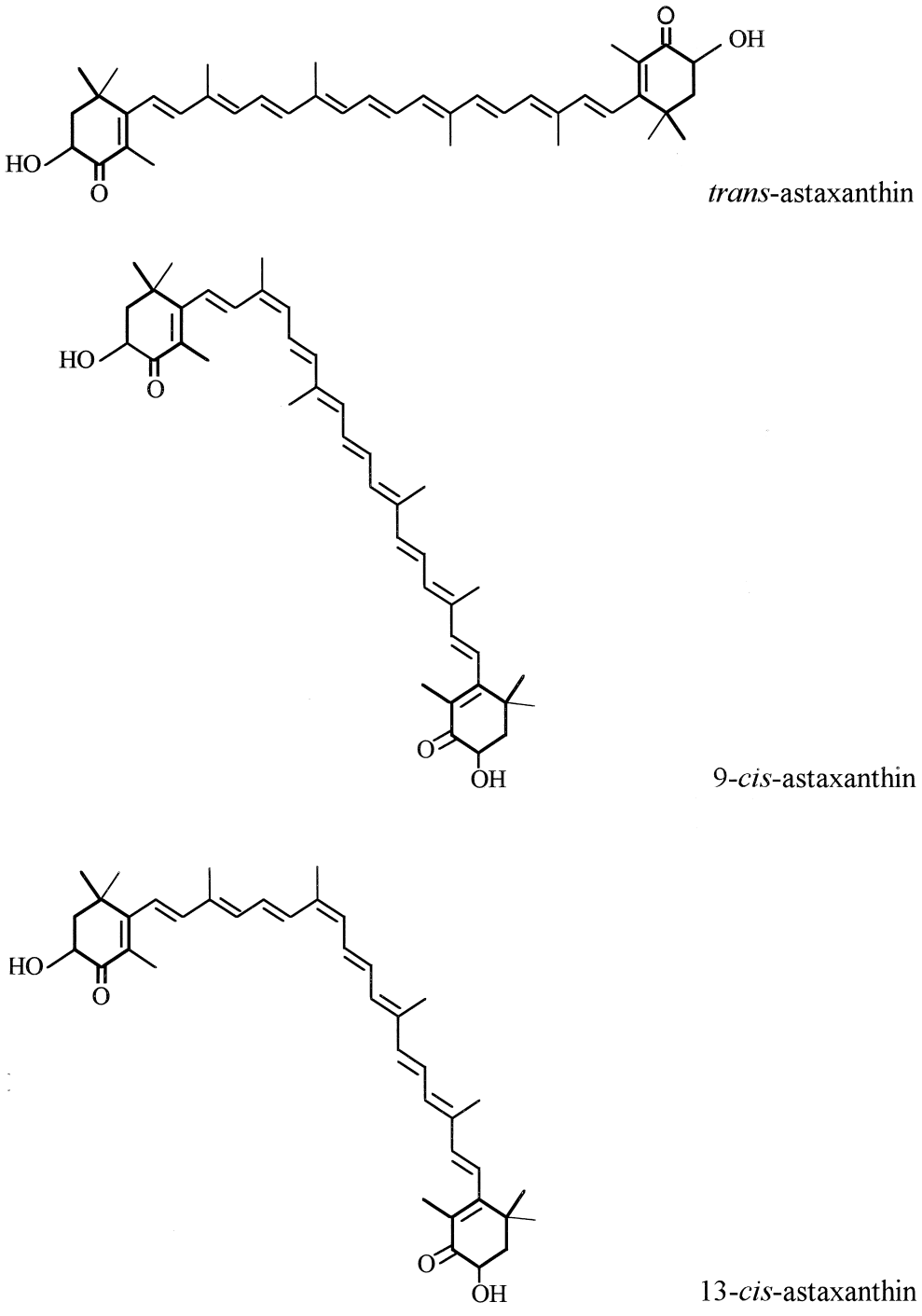

Fig. 1. Structures of *trans*-astaxanthin, 9-*cis*-astaxanthin, and 13-*cis*-astaxanthin.

