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Degradation and isomerization of chlorophyll a and β-carotene as affected by various heating and illumination treatments

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The degradation and isomerization of β-carotene and chlorophyll a as affected by oven-heating, reflux-heating, iodine-catalysed illumination, and non-iodine-catalysed illumination, were studied. Results showed that the degradations of both total β-carotene and chlorophyll a may fit the first-order model under either heating or illumination treatment. 13-*cis*-β-Carotene and 13,15-di-*cis*-β-carotene were the major *cis* isomers of β-carotene formed during oven heating, while 13-*cis*-β-carotene was favoured during reflux heating. For illumination with or without iodine as catalyst, 13,15-di-*cis*-β-carotene was the major *cis* isomer of β-carotene formed. The formation of 13,15-di-*cis*-β-carotene may be due to conversion of either 13-*cis*- or 15-*cis*-β-carotene. No epimerization of chlorophyll a was observed as a result of illumination.

INTRODUCTION

Carotenoids, an important class of biological compounds which are widely distributed in foods, have received considerable attention in the past decade because of their beneficial effects to human health. Epidemiological studies have shown that the consumption of fruits and vegetables high in carotenoid content can elevate all-*trans*-β-carotene levels in the blood, which in turn can be protective against some fatal diseases such as skin and stomach cancers (Mathews-Roth, 1985; Krinsky, 1989; Moon, 1989; Block, 1991; Cutler, 1991). In addition to being a vitamin A precursor, all-*trans*-β-carotene is also an effective antioxidant because of the presence of a long chain of conjugated carbon–carbon double bonds (Burton and Ingold, 1984; Fakourelis *et al.*, 1987; Yen and Chen, 1995). Moreover, all-*trans*-β-carotene was reported to be able to enhance immune response in rats (Bendich, 1989).

Most β-carotene is naturally present in the *trans* form; however, there are still significant amounts of the *cis* forms of β-carotene in foods. The *cis* isomers of β-carotene in foods may be formed during extraction or chromatography. It has been reported that the chlorinated solvents can promote isomerization of *trans* conjugated polyenes such as β-carotene during extraction (Pesek *et al.*, 1990). Also, the isomerization of β-carotene was found to be higher in non-polar solvents than in polar solvents (Pesek *et al.*, 1990). In addition, some authors postulated that the chromatography procedure may induce formation of *cis* isomers of β-carotene (Khachik *et al.*, 1986; Chen and Chen, 1993; Chen *et al.*, 1994, 1995). Due to the fact that the formation of *cis*-β-carotene could lower vitamin A activity and decrease colour intensity (Sweeney and Marsh, 1971; Schwartz and Patroni-Killam, 1985; Chen *et al.*, 1995), the National Research Council of the United States (Food and Nutrition Board, 1980) suggested that the *cis* forms of β-carotene in foods should be quantified. The major *cis* forms of β-carotene in foods include 9-*cis* and 13-*cis* (Chandler and Schwartz, 1987; Pesek and Warthesen, 1990; Pesek *et al.*, 1990; Chen and Chen, 1993). In addition, some other *cis* forms of β-carotene such as 15-*cis* and 13,15-di-*cis* were also present (Tsukida *et al.*, 1982; Koyama *et al.*, 1988; Chen and Chen, 1994; Chen *et al.*, 1994). The mechanism of formation and conversion of *cis* isomers of β-carotene during heating and illumination has been adequately discussed (Pesek and Warthesen, 1990; Pesek *et al.*, 1990; Chen *et al.*, 1994). Pesek *et al.* (1990) observed that during storage of all-*trans*-β-carotene solution in the dark at 45°C, 9-*cis*-β-carotene could be converted to 13-*cis*-β-carotene only after it had changed to all-*trans*-β-carotene. This result implied that the direct conversion between 9-*cis*- and 13-*cis*-β-carotene is not possible. In a later study, Chen *et al.* (1994) further reported that 13-*cis*-β-carotene can be converted to 13,15-di-*cis*-β-carotene during illumination of all-*trans*-β-carotene crystals. The effect of illumination on the isomerization

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of all-*trans*-β-carotene in plant tissue and thylakoid membrane was conducted by Ashikawa *et al.* (1986), and the authors found that a reversible decrease in the relative amounts of 15-*cis*- and 13-*cis*-β-carotene occurred for the membrane, while the 9-*cis* isomer remained unchanged. In contrast, other studies have found that 9-*cis*-β-carotene was formed in greater amount than either 13-*cis*- or 15-*cis*-β-carotene during illumination of all-*trans*-β-carotene in the presence of the photosensitizer chlorophyll a (Jensen *et al.*, 1982; O'Neil and Schwartz, 1995). As these results are contradictory, the photoisomerization of all-*trans*-β-carotene, in the presence of chlorophyll a during illumination, needs to be further investigated. In addition to illumination, the effects of direct heating on degradation and isomerization of all-*trans*-β-carotene crystals were investigated (Chen *et al.*, 1994). However, incomplete heat transfer may occur during oven-heating of all-*trans*-β-carotene crystals. Thus, some authors have used reflux-heating instead (Zechmeister, 1944). It was also reported that the mechanisms for degradation and isomerization of all-*trans*-β-carotene by oven-heating and reflux-heating may be completely different (Zechmeister, 1944). Therefore, it is necessary to determine the effects of oven-heating and reflux-heating on the degradation and isomerization of all-*trans*-β-carotene.

