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Relationship between chlorophyll a and β-carotene in a lipid-containing model system during illumination

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The relationship between chlorophyll a (Chl a) and β-carotene during illumination 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 exposed to a 40 w light for 3 h. Isomerization and degradation reations 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 separated and detected. Both the degradations of total amount of Chl a and β-carotene fit the first-order model. The degradation rate of total amount of β-carotene was highest in methyl stearate, followed by in methyl oleate and in methyl linoleate, while a reverse order was observed for the degradation rate of total amount of Chl a. In the presence of three fatty acid esters, Chl a is more susceptible to isomerization and degradation than β-carotene, and the degradation rates for both Chl a and β-carotene are significantly different ($p < 0.05$). © 1998 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

Both carotenoids and chlorophylls are important components of edible vegetable oil; though present in minute amount, they can be responsible for colour and oxidation stability of oil (Endo *et al.*, 1984, 1985; Usuki *et al.*, 1984; Fakourelis *et al.*, 1987). The beneficial effects of carotenoids and chlorophylls to human health have been well established (Mathews-Roth, 1981, 1982, 1985; Krinsky, 1989; Ziegler, 1989; Sheer, 1991). In addition, carotenoids, such as β-carotene, are reported to possess light-filtering effects, and thus minimize the photooxidation of oil during illumination (Fakourelis *et al.*, 1987; Lee and Min, 1988, 1990). Unlike carotenoids, chlorophylls such as chlorophyll a (Chl a) have been shown to be photosensitizers; i.e. after absorption of energy Chl a can transfer it to triplet oxygen to form a more reactive singlet oxygen, which then reacts with unsaturated fatty acids or β-carotene (Foote and Denny, 1968; Foote *et al.*, 1970a,b; Rawls and Santen, 1970; Carlsson *et al.*, 1976; Terao and Matsushita, 1977; Jensen *et al.*, 1982; Endo *et al.*, 1984, 1985; Usuki *et al.*, 1984; Kiritsakis and Dugan, 1985; Lee and Min, 1988, 1990; O'Neil and Schwartz, 1995). The results of these studies also showed that, in the presence of β-carotene, the oxidation stability of oil under light storage can be greatly increased because β-carotene itself could reduce the photosensitized oxidation by a combination of excited triplet-sensitizer quenching and singlet oxygen quenching. In a study dealing with the photosensitized isomerization of β-carotene in the presence of Chl a, Jensen *et al.* (1982) suggested that the mechanism of triplet β-carotene isomerization should be due to triplet energy transfer from Chl a to β-carotene. This result was further confirmed by O'Neil and Schwartz (1995), who reported that light alone did not photoisomerize β-carotene into 9-cis isomer to the extent observed when chlorophyll compounds are present. Instead, the isomerization is thought to occur following the quenching of the excited triplet state of chlorophyll compounds by β-carotene.

The quenching effect of β-carotene on excited Chl a during illumination was reported to be dependent upon concentrations of both (Jensen *et al.*, 1982; O'Neil and Schwartz, 1995). Kiritsakis and Dugan (1985) pointed out that the photosensitized oxidation of edible oil containing 6 ppm Chl a can be retarded in the presence of 100 ppm β-carotene. This result implied that Chl a may possess prooxidant or antioxidant ability towards edible oil during storage. Endo *et al.* (1984) studied the effect of various chlorophyll concentrations on the stability of methyl linoleate during illumination and found that, with chlorophyll concentration at 20 ppm and above, the peroxide value increased sharply, indicating that photosensitized oxidation is the main reaction. In

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contrast, some researchers reported that chlorophyll may possess antioxidant ability during storage of oil in the dark (Matsushita and Iwami, 1965; Endo *et al.*, 1984; Usuki *et al.*, 1984).