$$\ln\{[(K_{iso}+1)\,c - C_0]/K_{iso}C_0\} = -(k_1 + k_2)\,t \quad (5)$$

Eq. (5) can also be written as:

$$c = C_0\,[1 + K_{iso}e^{-(k_1+k_2)\,t}]/(K_{iso}+1) \quad (6)$$

or

$$c = C_{cis}e^{-(k_1+k_2)\,t} + C_{trans} \quad (7)$$

Graphical methods can be employed to test the first-order equation for a reversible reaction and obtain the rate constant. If the isomerization reaction of *trans*-astaxanthin in organic solvents is a first order reversible reaction, according to Eq. (5), a plot of $\ln\{[(K_{iso}+1)\,c - C_0]/K_{iso}\,C_0\}$ versus t will give a straight line with a slope equal to $-(k_1+k_2)$.

Absolute temperature (T)-dependence of the rate constant (k) of the isomerization reaction can be described by the Arrhenius equation:

$$\ln k = -\frac{E_a}{RT} + \ln k_0 \quad (8)$$

Activation energy (E_a) can be estimated on the basis of linear regression analysis of $\ln k$ vs. $1/T$. In Eq. (8), R is universal gas constant (8.314 J K^{-1} mol^{-1}).

3. Results and discussion

In order to further improve the separation of 9-*cis*-astaxanthin and 13-*cis*-astaxanthin and to reduce the analysis time, a modified HPLC method was developed. A Waters Symmetry C_{18} column, which was shorter than a Beckman Ultrasphere C_{18} column used in our previous analysis (Yuan & Chen, 1999), and a modified mobile phase, in which the water content was increased from 4.5 to 7.0%, were applied. Further increasing the content of water in the mobile phase could improve the separation of astaxanthins by increasing their retention times. As shown in Figs. 2–4, although the content of water was increased, the retention time of astaxanthins on a Symmetry C_{18} column was less than that on an Ultrasphere C_{18} column (Yuan & Chen, 1999), and then the analysis time was shortened. The separations of *trans*-astaxanthin and *cis*-astaxanthins were similar in the two methods, but the separation of 9-*cis* and 13-*cis*-astaxanthins was improved by using the modified method (Figs. 2, 3 and 4b).

Different organic solvents have been used to investigate the isomerization of *trans*-astaxanthin in solutions and the results have shown that *trans*-astaxanthin dissolved in organic solvents could easily isomerize to its *cis*-isomers, mainly the 9-*cis*-astaxanthin and 13-*cis*-astaxanthin (Yuan & Chen, 1999). In the present experiment, the isomerization of *cis*-astaxanthins, which were prepared by semipreparative HPLC from the *cis-trans* mixture of astaxanthin, was also studied. It would be much easier to understand the isomerization mechanisms of *trans*-astaxanthin while a purified *cis*-isomer (9-*cis* or 13-*cis*-astaxanthin) was used as a starting compound for isomerization. But, it was difficult to obtain a single 9-*cis*- or 13-*cis*-astaxanthin, especially in enough amounts, by the present semipreparative HPLC method, in which 9-*cis*- or 13-*cis*-astaxanthin could not be separated well and only the mixture of two *cis*-isomers was obtained (Yuan & Chen, 2000). Two mixture solutions, in which the relative contents of 9-*cis*-astaxanthin were, respectively, 44 and 70%, were obtained. Fig. 2 shows the isomerization process of a mixture of 13-*cis*-astaxanthin (56%) and 9-*cis*-astaxanthin (44%) dissolved in dichloromethane after 7 (Fig. 2a) and 23 days (Fig. 2b) of isomerizations at ambient temperature. Fig. 3 shows the isomerization process of a mixture of *cis*-astaxanthins, in which the concentration of 9-*cis*-astaxanthin (70%) was much higher than that of 13-*cis*-astaxanthin (30%), after 1 (Fig. 3a) and 23 days (Fig. 3b) of isomerizations at ambient temperature. The results indicate that *cis*-astaxanthins in solution could also isomerize to produce *trans*-astaxanthin and further support the fact that the isomerization reaction of *trans*-carotenoids to *cis*-

