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# Relationship between chlorophyll *a* and β-carotene in a lipid-containing model system during heating

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The relationship between chlorophyll *a* (Chl *a*) and β-carotene during heating in the presence of fatty acid esters, methyl stearate, methyl oleate and methyl linoleate, was studied. Mixtures of Chl *a*, β-carotene and fatty acid esters were heated at 60°C and 120°C for varied lengths of time. Isomerization and degradation reactions of Chl *a* and β-carotene were monitored using HPLC with diode array detection. Three isomers of Chl *a* and four *cis* isomers of β-carotene were detected. Both the degradations of total amounts of Chl *a* and β-carotene during heating fit the first-order model. The degradation rates of total amounts of Chl *a* and β-carotene were highest in methyl stearate, followed by in methyl oleate and methyl linoleate. In the presence of fatty acid esters Chl *a* is more susceptible to isomerization and degradation than β-carotene during heating. © 1998 Elsevier Science Ltd. All rights reserved

## INTRODUCTION

The major pigments in edible vegetable oil include carotenoids and chlorophylls, both of which can be responsible for colour and oxidation stability of oil. (Endo *et al.*, 1984, 1985; Usuki *et al.*, 1984; Fakourelis *et al.*, 1987). In addition, the presence of a small amount of free fatty acid in vegetable oil can also be responsible for formation of off-flavour caused by lipid oxidation (Frankel, 1985; Warner and Frankel, 1987). Therefore, the elucidation of the relationship between carotenoids and chlorophylls in the presence of saturated and unsaturated fatty acids during heating of oil is very important.

Carotenoids can not only lose provitamin A activity but also colour intensity because of formation of *cis* isomers during heating (Chen and Chen, 1993; Chen *et al.*, 1995). Likewise, chlorophylls can be degraded or converted to pheophytins, pyropheophytins or chlorophyll oxidation products, depending on temperature and length of treatment by heating (Schwartz *et al.*, 1981; Khachik *et al.*, 1986; Schwartz and Lorenzo, 1991; Chen and Chen, 1993). The formation of pheophytins or pyropheophytins from chlorophylls is often accompanied by a colour change from green to olive-brown (Khachik *et al.*, 1986; Chen and Chen, 1993). However, in the presence of saturated and unsaturated fatty acids, the degradation and isomerization patterns of carotenoids and chlorophylls may be completely different during heating of vegetable oil. Baloch *et al.* (1977) studied the effect of heating on β-carotene stability in palm oil and found that the concentrations of β-carotene and unsaturated fatty acids, oleic acid and linoleic acid, decreased with increasing temperature. However, the decreased amount of β-carotene was greater than that of linoleic acid, indicating that the former is more susceptible to oxidation than the latter. Hdieani *et al.* (1992) further reported that β-carotene can be completely destroyed at 200°C for 60 minutes. The effect of heating on stability of chlorophylls in cottonseed oil was studied by Taha *et al.* (1988), who found that chlorophylls can be completely destroyed at 180°C for 60 min. Also, the formation rate of peroxide was found to be lower than that of the degradation rate. As no information is available as to the effect of heating on the isomerization and degradation of carotenoids and chlorophylls in edible vegetable oil, it is necessary to elucidate the relationship between β-carotene and chlorophyll *a* in the presence of fatty acid esters during heating. The purposes of this study were to determine the isomerization and degradation of Chl *a* and β-carotene in the presence of fatty acid esters and postulate their relationships during heating.

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## MATERIALS AND METHODS

### Materials

Chlorophyll *a* (Chl *a*) standard with a purity of approx. 100% and all-*trans*-$\beta$-carotene standard with a purity of 95% were purchased from Sigma Co. (St Louis, MO, USA). Methyl stearate, methyl oleate and methyl linoleate were obtained from Nucheck Co. (Elysian, MN, USA). HPLC-grade solvents including methanol, tetrahydrofuran, acetone, *n*-hexane and acetonitrile were from Mallinckrodt Co. (Paris, Ky, USA). Solvents used for extraction including *n*-hexane, acetone and methanol were analytical grade, and were from Mallinckrodt Co. HPLC-grade solvents and deionized water were filtered through a 0.2-μm membrane filter and degassed by sonication prior to use. Sep-Pak $C_{18}$ cartridge containing 500 mg packing material was from J. T. Baker Co. (Phillipsburg, NJ, USA). A Vydac 201TP54 $C_{18}$ column (250×4.6 mm i.d.) packed with 5 $\mu$m particle size (Hesperia, CA, USA) was used. Chlorophyll *a*′ (Chl *a*′) standard was prepared using a method described by Katz *et al*. (1968).