Chlorophylls are also an important class of biological compounds that are widely distributed in green plants. The major chlorophylls in foods include chlorophyll a and chlorophyll b. Chlorophyll a was reported to be present at a concentration two to three times higher than chlorophyll b (Schwartz and Lorenzo, 1990). Due to the presence of a long chain of conjugated carbon–carbon double bonds, chlorophylls are also susceptible to heat, oxygen, light and acid degradations. The epimerization of chlorophyll a to chlorophyll a′, the C-10 epimer of chlorophyll a, can occur at room temperature, and is even faster during heating (Schwartz and Lorenzo, 1990; Chen and Cheng, 1993). It has been well established that chlorophyll a can be a photosensitizer, i.e. after absorption of energy, chlorophyll a can transfer energy to triplet oxygen to form a more active singlet oxygen, which then reacts with all-*trans*-β-carotene and results in subsequent isomerization of triplet all-*trans*-β-carotene (Foote and Denny, 1968; Foote *et al.*, 1970*a*; Foote *et al.*, 1970*b*; Carlsson *et al.*, 1976; Jensen *et al.*, 1982; Kiritsakis and Dugan, 1985; Lee and Min, 1988, 1990; O'Neil and Schwartz, 1995). The results of these studies also showed that the quenching of the excited triplet state of chlorophyll a by β-carotene can result in the subsequent isomerization of triplet β-carotene during illumination. However, Jensen *et al.* (1982) reported that, with all-*trans*-β-carotene and chlorophyll a concentrations at a certain ratio, this effect did not occur. Moreover, it has been reported that the isomerization of all-*trans*-β-carotene in the presence of chlorophyll a was minimal during microwave cooking of vegetables, indicating that chlorophyll a may inhibit all-*trans*-β-carotene isomerization during heating (Khachik *et al.*, 1986; Chen and Chen, 1993). The purposes of this study were (1) to determine the effects of oven-heating and reflux-heating on degradation and isomerization of all-*trans*-β-carotene, and (2) to study the degradation and isomerization of all-*trans*-β-carotene in the presence of the photosensitizer, chlorophyll a, during illumination.

MATERIALS AND METHODS

Materials

Standards, including all-*trans*-β-carotene and chlorophyll a, were purchased from Sigma Co. (St. Louis, MO, USA). Organic solvents used for dissolving β-carotene and chlorophyll a, including hexane, acetone and pyridine, were analytical grade, and were from Mallinckrodt Co. (Paris, Ky, USA). The HPLC-grade solvents, including methanol, acetonitrile, methylene chloride and tetrahydrofuran, were also from Mallinckrodt Co. Chlorophyll a′ was prepared from chlorophyll a using a method described by Katz *et al.* (1968).

Instrumentation

The HPLC instrument consists of a SSI 222D pump (Scientific System Inc., State College, PA, USA), a Linear 206 PHD photodiode-array detector (Linear Instrument, Reno, NA, USA), and an Axxiom 727 dual-channel chromatography data system (Axxiom Chromatography Inc., Calabasas, CA, USA). A Beckman DU-70 Double-Beam Spectrophotometer (Irvine, CA, USA) was used to determine absorption spectra. An Advantec SF-2120 fraction collector (Tokyo, Japan) was used to collect eluates. A Vydac 201TP54 polymeric C_{18} column (25 cm×4.6 mm I.D.) (Hesperia, CA, USA) containing 5 μm particle size was used.

Oven heating of all-*trans*-β-carotene

Ten mg of all-*trans*-β-carotene crystals were dissolved in 100 ml hexane, of which 20 ml was poured into a small aluminium-foil cup. After evaporation of hexane with nitrogen gas, the aluminium-foil cup was unfolded so that all-*trans*-β-carotene could be spread uniformly on the surface of the cup. Then the cup was placed in the oven and heated at 50, 100, 125 or 150°C for 5, 10, 15, 20, 25 and 30 min. After heating, the all-*trans*-β-carotene crystals were redissolved in 20 ml hexane and filtered through a 0.2 μm membrane filter, and 20 μl of sample was collected for HPLC analysis.

Reflux heating of all-*trans*-β-carotene

Ten mg of all-*trans*-β-carotene crystals were poured into a reflux flask containing 300 ml hexane, to which a

condenser was connected and ice water was passed through this tube to enhance the cooling effect. The flask temperature was controlled at 70°C, and the solution was heated for 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 70, 80, 90, 100 and 140 min. Before collecting samples, the flask was removed and immersed in ice water so that the degradation or isomerization of all-*trans*-β-carotene could be terminated. Ten-ml samples were taken from the flask at intervals after heating, and hexane was added to bring the volume to 300 ml, and the flask was reheated. The 10 ml samples taken from the flask were filtered through a 0.2-μm membrane filter, and 20 μl of samples were collected for HPLC analysis.

Iodine-catalysed illumination of all-*trans*-β-carotene

A method similar to that described by Chen *et al.* (1994) was used. Three mg of all-*trans*-β-carotene crystals were dissolved in 100 ml hexane and 60 μg iodine (2%) was added to the flask. The flask was illuminated in a cold chamber (−5.4°C) under fluorescent light for 2, 4, 6, 8, 10, 13, 15, 20, 25, 30 and 35 min. The fluorescent tube (General Electric 20 W) was suspended 20 cm above the flask where the light intensity measured 2000 lux. After illumination, samples were filtered through a 0.2-μm membrane filter, and 20 μl of sample was collected for HPLC analysis.

Illumination of all-*trans*-β-carotene

Three mg of all-*trans*-β-carotene crystals were dissolved in 100 ml hexane in a flask, and the flask was illuminated in a cold chamber (−5.4°C) for 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 h under fluorescent light. The fluorescent tube (General Electric 20 W) was suspended approximately 20 cm above the flask, where the light intensity measured 2000 lux. After illumination, the solution was filtered through a 0.2-μm membrane filter, and 20 μl of sample was collected for HPLC analysis.