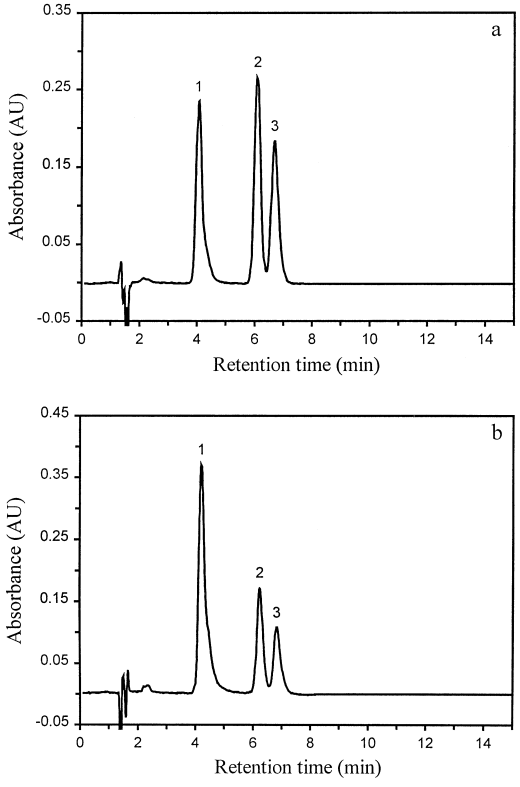

Fig. 2. Typical chromatograms of a mixture of 13-*cis*-astaxanthin (56%) and 9-*cis*-astaxanthin (44%) dissolved in dichloromethane after 7 days (a) and 23 days (b) of isomerizations at ambient temperature. Peaks: 1, *trans*-astaxanthin; 2, 9-*cis*-astaxanthin; 3, 13-*cis*-astaxanthin.

isomers was a reversible reaction (Subagio et al., 1998; Yuan & Chen, 1999).

In the isomerization mixture of *cis*-astaxanthin to *trans*-astaxanthin (Fig. 3), a peak in the tail of the *trans*-astaxanthin peak, which was not separated from *trans*-astaxanthin (peak 1) and had a maximum absorption wavelength of 470 nm, was found and tentatively identified as 7-*cis*-astaxanthin. The peak could also be seen in Figs. 2 and 4b, but it was relatively small in comparison with that in Fig. 3, indicating that this *cis*-isomer was formed perhaps from 9-*cis*-astaxanthin. If a purified 9-*cis*-astaxanthin could be obtained, it would be helpful to confirm whether 7-*cis*-isomer was formed from 9-*cis*-astaxanthin or not. Therefore, the method to prepare purified *cis*-astaxanthins should be studied further.

According to Bjerkeng and co-workers (Bjerkeng et al., 1997), in the quasi-equilibrium mixture, *trans*-astaxanthin, 9-*cis*-astaxanthin, 13-*cis*-astaxanthin, and a small amount of di-*cis*-astaxanthins were present. As in different solvents (Yuan & Chen, 1999), at different temperatures the relative contents of 9-*cis*- and 13-*cis*-astaxanthins formed during isomerization were also different. As shown in Fig. 4a, at 10°C, 13-*cis*-astaxanthin was the main *cis*-isomer from *trans*-astaxanthin, but 9-*cis*-astaxanthin was almost not detected. At the beginning of isomerization or under a mild isomerization condition, for example, at 10°C (Fig. 4a), no di-*cis*-astaxanthin or other *cis*-isomer peaks were found. However, in Fig. 4b, a very small peak at ~5 min was detected, indicating that some other *cis*-isomers could be formed in addition to 9-*cis*-astaxanthin and 13-*cis*-astaxanthin under a strong isomerization condition (for example, at 70°C for 10 h).