### Instrumentation

The HPLC instrument consists of an SSI 222D pump (Scientific System Inc., State College, PA, USA) and a Linear 206 photodiode array detector (Linear Instrument, Reno, USA). An Advantec SF-2120 fraction collector (Tokyo, Japan) was used to collect eluates. The data were stored and processed with an Axxiom 727 dual-channel chromatography data system (Axxiom Chromatography Inc., Calabasas, CA, USA). Spectrophotometric determinations were made with a Beckman DU-70 double-beam Spectrophotometer (Irvine, CA, USA). The funnel shaker (Type VS-6) was from Hsiang-Tai Co. (Taipei, Taiwan).

### Heating of Chl *a* and $\beta$-carotene in the presence of fatty acid esters

One working solution of 1000 $\mu$g ml$^{-1}$ Chl *a* was prepared by dissolving 10 mg Chl *a* in 10 ml acetone, while three working solutions of 1000 $\mu$g ml$^{-1}$, $\beta$-carotene containing fatty acid esters were prepared by dissolving 1 mg $\beta$-carotene in 1 ml methyl oleate, 1 ml methyl linoleate, and 1 ml *n*-hexane containing 1 g methyl stearate, respectively. Aliquots of 60 $\mu$l Chl *a* and 60 $\mu$l $\beta$-carotene were mixed in a 15-ml test tube with a screw cap on the top, and 880 $\mu$l of methyl stearate, methyl oleate and methyl linoleate each was added to bring about a total volume of 1000 $\mu$l for each methyl ester solution. A total of 84 tubes were used, of which 42 were heated at 60°C for 0, 5, 10, 20, 30, 60 and 90 min and the other 42 heated at 120°C for 0, 1, 2, 5, 10, 15 and 30 min in an oven. All the sample tubes were wrapped with aluminium foil and stored at refrigerated temperature prior to heating. Six tubes, which contained the solution of methyl stearate, methyl oleate and methyl linoleate in duplicate, were randomly collected at time intervals and inserted into an ice box to terminate the reaction. After heating all the treated samples were dissolved in 3 ml *n*-hexane for extraction.

### Extraction of Chl *a*, $\beta$-carotene and their isomers

A mixture of 0.5 g magnesium oxide and 0.5 g diatomaceous earth was poured onto a Sep-Pak $C_{18}$ cartridge about 0.5 cm high. A 3 ml sample was poured into the Sep-Pak $C_{18}$ cartridge, which was previously activated by 6 ml methanol and 12 ml *n*-hexane. The solutions containing all-*trans*-$\beta$-carotene and its *cis* isomers, and methyl stearate, methyl oleate, and methyl linoleate were first eluted with 24 ml *n*-hexane. Chl *a* and its isomers were next eluted with 6 ml acetone. The first portion of eluate was poured into a 250-ml flask, and 30 ml of methanolic potassium hydroxide (40%), 5 ml of tetrahydrofuran, and 10 ml of *n*-hexane were added and stirred vigorously at room temperature for saponification for 2 h using a funnel shaker. Then the mixture was poured into a separatory funnel, and 100 ml deionized water was added to the funnel four times to remove saponifiables and water-soluble impurities. The upper layer of solution was collected and filtered through anhydrous sodium sulfate. After evaporation of solvent with nitrogen gas, the sample was dissolved in methanol–acetonitrile–tetrahydrofuran (57:42:1, v/v/v) and filtered through a 0.2-$\mu$m membrane filter. A 20 $\mu$l sample was collected for HPLC analysis. A second portion of eluate was collected and the solvent was evaporated with nitrogen gas. Then the sample was dissolved in methanol–acetonitrile-deionized water (94:5:1, v/v/v) and filtered through a 0.2-$\mu$m membrane filter, and a 20 $\mu$l sample was collected for HPLC analysis.

### Identification and quantification of Chl *a*, $\beta$-carotene and their isomers

The identification of all-*trans*-$\beta$-carotene, Chl *a* and Chl *a*′ were conducted by comparison of retention time and absorption spectra with reference standards. In addition, the identification of *cis*-$\beta$-carotene isomers were based on spectral characteristics and Q ratios as described in several previous studies (Chen and Chen, 1993, 1994; Chen *et al*., 1994, 1995). Chl *a* isomers I and II were tentatively identified based on spectral characteristics and retention behaviour on the HPLC chromatogram as reported by Chen and Chen (1993). The quantitation was carried out by an external calibration method. Eight concentrations ranged from 10 to 100 ppm of all-*trans*-$\beta$-carotene, Chl *a* and Chl *a*′ were each prepared and the calibration curves for each was obtained by plotting area against concentration. The calibration curves gave good linearity for each ($r^2 = 0.9893$ for all-*trans*-$\beta$-carotene, 0.9974 for Chl *a*

and 0.9758 for Chl *a*′). As no standards of *cis*-β-carotene and Chl *a* isomers I and II are commercially available, *cis* isomers of β-carotene were calculated as all-*trans*-β-carotene while Chl *a* isomers I and II were calculated as Chl *a* equivalents. Based on a report by Chen *et al.* (1993), the purity of each peak was assessed by collecting spectra from the downslope, upslope and apex portions of the peak, and the spectra were normalized and overlaid to see if there was any difference in curve shape. The purity of each peak could be assessed to be close to 100% if no difference in curve shape was observed. Duplicate analyses were conducted and the mean values were determined. The rate constants of degradation of total amount of Chl *a* and β-carotene were also determined using a method described by Chen *et al.* (1994). Unlike β-carotene, only the degradation data of Chl *a* and its isomers during 120°C heating over a period of 15 min were collected for kinetic analysis because they were found to be completely destroyed after 120°C heating over 30 min.