Illumination of chlorophyll a

Three mg of chlorophyll a was dissolved in 100 ml acetone in a flask, and the flask was placed in a cold chamber (−5.4°C) and illuminated under fluorescent light for 1, 2, 4, 6, 7, 8, 9, 10, 12, 14, 16, 18 and 22 h. The fluorescent tube (General Electric 20 W) was suspended approximately 20 cm above the flask, where the light intensity measured 2000 lux. After illumination, the solution was filtered through a 0.2-μm membrane filter, and 20 μl of sample was collected for HPLC analysis.

Illumination of a mixture of all-*trans*-β-carotene and chlorophyll a

A mixture consisting of 6 mg of all-*trans*-β-carotene in 100 ml hexane and 6 mg of chlorophyll a in 100 ml acetone were placed in a flask. The flask was placed in a cold chamber (−5.4°C) and illuminated under fluorescent light for 1, 2, 3, 6, 8, 9, 10, 11, 13, 14, 15, 16, 17, 18, 19, 20, 21 and 22 h. The fluorescent tube (General Electric 20 W) was suspended approximately 20 cm above the flask, where the light intensity measured 2000 lux. After illumination, the solution was filtered through a 0.2-μm membrane filter, and 20 μl of sample was collected for HPLC analysis.

HPLC analysis of β-carotene-and its *cis* isomers, and chlorophyll a

A ternary solvent system of methanol–methylene chloride–tetrahydrofuran (92:1:3, v/v/v) with sample solvent hexane (100%) was developed to separate β-carotene and its four *cis* isomers, including 9-*cis*-, 13-*cis*-, 15-*cis*- and 13, 15-di-*cis*-β-carotene. The other solvent system consists of methanol–methylene chloride–tetrahydrofuran (92:5:3, v/v/v) with sample solvent hexane–acetone (50:50, v/v), which was used to separate β-carotene and its four *cis* isomers, and chlorophyll a simultaneously. For both systems, the flow rates were 1.0 ml/min with sensitivity at 0.2 AUFS. A detection wavelength of 450 nm was used to detect β-carotene and its four *cis* isomers, and 660 nm was used to detect chlorophyll a. The injection volume was 20 μl. Both all-*trans*-β-carotene and chlorophyll a were identified by cochromatography with added standards, and comparison of absorption spectra with reference standards. In addition, *cis* isomers of β-carotene were identified based on spectral characteristics and Q ratio as described in a previous study (Chen *et al.*, 1994). Nevertheless, the identification of 13,15-di-*cis*-β-carotene is only tentative because of lack of a reference standard. Also, the positive identification of this di-*cis* isomer by a more advanced instrument such as NMR has been difficult because only a minute amount was present in the sample. The quantitation of each pigment was carried out using an absolute calibration curve as described by Chen *et al.* (1994). As no *cis*-β-carotene standards are available, *cis* isomers of β-carotene were calculated as all-*trans*-β-carotene equivalents. Duplicate analyses were conducted and mean values determined. The data were also subjected to analysis of variance and Duncan's multiple range test with the use of a statistical analysis system (SAS/STAT, 1985).

RESULTS AND DISCUSSION

Separation of β-carotene and its *cis* isomers, chlorophyll a

Due to the fact that the column to column variability is greater for the polymeric column than for the monomeric column (Epler and Sander, 1992; Chen *et al.*, 1995), the solvent systems used for separation of β-carotene and its *cis* isomers, and chlorophyll a have to be

investigated. In the beginning a binary solvent system of methanol–methylene chloride (99:1, v/v) with sample solvent methanol–methylene chloride (45:55, v/v) was employed (Chen *et al.*, 1995), however, the separation was not satisfactory. By adding tetrahydrofuran as modifier and changing sample solvent to hexane (100%), β-carotene and its four *cis* isomers, 9-*cis*-, 13-*cis*-, 15-*cis*-, and 13,15-di-*cis*-β-carotene were adequately resolved by methanol–methylene chloride–tetrahydrofuran (92:1:3, v/v/v) with flow rate at 1.0 ml/min and detection at 450 nm. The k′ values (capacity factor) for all peaks were ideally controlled between 3.2 (13, 15-di-*cis*-β-carotene) and 5.9 (9-*cis*-β-carotene). Likewise, the simultaneous separation of β-carotene and its *cis* isomers, and chlorophyll a was achieved by a ternary solvent system of methanol–methylene chloride–tetrahydrofuran (92:5:3, v/v/v) with sample solvent hexane–acetone (50:50, v/v) and flow rate at 1.0 ml/min, and detection at 450 nm. The k′values for all peaks were also ideally controlled between 0.8 (chlorophyll a) and 5.2 (9-*cis*-β-carotene). It has been well established that the k′value should be controlled between 2 and 10 to obtain an ideal separation (Dolan, 1990).