In the present experiment, the concentrations (%) of *trans*-astaxanthin (C_{trans}), 9-*cis*-astaxanthin ($C_{9\text{-}cis}$), and 13-*cis*-astaxanthin ($C_{13\text{-}cis}$) at the quasi-equilibrium were 50.3, 25.5, and 24.2% (Fig. 2b), respectively. The content of *trans*-astaxanthin (50.3%) was slightly in excess of that (49.9%) reported for the quasi-equilibrium mixture of astaxanthin by others (Bjerkeng et al., 1997). Although the relative content of 9-*cis*-astaxanthin was different from that reported by Bjerkeng and co-workers (Bjerkeng et al., 1997), the total content of *cis*-astaxanthins was almost consistent. Therefore, the equilibrium constant for the reversible isomerization reaction could be calculated by Eq. (2):

$$K_{\text{iso}} = k_1/k_2 = C_{cis}/C_{trans} = (25.5 + 24.2)/50.3 = 0.9881$$

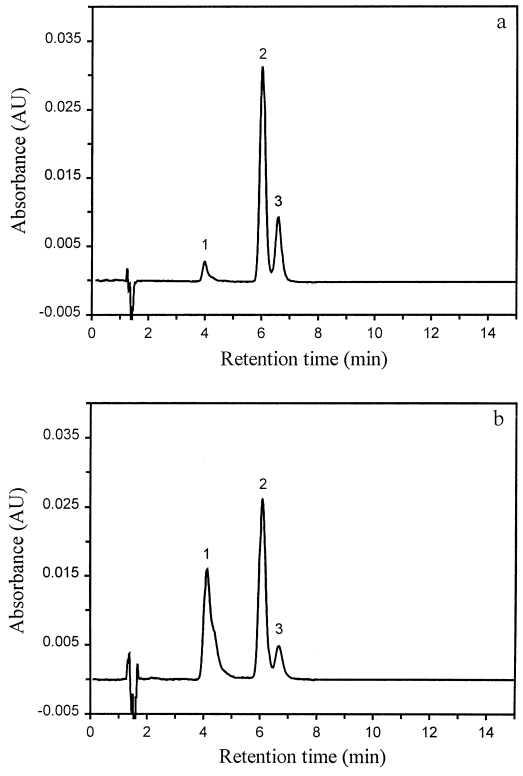

Fig. 3. Typical chromatograms of a mixture of 9-*cis*-astaxanthin (70%) and 13-*cis*-astaxanthin (30%) dissolved in dichloromethane after 1 day (a) and 23 days (b) of isomerization at ambient temperature. Peaks: 1, *trans*-astaxanthin; 2, 9-*cis*-astaxanthin; 3, 13-*cis*-astaxanthin.

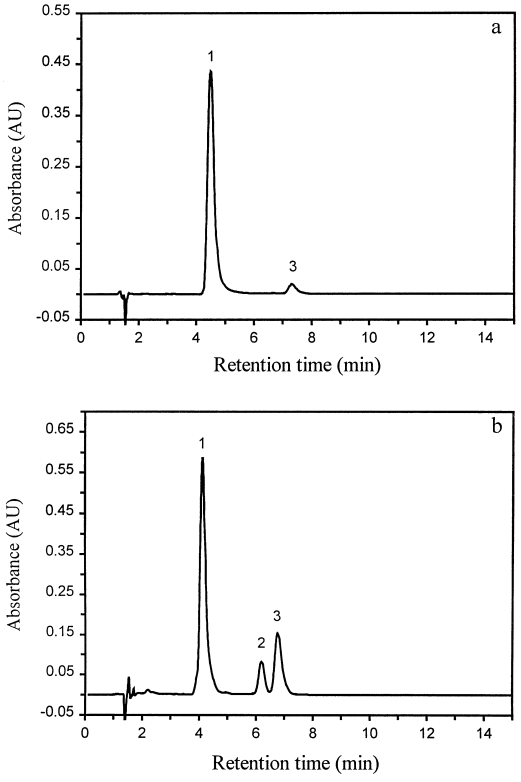

Fig. 4. Typical chromatograms of *trans*-astaxanthin in a mixture of (a) dichloromethane (25%) and methanol (75%) at 10°C for 750 h of isomerization and (b) in dimethyl sulfoxide at 70°C for 10 h of isomerization. Peaks: 1, *trans*-astaxanthin; 2, 9-*cis*-astaxanthin; 3, 13-*cis*-astaxanthin.