## RESULTS AND DISCUSSION

### HPLC separation of β-carotene, Chl *a* and their isomers

Figure 1 shows the HPLC chromatogram of all-*trans*-β-carotene and its four *cis* isomers, 9-*cis*-, 13-*cis*-, 15-*cis*- and 13,15-di-*cis* β-carotene in the presence of Chl *a* and methyl oleate during heating at 120°C for 30 min. Separation was complete within 12 min by employing a ternary solvent system of methanol–acetonitrile–tetrahyrofuran (57:42:1, v/v/v) with flow rate at 1.0 ml $min^{-1}$, detection wavelength at 450 nm and sensitivity at 0.02 AUFS. Figure 2 shows the HPLC chromatogram of Chl *a* and its three isomers, Chl *a*′, Chl *a* isomer I and Chl *a* isomer II in the presence of β-carotene and methyl oleate during heating at 60°C for 90 min. Separation was complete within 13 min by employing a ternary solvent system of ethanol–acetonitrile–water (94:5:1, v/v/v) with flow rate at 0.8 ml $min^{-1}$, detection wavelength at 660 nm and sensitivity at 0.02 AUFS. The purity of each peak was assessed to be close to 100% as no difference in curve shape was observed.

### Percentage change of β-carotene and its *cis* isomers during heating in the presence of fatty acid esters and Chl *a*

Table 1 shows the percentage changes of β-carotene and its *cis* isomers in the presence of fatty acid esters and Chl *a* during heating at 60°C for 90 min. β-carotene standard was found to contain 84% all-*trans*, 3.2% 13,15-di-*cis*, 1.3% 15-*cis*, 4.1% 9-*cis* and 7.4% 13-*cis* prior to heating. With the exception of all-*trans*- and

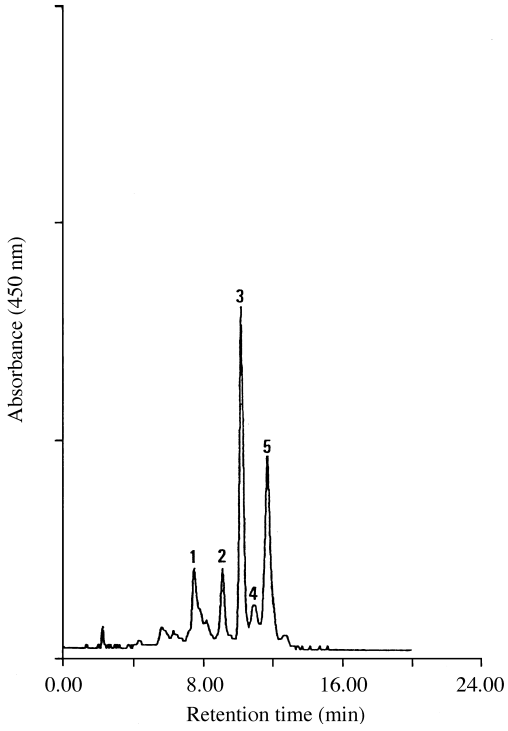


**Fig. 1.** HPLC chromatogram of all-*trans*-β-carotene and its *cis* isomers in the presence of Chl *a* and methyl oleate during heating at 120°C for 30 min. Chromatographic condition described in text. Peaks: 1 = 13,15-di-*cis*-β-carotene, 2 = 15-*cis*-β-carotene, 3 = all-*trans*-β-carotene, 4 = 9-*cis*-β-carotene, 5 = 13-*cis*-β-carotene.