Oven heating of all-*trans*-β-carotene

All-*trans*-β-carotene standard was found to contain trace amounts of 9-*cis*-, 13-*cis* and 15-*cis*-β-carotene, and was used without further purification. No significant concentration change ($p > 0.05$) was found for 9-*cis*-, 13-*cis*- or 15-*cis*-β-carotene after oven-heating of all-*trans*-β-carotene crystals at 50 and 100°C for 0, 5, 10, 15, 20, 25 and 30 min. However, the degradation of all-*trans*-β-carotene became significant ($p < 0.05$) after heating at 50 and 100°C for 25 and 10 min, respectively. Figure 1 shows the concentration changes of all-*trans*-β-carotene and its *cis* isomers during heating at 125°C over a period of 30 min. The formation of 13,15-di-*cis*-β-carotene was not observed until all-*trans*-β-carotene was heated at 125°C for 20 min. This result seems to be contradictory to a report by Chen *et al.* (1994), who found that 13,15-di-*cis*-β-carotene was not formed even after 150°C oven-heating for 30 min. This inconsistency can be attributed to the difference in methods of sample handling. As all-*trans*-β-carotene crystals were placed in the vial and heated in the oven for the latter method, incomplete heat penetration may occur for the interior portion of sample crystal. In contrast, in this study all-*trans*-β-carotene crystal was uniformly spread on the surface of aluminium-foil cup, and thus complete heat penetration was possible for the whole sample. It has been reported that the activation energy required for formation of a di-*cis* carotenoid should be higher than that of a mono-*cis* carotenoid (Zechmeister, 1944; Chen *et al.*, 1994). Thus, the more the energy supply, the more the formation of 13,15-di-*cis*-β-carotene. The amount of 15-*cis*-β-carotene did not show significant change ($p > 0.05$) until heating time reached 20 min, and only minor loss (0.2 μg/ml) was observed after 125°C heating for 30 min. Likewise, the amount of 13-*cis*-β-carotene did not increase until heating time reached 20 min, and the increased level was 1.2 μg/ml after 30 min heating. A similar trend was observed for 9-*cis*-β-carotene; however, only a minor increase (0.37 μg/ml) was observed after 30 min heating. The formation of 9-*cis*- or 13-*cis*-β-carotene may be due to conversion of all-*trans*- or 15-*cis*-β-carotene. It has been well established that 15-*cis*-β-carotene can be converted to 9-*cis*- or 13-*cis*-β-carotene only after it is changed to all-*trans*-β-carotene (Jensen *et al.*, 1982; Chen *et al.*, 1994). Figure 2 shows the concentration changes of β-carotene and its *cis* isomers during heating at 150°C over a period of 30 min. The concentration change was quite similar to that in Fig. 1 with the exception that 13,15-di-*cis*-β-carotene was formed after 150°C heating for 10 min. This result implied that the higher the temperature, the shorter the times required for formation of a di-*cis* carotenoid. Figure 3 shows the first-order plot for β-carotene degradation during oven-heating at various temperatures. The degradation rate constants were 0.001, 0.002, 0.018 and 0.026 (min^{-1}) for heating temperatures of 50, 100, 125 and 150°C, respectively. This result indicated that the higher the temperature, the faster degradation rate of total β-carotene. Figure 4 shows the plot of ln(k) versus ($1/T$) for total β-carotene degradation under oven-heating. A linear regression curve with high

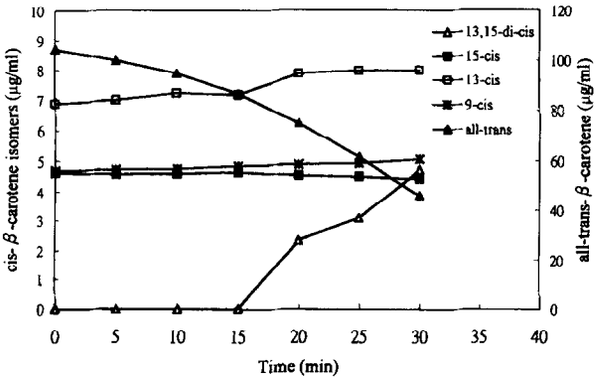

Fig. 1. Concentration changes of β-carotene and its *cis* isomers during oven-heating at 125°C for varied lengths of time.

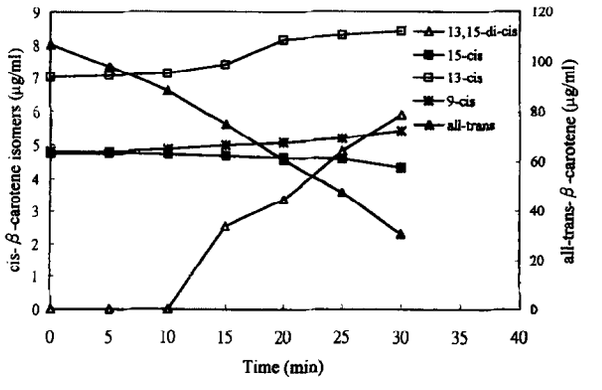

Fig. 2. Concentration changes of β-carotene and its *cis* isomers during oven-heating at 150°C for varied lengths of time.

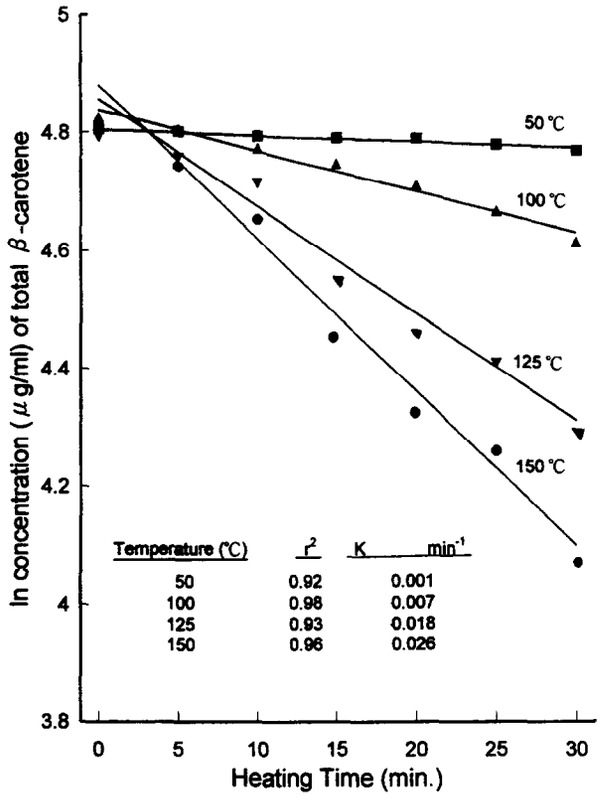

Fig. 3. First-order plot for the degradation of total β-carotene during oven-heating at various temperatures.