For a first order reversible isomerization reaction, when the initial concentration (%) at $t=0$ is $C_0=100\%$, Eq. (5) becomes:

$$\ln(0.020c - 1.012) = -(k_1 + k_2)\, t \quad (9)$$

The experimental data at different temperatures were obtained by determining the concentrations of astaxanthins over time. The results of the linear regression analysis showed that the plots of $\ln(0.020c-1.012)$ versus time t gave a straight line, indicating that the isomerization reaction of *trans*-astaxanthin in organic solvents was a first order reversible reaction. Table 1 shows the first-order reaction rate constants (h^{-1}) and correlation coefficients for the reversible isomerization reactions of *trans*-astaxanthin in dimethyl sulfoxide at 20–70°C. Because the freezing point of dimethyl sulfoxide is at 18.0–18.4°C, a mixture of dichloromethane (25%) and methanol (75%) was used as the solvent for the isomerization reaction at a low temperature (e.g. 10°C). The reaction rate constant (h^{-1}) for the isomerization reaction at 10°C is also shown in Table 1. As can be seen from Table 1, correlation coefficients for the linear regression analysis were situated between 0.9807 and 0.9990 for the isomerization reactions at different temperatures, indicating that the isomerization reaction of *trans*-astaxanthin could adequately be represented by the first-order reversible reaction kinetics.

Table 1
First-order reversible reaction rate constants (h^{-1}) of the isomerization reaction of *trans*-astaxanthin in dimethyl sulfoxide at different temperature

Temperature (°C)	k_1+k_2	r
10[a]	0.0002	0.9990
20	0.0022	0.9952
30	0.0083	0.9944
35	0.0146	0.9947
40	0.0273	0.9944
45	0.0410	0.9916
50	0.0896	0.9952
60	0.2484	0.9807
70	0.7643	0.9852

[a] Dissolved in a mixture of dichloromethane (25%) and methanol (75%).

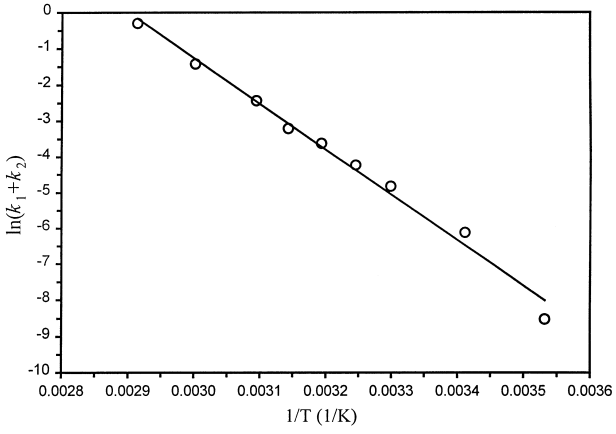

Fig. 5. Relationship between the isomerization rate constants and reaction temperatures.

Temperature dependence of the isomerization reaction rate constants could adequately be described by the Arrhenius equation [Eq. (8)]. Fig. 5 shows a linear relationship between $\ln(k_1+k_2)$ and reciprocal absolute temperature $(1/T)$: $\ln(k_1+k_2) = -12732/T + 36.952$ ($r=0.9948$). Therefore, corresponding activation energy equalled 105.8 ± 4.2 kJ/mol for the isomerization reaction of *trans*-astaxanthin.

The change in the concentration (%) of *trans*-astaxanthin in organic solvents during the isomerization reaction could be represented by Eqs. (6) and (7), which was obtained by using the first-order reversible reaction kinetics model. As shown in Figs 6 and 7, the experiment data fitted the model ($c = 49.7 \times e^{-(k_1+k_2)t} + 50.3$) quite well.