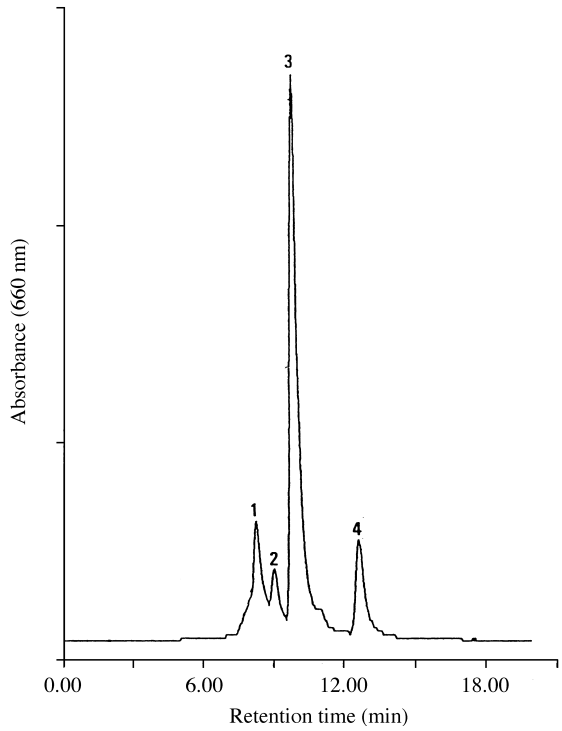


**Fig. 2.** HPLC chromatogram of Chl *a* and its isomers in the presence of β-carotene and methyl oleate during heating at 60°C for 90 min. Chromatographic condition described in text. Peaks: 1 = Chl *a* isomer I, 2 = Chl *a* isomer II, 3 = Chl *a*, 4 = Chl *a*′.

**Table 1. Percentage changes of all-*trans*-β-carotene and its *cis* isomers in the presence of Chl *a* and fatty acid esters during heating at 60 and 120°C for varied lengths of time[a]**

| Temp | Time (min) | Methyl stearate | | | | | Methyl oleate | | | | | Methyl linoleate | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | β-Carotene | | | | | β-Carotene | | | | | β-Carotene | | | | |
| | | 13,15-di-*cis* | 15-*cis* | all-*trans* | 9-*cis* | 13-*cis* | 13,15-di-*cis* | 15-*cis* | all-*trans* | 9-*cis* | 13-*cis* | 13,15-di-*cis* | 15-*cis* | all-*trans* | 9-*cis* | 13-*cis* |
| 60°C | 0 | 3.2 | 1.3 | 84.0 | 4.1 | 7.4 | 3.2 | 1.3 | 84.0 | 4.1 | 7.4 | 3.2 | 1.3 | 84.0 | 4.1 | 7.4 |
| | 5 | 4.2 | 1.7 | 80.5 | 1.2 | 12.4 | 2.2 | 2.6 | 83.9 | 2.4 | 8.9 | 3.0 | 2.1 | 83.3 | 3.4 | 8.2 |
| | 10 | 3.7 | 7.0 | 70.3 | 1.2 | 17.8 | 2.3 | 3.1 | 83.8 | 1.7 | 9.1 | 3.5 | 1.8 | 69.8 | 2.7 | 22.2 |
| | 20 | 4.1 | 3.4 | 59.9 | 0.7 | 31.9 | 3.9 | 4.6 | 71.7 | 1.0 | 18.8 | 3.7 | 2.2 | 63.8 | 1.0 | 29.3 |
| | 30 | 3.9 | 5.4 | 51.8 | 1.2 | 37.7 | 4.9 | 5.2 | 68.2 | 1.2 | 20.5 | 4.4 | 2.1 | 61.1 | 1.2 | 31.2 |
| | 60 | 4.5 | 5.0 | 50.4 | 1.7 | 38.4 | 4.8 | 1.1 | 57.8 | 1.2 | 35.1 | 4.9 | 3.1 | 59.4 | 1.3 | 31.3 |
| | 90 | 3.9 | 4.9 | 47.5 | 2.7 | 41.0 | 4.8 | 1.1 | 54.6 | 1.3 | 38.2 | 9.2 | 5.0 | 56.5 | 1.3 | 28.0 |
| 120°C | 0 | 3.2 | 1.3 | 84.0 | 4.1 | 7.4 | 3.2 | 1.3 | 84.0 | 4.1 | 7.4 | 3.2 | 1.3 | 84.0 | 4.1 | 7.4 |
| | 1 | 6.3 | 9.2 | 69.0 | 5.4 | 10.1 | 5.8 | 12.2 | 53.0 | 14.9 | 14.1 | 14.2 | 8.8 | 62.0 | 6.7 | 8.3 |
| | 2 | 6.5 | 20.8 | 63.0 | 4.0 | 5.7 | 15.9 | 11.1 | 52.0 | 8.8 | 12.2 | 14.1 | 4.6 | 61.0 | 9.8 | 10.5 |
| | 5 | 8.4 | 14.2 | 54.0 | 9.2 | 14.2 | 16.0 | 10.0 | 52.0 | 7.9 | 14.1 | 12.7 | 10.7 | 60.0 | 6.2 | 10.4 |
| | 10 | 19.1 | 16.2 | 39.3 | 14.1 | 11.3 | 13.0 | 9.8 | 47.2 | 11.2 | 18.8 | 16.0 | 5.4 | 56.0 | 8.6 | 14.0 |
| | 15 | 19.3 | 16.5 | 37.7 | 8.4 | 18.1 | 13.5 | 10.2 | 46.1 | 11.5 | 18.7 | 17.9 | 5.1 | 52.2 | 8.8 | 16.0 |
| | 30 | 19.1 | 16.3 | 33.2 | 12.2 | 19.2 | 13.2 | 10.1 | 47.0 | 11.0 | 18.7 | 19.7 | 7.2 | 51.0 | 7.7 | 14.4 |

[a]Averages of duplicate analyses.