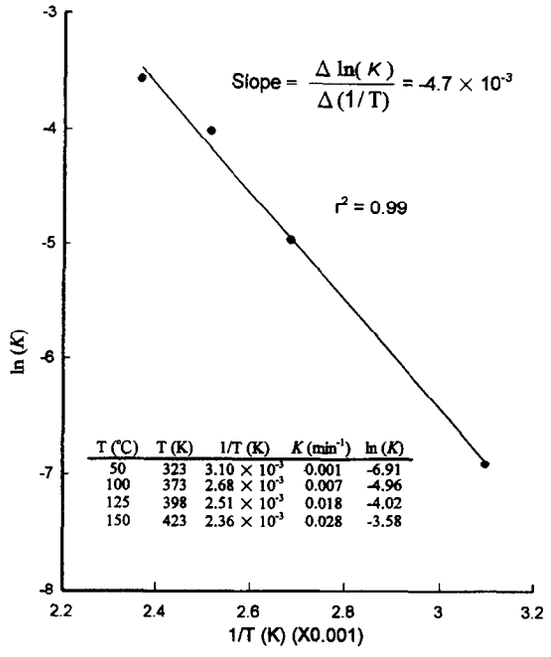

Fig. 4. Plot of ln(K) versus ($1/T$) for total β-carotene degradation during oven-heating.

correlation coefficient ($r^2 = 0.99$) and slope -4.7×10^{-3} (K) was obtained. According to the Arrhenius equation:

$$\ln(k) = -(Ea/R)(1/T) + \ln(A)$$

where Ea is activation energy (J/mol) and R is the ideal gas constant (8.3148 J/Kmol).

The activation energy for total β-carotene degradation was calculated as 3.9×10^4(J/mol).

Reflux heating of all-*trans*-β-carotene

Figure 5 shows the concentration change of all-*trans*-β-carotene and its *cis* isomers during reflux heating. No formation of 13,15-di-*cis*-β-carotene was observed, mainly because of the low temperature (70°C) used. The only significant increase ($p < 0.05$) was observed for 15-*cis*-β-carotene after heating time reached 25 min and above, and the increased amount was not significant ($p > 0.05$) after 60 min heating. The all-*trans*-β-carotene concentration decreased along with increasing heating time, and remained non-significant change ($p > 0.05$) with heating time reaching 70 min and above, indicating that the whole system approached equilibrium. For 13-*cis*-β-carotene, it increased consistently over a period of 15–60 min, and showed no change ($p > 0.05$) thereafter. The same trend was also observed for 9-*cis*-β-carotene. From Fig. 5 it can be found that 13-*cis*-β-carotene was formed in largest amount, followed by 9-*cis*- and 15-*cis*-β-carotene. Similar results were demonstrated by Zechmeister (1944), who found that 13-*cis*-β-carotene was formed at a concentration twice that of 9-*cis*-β-carotene after reflux-heating of all-*trans*-β-carotene solution. The author also reported that, with reflux-heating, the whole system could reach equilibrium within 30–60 min. From the preceding result it can be found that reflux heating is more likely to cause β-carotene isomerization, while oven-heating is more likely to cause β-carotene degradation. This phenomenon can be attributed to differences in degradation mechanisms as affected by temperature and physical state of the reaction system. Nevertheless, some other factors should also be considered. During reflux-heating, hexane was used as a heating medium, and thus the chance for oxygen to react with all-*trans*-β-carotene would be less than that by oven-heating. Also, non-polar solvents, such as hexane, were reported to promote all-*trans*-β-carotene isomerization more than polar solvents (Pesek *et al.*, 1990).

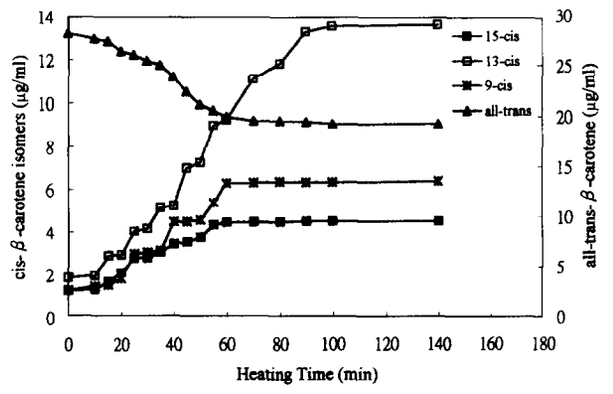

Fig. 5. Concentration changes of β-carotene and its *cis* isomers during reflux-heating.