Since

$$C_{trans} = C_0 - C_{cis} = C_0 - K_{iso} C_{trans}$$

substitution of this equation and Eq. (2) into Eq. (3) leads to:

$$dc/dt = -(k_1 + k_2)(c - C_{trans}) \quad (10)$$

Eq. (10) indicates that the rate for isomerization of *trans*-astaxanthin (dc/dt) is proportional to the distance removed from equilibrium ($c-C_{trans}$), and equilibrium is approached with a rate constant equal to k_1+k_2 (Drenth & Kwart, 1980). As shown in Figs. 6 and 7, under a mild condition for isomerization, for example,

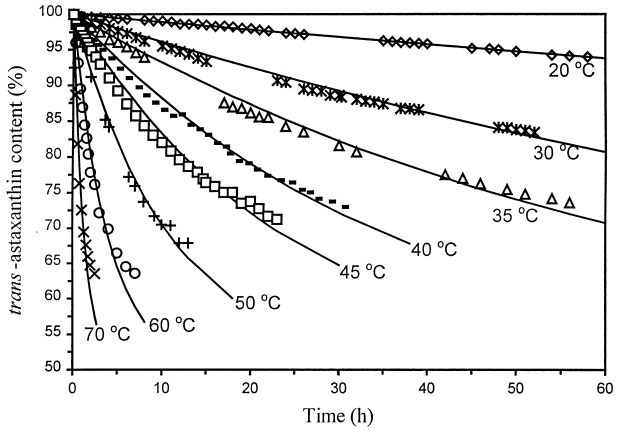

Fig. 6. Changes in concentration (%) of *trans*-astaxanthin during the isomerization process of *trans*-astaxanthin in dimethyl sulfoxide at different temperatures between 20 and 70°C. The symbols and the lines represent the experimental data and the calculated values by the kinetics model proposed, respectively.

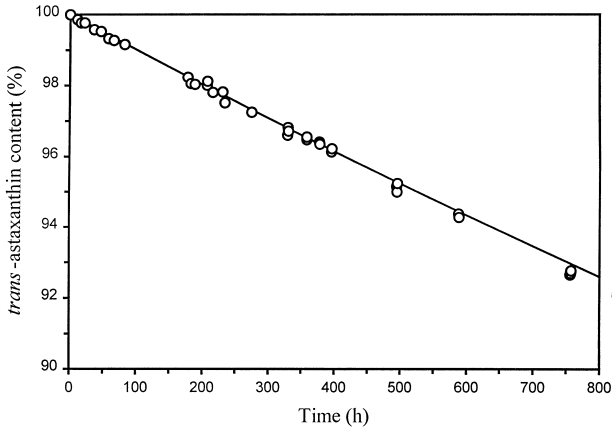

Fig. 7. Change in concentration (%) of *trans*-astaxanthin during the isomerization process of *trans*-astaxanthin in a mixture of dichloromethane (25%) and methanol (75%) at 10°C. The symbol and the line represent the experimental data and the calculated values by the kinetics model proposed, respectively.

at a low temperature (< 35°C), the isomerization reaction was slow and the decrease of *trans*-astaxanthin concentration was small. The plots of the content (%) of *trans*-astaxanthin versus time appear to be a straight line, indicating that the isomerization reaction in the initial stages at low temperature (< 35°C) might be represented approximately by zero-order reaction kinetics (Yuan & Chen, 1999).

In conclusion, in organic solvents, *trans*-astaxanthin could be easily isomerized to 13-*cis*-astaxanthin and 9-*cis*-astaxanthin and *cis*-astaxanthins could also be isomerized to produce *trans*-astaxanthin and the other *cis*-astaxanthins. At high temperature, some other *cis*-isomers might be produced in addition to 9-*cis*- and 13-*cis*-astaxanthins. The isomerization of *trans*-astaxanthin to *cis*-astaxanthins appears to be a first-order reversible reaction and could be represented by the first-order reversible reaction kinetics model.

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