13-*cis*-β- carotene, the amounts of 9-*cis*-, 15-*cis*- and 13,15-di-*cis*-β-carotene did not show consistent change during heating in the presence of methyl stearate and Chl *a*. After heating for 90 min, the amount of all-*trans*-β-carotene decreased by 36.5% while the amount of 13-*cis*-β-carotene increased by 33.6%. This result implied that 13-*cis*-β-carotene was the major β-carotene isomer formed during heating. The degradation of the total amount of β-carotene in the presence of methyl stearate and Chl *a* during heating may fit the first-order model because a linear correlation ($r^2 = 0.9368$) was observed for the plot of the logarithm of the total β-carotene concentration vs time, and the degradation rate constant was 0.022 ($min^{-1}$).

The percentage changes of β-carotene and its *cis* isomers during heating in the presence of methyl oleate and Chl *a* at 60°C for 90 min are also shown in Table 1. After 90 min heating the amounts of all-*trans*-, 9-*cis*- and 15-*cis*-β-carotene decreased by 29.4, 2.8 and 0.2%, respectively, while the amounts of 13-*cis*-β-carotene increased by 30.8% and 13,15-di-*cis*-β-carotene increased by 1.6%. The formation of 13,15-di-*cis*-β-carotene may be due to conversion of 13-*cis*-β-carotene or 15-*cis*-β-carotene as reported by Chen *et al.* (1994, 1995). As the conversion between all-*trans*-β-carotene and its mono-*cis* isomers was reported to be reversible, the decreases in 9-*cis*- and 15-*cis*-β-carotene implied that both may be converted to all-*trans*-β-carotene or undergo degradation simultaneously during heating (Chen *et al.*, 1994). Likewise, 13-*cis*-β-carotene may also be converted to all-*trans*- or 13,15-di-*cis*-β-carotene, or undergo degradation simultaneously. This would explain why some inconsistent percentage changes were observed for 9-*cis*-, 15-*cis*- and 13,15-di-*cis*-β-carotene during heating. Furthermore, it may be postulated that 15-*cis*-β-carotene is more susceptible to colliding with other molecules to form another mono-*cis* or di-*cis* isomer, because 15-*cis*-β-carotene is a larger molecule and occupies more space than 9-*cis*- or 13-*cis*-β-carotene. It was also observed that the presence of methyl oleate may facilitate formation of the di-*cis* isomer of β-carotene during heating. The degradation of the total amount of β-carotene in the presence of methyl oleate and Chl *a* during heating may fit the first-order model because a linear correlation ($r^2 = 0.9689$) was observed for the plot of the logarithm of the total β-carotene concentration vs time, and the degradation rate constant was 0.013 ($min^{-1}$).

The percentage changes of β-carotene and its *cis* isomers during heating in the presence of methyl linoleate and Chl *a* at 60°C for 90 min are shown in Table 1. Only minor changes were observed for 9-*cis*-β-carotene and 15-*cis*-β-carotene during heating. In contrast, the amounts of all-*trans*-β-carotene decreased by 27.5% after 90 min heating, while the amounts of 13-*cis*-β-carotene and 13,15-di-*cis*-β-carotene increased by 20.6 and 6.0 %, respectively. This result implied that the presence of methyl linoleate may facilitate formation of 13,15-di-*cis*-β-carotene during heating. The degradation of the total amount of β-carotene in the presence of methyl linoleate and Chl *a* during heating may fit the first-order model because a linear correlation ($r^2 = 0.9440$) was observed for the plot of the logarithm of the total β-carotene concentration vs time, and the degradation rate constant was 0.006 ($min^{-1}$).

By comparison of the results shown above, it can be found that, in the presence of Chl *a*, the degradation rate of the total amount of β-carotene was highest in methyl stearate, followed by in methyl oleate and methyl linoleate. This is probably because methyl linoleate can compete with β-carotene for oxygen, and thus less oxygen was available to react with β-carotene

in the presence of methyl linoleate during heating, which in turn results in the lowest degradation rate.