Therefore, the reflux-heating of all-*trans*-β-carotene solution would more likely undergo isomerization than degradation. In addition, a sudden cooling step was used for reflux heating, which would make the backward reaction (*trans*←*cis*) proceed faster than the forward reaction (*trans*→*cis*), and, finally an equilibrium state will be reached for all-*trans*-β-carotene. Moreover, the amount of all-*trans*-β-carotene should be higher than that of the *cis* forms after equilibrium is reached for the whole system, mainly because all-*trans*-β-carotene itself is also an intermediate during isomerization of all-*trans*-β-carotene (Pesek and Warthesen, 1990; Pesek *et al.*, 1990; Chen *et al.*, 1994).

Iodine-catalysed illumination of all-*trans*-β-carotene

Figure 6 shows the concentration changes of β-carotene and its *cis* isomers during iodine-catalysed illumination for 35 min. The formation of 13,15-di-*cis*-β-carotene was not observed until illumination time reached 10 min, and the amount increased with increasing exposure time thereafter. The formation of 13,15-di-*cis*-β-carotene may be due to conversion of 13-*cis*- or 15-*cis*-β-carotene as, reported by Chen *et al.* (1994). For 15-*cis*-β-carotene, it did not show significant increase ($p > 0.05$) until illumination time reached 13 min and above, and showed no significant change ($p > 0.05$) with exposure time 20–35 min. Like oven-heating, the degradation of total β-carotene may also fit the first-order model. As no significant change ($p > 0.05$) occurred after exposure time reached 25 min, this result implied that the whole system reached equilibrium. Chandler and Schwartz (1987) studied the effect of iodine-catalysed illumination on all-*trans*-β-carotene isomerization and found that the system equilibration could be reached within 76 min. Apparently the difference in illumination time for reaching system equilibrium can be attributed to illumination mode, light intensity and presence of catalyst. The degradation of total β-carotene during iodine-catalysed illumination may also fit the first-order model. A large increase (9.1 μg/ml) was observed for 13-*cis*-β-carotene after illumination time reached 13 min, and then decreased insignificantly ($p > 0.05$) thereafter.

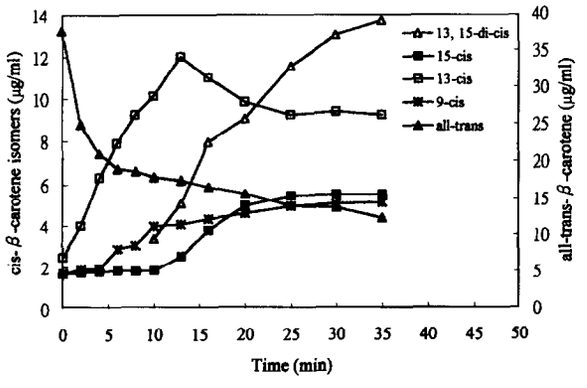

Fig. 6. Concentration changes of β-carotene and its *cis* isomers during iodine-catalysed illumination for 35 min.

A consistent increase was found for 9-*cis*-β-carotene; however, no significant change occurred after 25 min illumination. From Fig. 6 it can be seen that 13,15-di-*cis*-β-carotene was formed in largest amount, followed by 13-*cis*-, 9-*cis*-, and 15-*cis*-β-carotene. Zechmeister (1944) reported that the activation energy required for isomerization of all-*trans*-β-carotene would be less for the central double bond. Thus, 15-*cis*-β-carotene would be more easily formed than either 9-*cis*-, 13-*cis*- or 13,15-di-*cis*-β-carotene. This would be contradictory to the result in this study, which showed that 13,15-di-*cis*-β-carotene was favoured during iodine-catalysed illumination. This may be explained as follows: compared to 9-*cis*- or 13-*cis*-β-carotene, 15-*cis*-β-carotene is a larger molecule which occupies more space, and thus can collide with other molecules more easily. Therefore, the unstable nature of 15-*cis*-β-carotene means that it can be converted to the other forms of *cis* isomers more easily than either 9-*cis*- or 13-*cis*-β-carotene. This would account for the minimal amount of 15-*cis*-β-carotene formed during iodine-catalysed illumination. Theoretically, the conversion energy for formation of di-*cis*-β-carotene should be higher than that of mono-*cis*-β-carotene. However, in this study the activation energy for formation of 13,15-di-*cis*-β-carotene was lowered because of the presence of catalyst iodine, and a large increase of 13,15-di-*cis*-β-carotene was thus observed. From the molecular point of view, the formation of 13,15-di-*cis*-β-carotene is possible because of the presence of two *cis* bonds, which can elongate the long chain of conjugated carbon–carbon double bonds of 13-*cis*- or 15-*cis*-β-carotene, and thus increase the stability of this molecule.

Illumination of all-*trans*-β-carotene

Figure 7 shows the concentration changes of β-carotene and its isomers during illumination. 13,15-di-*cis*-β-carotene was not detected until the illumination time reached 4 h, and the amounts increased thereafter. However, no significant difference ($p > 0.05$) was observed for exposure time of 12–20 h. The same trend was observed for 15-*cis*-, 13-*cis*- and 9-*cis*-β-carotene with the exception that the amounts of both 13-*cis*- and 9-*cis*-β-carotene decreased after the illumination time

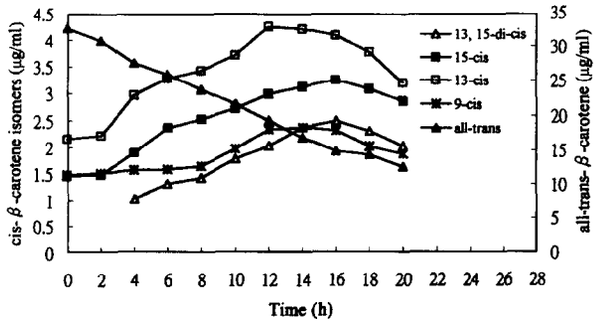

Fig. 7. Concentration changes of β-carotene and its *cis* isomers during illumination at −5.4°C for 20 h.

reached 18 h. By comparison of iodine-catalysed illumination and non-iodine-catalysed illumination of all *trans*-β-carotene, it can be found both 13-*cis*- and 13,15-di-*cis*-β-carotene were formed in greater amount by the former method. This is probably because iodine-catalysed illumination causes more all-*trans*-β-carotene isomerization, while the non-iodine-catalysed illumination causes more all *trans*-β-carotene degradation. The degradation of all-*trans*-β-carotene during illumination may also fit the first-order model (Fig. 8).