The percentage changes of β-carotene and its *cis* isomers during heating in the presence of methyl stearate and Chl *a* at 120°C for 30 min are shown in Table 1. After 120°C heating for 30 min, the amounts of all-*trans*-β-carotene decreased by 50.8%. In contrast, the amounts of 13-*cis*-, 9-*cis*-, 15-*cis*-, and 13,15-di-*cis*-β-carotene increased by 11.8, 8.1, 15.0 and 15.9%, respectively. It was also found that, with the exception of 13-*cis*-β-carotene, the formations of 9-*cis*-, 15-*cis*- and 13,15-di-*cis*-β-carotene were greater at 120 than 60°C. The large increase of 13,15-di-*cis*-β-carotene at 120°C can be attributed to the conversion of 13-*cis*-β-carotene. The inconsistent change of 15-*cis*-β-carotene occurred during the initial period of heating, and then gradually approached equilibrium. It is also possible that 15-*cis*-β-carotene can be an intermediate product during formation of 13,15-di-*cis*-β-carotene. The degradation rate of total amount of β-carotene in the presence of methyl stearate and Chl *a* during heating may fit the first-order model because a linear correlation ($r^2 = 0.9195$) was observed for the plot of the logarithm of the total β-carotene concentration vs time, and the degradation rate constant was 0.082 ($min^{-1}$).

The percentage changes of β-carotene and its cis isomers during heating in the presence of methyl oleate and Chl *a* at 120°C for 30 min are shown in Table 1. After 120°C heating for 30 min, the amount of all-*trans*-β-carotene decreased by 37% while the amounts of 9-*cis*-, 13-*cis*-, 15-*cis*- and 13,15-di-*cis*-β-carotene increased by 6.9, 11.3, 8.8 and 10.0%, respectively. However, inconsistent percentage changes were observed for these isomers during heating. This result indicated that the reversible conversion between all-*trans*-β-carotene and its mono-*cis* isomer is a very complex phenomenon. The degradation rate of total amount of β-carotene in the presence of methyl oleate and Chl *a* during heating may fit the first-order model because a linear correlation ($r^2 = 0.9595$) was observed for the plot of logarithm of the total β-carotene concentration vs time and the degradation rate constant was 0.042 ($min^{-1}$).

The percentage changes of β-carotene and its *cis* isomer during heating in the presence of methyl linoleate and Chl *a* at 120°C for 30 min are shown in Table 1. The amount of all-*trans*-β-carotene decreased by 33% after 120°C heating for 30 min, while the amounts of 9-*cis*-, 13-*cis*-, 15-*cis*-, and 13,15-di-*cis*-β-carotene increased by 3.6, 7.0, 5.9 and 16.5%, respectively. Nevertheless, inconsistent percentage changes were observed for these isomers during heating. The degradation rate of total amount of β-carotene in the presence of methyl linoleate and Chl *a* during heating may fit the first-order model because a linear correlation ($r^2 = 0.9103$) for the plot of logarithm of the total β-carotene concentration vs time was observed, and the degradation rate constant was 0.019 ($min^{-1}$).

By comparison of the results shown above, it can be found that, in the presence of Chl *a*, the degradation rate of total amount of β-carotene was highest in methyl stearate, followed by in methyl oleate and methyl linoleate. As explained above, in the presence of methyl linoleate less oxygen was available to react with β-carotene, which in turn results in the lowest degradation rate. In addition, it has been reported that methyl linoleate is more susceptible to reacting with free radicals than β-carotene (Arya *et al*., 1979; Carnevale *et al*., 1979; Ramakrishnan and Francis, 1979), and hence the reaction between β-carotene and free radicals can be minimized in the presence of methyl linoleate. In contrast to this result, Baloch *et al*. (1977) reported that β-carotene is more unstable than linoleic acid, and thus linoleic acid can be protected in the presence of β-carotene during heating. This difference can be attributed to the types of samples used, i.e the authors (Baloch *et al*., 1977) used palm oil as reference samples, which may contain many undesirable components, and thus the results can be greatly affected.

### Percentage changes of Chl *a* during heating in the presence of β-carotene and fatty acid esters

Table 2 shows the percentage changes of Chl *a* and its isomers during heating in the presence of β-carotene and fatty acid esters at 60°C for 90 min. Chl *a* was found to contain 9.1% Chl *a* isomer I and 8.2 % Chl *a* isomer II prior to heating. The amount of Chl *a* decreased with increasing heating time, and the decreased percentage was 21.7% after 90 min heating in the presence of methyl stearate and β-carotene. In contrast, the amounts of Chl *a* isomer I and Chl *a*′ increased by 9.9 and 14.0%, respectively. For Chl *a* isomer II, it showed inconsistent percentage change during heating, indicating that this isomer may be converted back to Chl *a* or undergo degradation simultaneously. It was also found that Chl *a*′ can be more easily formed than the other two isomers. As Chl *a* isomers I and II are probably oxidation products formed during heating of Chl *a* (Chen and Chen, 1993), it can be inferred from this study that Chl *a* can be more susceptible to epimerization than oxidation during heating. The degradation rate of total amount of Chl *a* during heating in the presence of methyl stearate and β-carotene during heating may fit the first-order model because a linear correlation ($r^2 = 0.9749$) for the plot of logarithm of total Chl *a* concentration vs time was observed, and the degradation rate constant was 0.019 ($min^{-1}$).

The percentage changes of Chl *a* and its isomers during heating in the presence of β-carotene and methyl oleate at 60°C for 90 min are shown in Table 2. The amounts of Chl *a* and Chl *a* isomer II decreased by 15.5 and 6.1% after 90 min heating, respectively, while the amounts of Chl *a*′ and Chl *a* isomer I increased by 17.2 and 4.4%. With heating time at 60 min the whole system gradually approached equilibrium. The degradation of total amount of Chl *a* in the presence of β-carotene and methyl oleate during heating may fit the first-order

**Table 2. Percentage changes of Chl *a* and its isomers in the presence of β–carotene and fatty acid esters during heating at 60 and 120°C for varied lengths of time[a]**

| Temp | Time (min) | Methyl sterarate | | | | Methyl oleate | | | | Methyl linoleate | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Chl *a* isomer I | Chl *a* isomer II | Chl *a* | Chl *a*′ | Chl *a* isomer I | Chl *a* isomer II | Chl *a* | Chl *a*′ | Chl *a* isomer I | Chl *a* isomer II | Chl *a* | Chl *a*′ |
| 60°C | 0 | 9.1 | 8.2 | 82.7 | 0.0 | 9.1 | 8.2 | 82.7 | 0.0 | 9.1 | 8.2 | 82.7 | 0.0 |
| | 5 | 16.2 | 12.1 | 71.0 | 0.7 | 10.5 | 6.2 | 78.9 | 4.4 | 5.2 | 1.6 | 82.9 | 10.3 |
| | 10 | 15.1 | 9.4 | 69.4 | 6.1 | 10.6 | 6.1 | 76.3 | 7.0 | 4.6 | 1.6 | 82.5 | 11.3 |
| | 20 | 16.2 | 6.1 | 67.5 | 10.2 | 11.4 | 6.0 | 72.1 | 10.5 | 3.8 | 1.9 | 80.4 | 13.9 |
| | 30 | 17.0 | 5.8 | 66.0 | 11.2 | 12.5 | 4.5 | 68.0 | 15.0 | 3.3 | 1.6 | 77.5 | 17.6 |
| | 60 | 18.7 | 6.3 | 63.0 | 12.0 | 13.7 | 2.3 | 67.0 | 17.0 | 1.8 | 2.9 | 77.2 | 18.1 |
| | 90 | 19.0 | 6.0 | 61.0 | 14.0 | 13.5 | 2.1 | 67.2 | 17.2 | 1.4 | 1.5 | 72.0 | 25.1 |
| 120°C | 0 | 9.1 | 8.2 | 82.7 | 0.0 | 9.1 | 8.2 | 82.7 | 0.0 | 9.1 | 8.2 | 82.7 | 0.0 |
| | 1 | 31.4 | 5.1 | 47.9 | 15.5 | 8.4 | 5.2 | 79.1 | 7.3 | 15.4 | 16.2 | 58.9 | 9.5 |
| | 2 | 43.6 | 15.1 | 30.3 | 11.0 | 16.6 | 11.1 | 56.3 | 16.0 | 11.3 | 20.0 | 52.6 | 16.1 |
| | 5 | 58.4 | 20.0 | 10.1 | 11.5 | 16.0 | 10.5 | 44.1 | 29.4 | 8.1 | 12.3 | 46.1 | 33.5 |
| | 10 | 47.5 | 25.5 | 9.0 | 18.0 | 16.5 | 10.4 | 43.0 | 30.1 | 10.5 | 10.5 | 47.1 | 31.9 |
| | 15 | 56.7 | 19.3 | 5.0 | 19.0 | 22.7 | 6.3 | 41.0 | 30.0 | 12.7 | 6.3 | 46.5 | 34.5 |

[a]Averages of duplicate analyses.

model because a linear correlation ($r^2 = 0.9726$) for the plot of logarithm of total Chl *a* concentration vs time was observed, and the degradation rate constant was 0.014 ($min^{-1}$).

The percentage changes of Chl *a* and its isomers during heating in the presence of β-carotene and methyl linoleate at 60°C for 90 min are shown in Table 2. In contrast to the results shown above, the amount of Chl *a* isomer I decreased with increasing heating time, and the decreased percentage was 7.7% after 90 min heating. Chl *a* and Chl *a* isomer II showed the same trend with the decreased percentages 10.7 and 6.7%, respectively. Chl *a*′ was formed in highest amount (25.1%). The degradation of total amount of Chl *a* in the presence of β-carotene and methyl linoleate during heating may fit the first-order model because a linear correlation ($r^2 = 0.9898$) for the plot of the logarithm of total Chl *a* concentration vs time was observed, and the degradation rate constant was 0.010 ($min^{-1}$).