Illumination of chlorophyll a

Figure 9 shows the concentration changes of chlorophyll a during illumination at −5.4°C for 22 h. Only a minor amount of chlorophyll a was detected after 22 h illumination, indicating that chlorophyll a degradation could proceed to a considerable extent after prolonged exposure to light. Interestingly, no chlorophyll a epimerization was observed during illumination. To further demonstrate this result, chlorophyll a′, the C-10 epimer of chlorophyll a was prepared from chlorophyll a using a method described by Katz *et al.* (1968). As the solvent system developed in this study could resolve both chlorophyll a and chlorophyll a′, the absence of chlorophyll a′ peak on the HPLC chromatogram indicated that no epimerization occurred for chlorophyll a during illumination. It has been well known that the formation of chlorophyll a′ and can occur at room temperature, and is even faster during blanching (Schwartz *et al.*, 1981; von Elbe *et al.*, 1986; Schwartz and Lorenzo, 1990). Thus, many authors have observed the formation of chlorophyll a′ under various heating treatments (Khachik *et al.*, 1986; Chen and Chen, 1993). Unlike heating, chlorophyll a can act as a photosensitizer during photochemical reactions, i.e. it can absorb light energy and transfer it to triplet oxygen to induce formation of unstable singlet oxygen (Foote and Denny, 1968; Foote *et al.*, 1970*a*,*b*; Carlsson *et al.*, 1976; Brown *et al.*, 1980; Jensen *et al.*, 1982; Usuki *et al.*, 1984; Schwartz and Lorenzo, 1990; O'Neil and Schwartz, 1995). The highly reactive singlet oxygen then probably attacks the double bond between the fifth and sixth carbon of chlorophyll a, resulting in a subsequent shift of the position of the double bond and the formation of hydroperoxides, which are then further cleaved through oxygen-oxygen linkage to form degradation products. In addition, the low illumination temperature (−5.4°C) can minimize the effect of heat radiation to a considerable extent and hence the formation of chlorophyll a′ can be retarded, Fig. 10 shows the first-order plot for chlorophyll a degradation during illumination

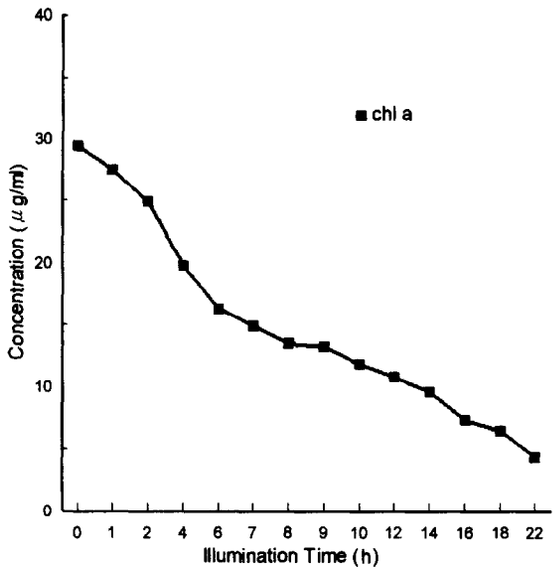

Fig. 9. Concentration changes of chlorophyll a during illumination at −5.4°C for 22 h.

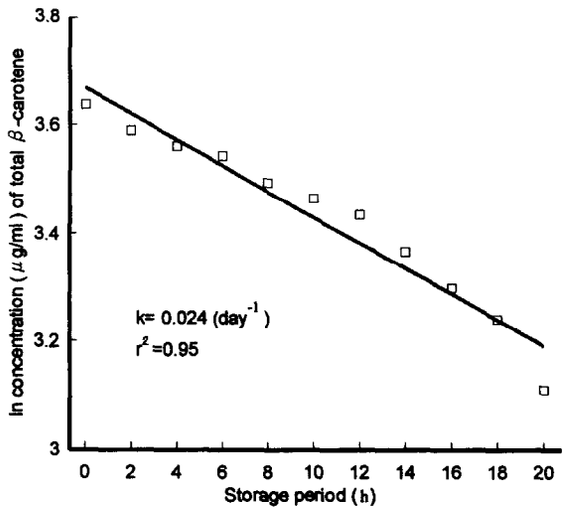

Fig. 8. First-order plot for the degradation of total β-carotene during illumination at −5.4°C for 20 h.