By comparison of the results shown above, it can be found that, in the presence of methyl linoleate and β-carotene the amount of Chl *a* decreased least, indicating that the presence of unsaturated fatty acid can minimize the degradation of Chl *a* during heating. In contrast, in the presence of methyl linoleate and β-carotene, the percentage increase of Chl *a*′ was largest, implying that the presence of unsaturated fatty acid can facilitate formation of Chl *a*′. For Chl *a* isomers I and II, the percentage changes were not consistent, probably because both are not only intermediate products formed during heating, but also can undergo degradation and reversible conversion simultaneously. In contrast to the formation of Chl *a*′, both Chl *a* isomers I and II were formed in greatest amount in methyl stearate, indicating that the presence of saturated fatty acid can facilitate the formation of these two isomers.

### Percentage changes of Chl *a* and its isomers during heating in the presence of fatty acid esters and β-carotene at 120°C for 15 min

The percentage changes of Chl *a* and its isomers during heating in the presence of methyl stearate and β-carotene at 120°C for 15 min are shown in Table 2. The amount of Chl *a* decreased by 77.7% while the amount of Chl *a*′ and Chl *a* isomer I increased by 19.0 and 47.6%, respectively, after 120°C heating for 15 min. For Chl *a* isomer II, it increased by 17.3% after 10 min heating, and then further decreased by 6.2% after prolonged heating for 15 min. The degradation of total amount of Chl *a* in the presence of methyl stearate and β-carotene during heating may fit the first-order model because a linear correlation ($r^2 = 0.9898$) was observed for the plot of the logarithm of total Chl *a* concentration vs time, and the rate constant was 0.16 ($min^{-1}$).

The percentage changes of Chl *a* and its isomers during heating in the presence of methyl oleate and β-carotene at 120°C heating for 15 min are shown in Table 2. The amount of Chl *a* decreased by 41.7% while the amounts of Chl *a*′ and Chl *a* isomer I increased by 30.0 and 13.6%, respectively after 120°C heating for 15 min. For Chl *a* isomer II, it increased by 2.9% after heating for 2 min, and further decreased by 4.8% after prolonged heating for 15 min. The degradation of total amount of Chl *a* in the presence of methyl oleate and β-carotene during heating may fit the first-order model because a linear correlation ($r^2 = 0.9587$) was observed for the plot of the logarithm of total Chl *a* concentration vs time, and the rate constant was 0.14 ($min^{-1}$).

The percentage changes of Chl *a* and its isomers during heating in the presence of methyl linoleate and β-carotene at 120°C heating for 15 min are shown in Table 2. The amount of Chl *a* decreased by 36.2% while

the amounts of Chl $a'$ increased by 34.5% after 120°C heating for 15 min. In contrast to the results shown above, the amount of Chl *a* isomer I increased by 6.3% after 1 min heating, and then decreased by 2.7% after prolonged heating for 15 min. The percentage change of Chl *a* isomer II showed the same trend with an increase of 11.8% after 2 min heating, and then decreased by 13.7% after 15 min heating. The degradation of total amount of Chl *a* in the presence of methyl linoleate and $\beta$-carotene during heating may fit the first-order model because a linear correlation ($r^2 = 0.9383$) was observed for the plot of the logarithm of total Chl *a* concentration vs time, and the rate constant was 0.12 ($min^{-1}$).

By comparison of the results shown above, it can be found that, in the presence of fatty acid esters and $\beta$-carotene, the percentage changes of Chl *a* and Chl $a'$ showed the trend as those during heating at 60°C, i.e the presence of saturated fatty acid can accelerate the degradation of Chl *a* and minimize formation of Chl $a'$ during heating. The inconsistent percentage changes of Chl *a* isomers I and II during heating indicated that both are probably intermediate products. It is also possible that both can undergo degradation and reversible conversion simultaneously. Nevertheless, both were formed in highest amount in methyl stearate, indicating that the presence of saturated fatty acid can facilitate formation of Chl *a* isomers I and II. From the preceding results it may be concluded that the higher temperature, the faster the degradation rate of Chl *a* and the greater formation of Chl $a'$, Chl *a* isomer I and Chl *a* isomer II. Chl *a* is more susceptible to oxidative degradation in the presence of saturated fatty acid than in the presence of unsaturated fatty acid during heating. The presence of saturated fatty acid can facilitate formation of Chl *a* isomers I and II while the presence of unsaturated fatty acid can facilitate formation of Chl $a'$. Chl *a* is more susceptible to oxidative degradation and isomerization than $\beta$-carotene in the presence of fatty acid esters, especially methyl stearate, during heating. Further research is necessary to elucidate the conversion mechanism between Chl *a* and its isomers during heating in the presence of $\beta$-carotene and fatty acid esters.

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