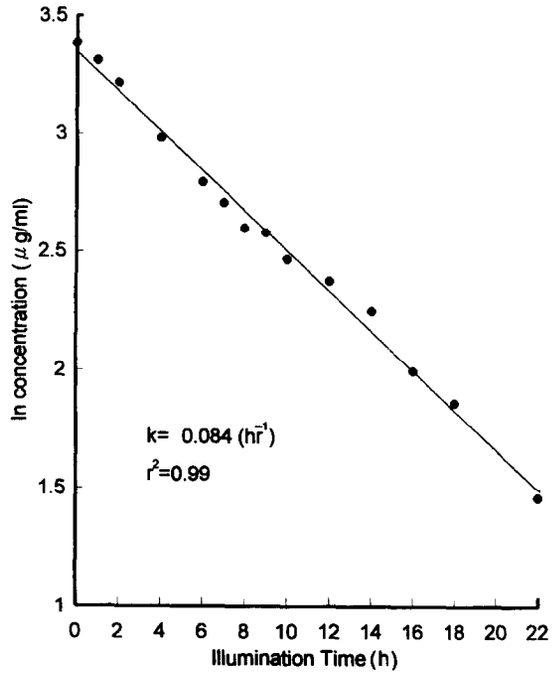

Fig. 10. First-order plot for chlorophyll a degradation during illumination at −5.4°C for 22 h.

at −5.4°C for 22 h. A linear regression curve with high correlation coefficient ($r^2 = 0.99$) between ln concentration and illumination time was observed. This result clearly showed that chlorophyll a degradation fits the first-order model, and the degradation rate was very fast as shown by k value [0.084 (hr^{-1})]. Similar results were reported by Schwartz and von Elbe (1983), and Schwartz and Lorenzo (1990).

Illumination of chlorophyll a and all-*trans*-β-carotene

Figure 11 shows the concentration changes of β-carotene and its *cis* isomers, and chlorophyll a during illumination at −5.4°C for 22 h. No significant concentration change ($p > 0.05$) occurred for chlorophyll a during illumination, indicating that the light energy absorbed by chlorophyll a could be transferred to triplet oxygen and all-*trans*-β-carotene, which in turn resulted in formation of 9-*cis*-, 13-*cis*-, 15-*cis*- and 13,15-di-*cis*-β-carotene. The formation of 13,15-di-*cis*-β-carotene was not observed until the illumination time reached 2 h, and the amount increased thereafter. No significant change ($p > 0.05$) was observed for either 9-*cis*- or 15-*cis*-β-carotene during illumination for 22 h. The degradation of all-*trans* β-carotene may also fit the first-order model. No significant concentration change ($p > 0.05$) of all-*trans*-β-carotene was observed after 18 h exposure, indicating that the whole system may, reach equilibrium at this stage. The same result was also found for iodine-catalysed illumination of all-*trans*-β-carotene. For 13-*cis*-β-carotene, a significant increase ($p < 0.05$) was found for exposure time 3–8 h, and the amount decreased thereafter. However, no significant difference ($p > 0.05$) was found after the exposure time reached 17 h and over. The inconsistent change of 13-*cis*-β-carotene may be due to interconversion between all-*trans*-, 13-*cis*-, or 13,15-di-*cis*-β-carotene. This result seems to be contradictory to some reports by Chandler and Schwartz (1987) and O'Neil and Schwartz (1995). The results of the former study showed that the amount of 9-*cis*-β-carotene increased in the beginning, and then decreased during iodine-catalysed illumination of all-*trans*-β-carotene for 76 min, while the latter study showed that the level of 9-*cis*-β-carotene increased at the beginning, then decreased, and again increased during chlorophyll a catalysed photoisomerization of all-*trans*-β-carotene. This difference may be due to light intensity, illumination mode, variety and concentration of catalyst, and chlorophyll a concentration. Jensen *et al.* (1982) demonstrated that the inhibition effect of excited singlet chlorophyll a by all-*trans*-β-carotene was dependent upon concentrations of both. From Fig. 11 it can also be found that the amount of *cis*-β-carotene formed does not equal to the amount of all-*trans*-β-carotene degraded. This is because, in addition to isomerization, all-*trans*-β-carotene can also be degraded to other smaller compounds. Also, the *cis* isomers of β-carotene may be degraded further or converted to other *cis* forms of β-carotene. Similar phenomena were observed in Figs 1, 2, 5–7.

In conclusion, 13-*cis*- and 13,15-di-*cis*-β-carotene were the major *cis* isomers of β-carotene formed during oven-heating while 13-*cis*-β-carotene was favoured during reflux-heating. For illumination with or without iodine as catalyst, 13,15-di-*cis*-β-carotene was the major *cis* isomer of β-carotene formed. The formation of 13,15-di-*cis*-β-carotene may be due to conversion of 13-*cis*- or 15-*cis*-β-carotene. The degradations of the total amount of both β-carotene and chlorophyll a may fit the first-order model during heating or illumination. No epimerization of chlorophyll a was observed as a result of illumination. Nevertheless, it should be noted that the results observed in this study may not be identical to those in real food systems because of presence of water, protein, fat, carbohydrate and other components.

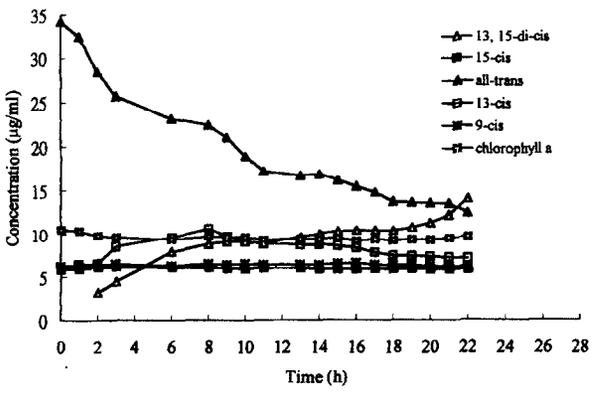

Fig. 11. Concentration changes of β-carotene and its *cis* isomers, and chlorophyll a during illumination at −5.4°C for 22 h.

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