Numerous published reports dealt with the effects of carotenoids or chlorophylls on the oxidation stability of oil during processing and storage (Foote and Denny, 1968; Foote *et al.*, 1970a,b; Endo *et al.*, 1984, 1985; Usuki *et al.*, 1984; Fakourelis *et al.*, 1987; Lee and Min, 1988, 1990; Yen and Chen, 1995). Very few reports have dealt with the stability of carotenoids and chlorophylls in edible oil during processing and storage. The degradation of β-carotene or Chl a in edible oil can be correlated well with the degree of unsaturation of fatty acids. Theoretically speaking, the high degree of unsaturation of oil can facilitate the degradation of β-carotene. However, Carnevale *et al.* (1979) observed that the degradation rate of β-carotene increased with increasing degree of saturation of edible oil. As these results are contradictory, it is necessary to study the degradation and isomerization of β-carotene and Chl a in a lipid-containing model system during illumination. The purposes of this stud were to determine the degradation and isomerization of β-carotene and Chl a, and postulate the relationship between them in the presence of methyl stearate, methyl oleate and methyl linoleate during illumination.

MATERIALS AND METHODS

Materials

Chlorophyll a (Chl a with a purity of approximately 100%) and all-trans-β-carotene (purity 95%) standards 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 also 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. A Sep-Pak C_{18} cartridge containing 500 mg packing material was from J. T. Baker Co, (Phillipsburg, NJ, USA). A Vydac 201TP54C_{18} column (25cm×4.6mm I.D.) packed with 5-μm particle size (Hesperia, CA, USA) was used. Chlorophyll a′ (Chl a′) standard was prepared using a method as 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, NA). 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).

Illumination of Chl a and all-trans-β-carotene in the presence of fatty acid esters

One working solution of 1000 μg/ml Chl a was prepared by dissolving 10 mg Chl a in 10 ml acetone, while three working solutions of 1000 μg/ml all-trans-β-carotene were prepared by dissolving 1 mg β-carotene in 1 ml methyl oleate, 1 ml methyl linoleate and 1 ml acetone containing 1 g methyl stearate individually. Aliquots of 60 μl Chl a and 60 μl all-trans-β-carotene were collected and mixed in a 15-ml test tube with a screw-cap on the top, and 880 μl of methyl stearate, methyl oleate and methyl linoleate each was added to bring about a total volume of 1000 μl for each treatment. A total of 36 tubes was used and luminated at 10°C for 0, 10, 30, 60 120 and 180 min. The fluorescent tube (General Electric 40 w) was suspended approximately 10 cm above the tube, where the light intensity measured about 5000 lux. All the sample tubes were stored at refrigerated temperature prior to illumination. Six tubes, which contained the solutions of methyl stearate, methy oleate and methyl linoleate in duplicate, were randomly collected at time intervals and inserted into an ice box to terminate the reaction. All the treated samples were then dissolved in 3 ml n-hexane for extraction.

Extraction of Chl a, β-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 up to about 0.5 cm high. Three ml of sample was poured into the Sep-Pak-C_{18} cartridge, which was previously activated by 6 ml methanol and treated with 12 ml n-hexane. The solutions containing all-trans-β-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 the 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-μm membrane filter. Twenty μl of sample

was collected for HPLC analysis. The 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-μm membrane filter, and 20 μl sample was collected for HPLC analysis.

Separation, identification and quantification of Chl a, β-carotene and their isomers

Two ternary solvent systems of methanol-acetonitrile-tetrahydrofuran (57:42:1, v/v/v) and methanol-acetonitrile-water (94:5:1, v/v/v) were developed to separate carotene and its cis isomers, and Chl a and its isomers, respectively, with detection wavelengths at 450 nm and 660 nm. A Vydac 201TP54 C_{18} column, with flow rates 1.0 ml/min for the former and 0.8 ml/min for the latter, was used. The sensitivity was 0.02 AUFS and the injection volume was 20 μl for both. β-Carotene, Chl a and Chl a′ were identified by comparison of retention times and absorption spectra of unknowns with reference standards. In addition, the identifications of cis-β-carotene isomers were based on spectral characteristics and Q ratios as described in some 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 using an external calibration method. Eight concentrations ranging from 10 to 100 ppm of all-trans-β-carotene and of Chl a each were prepared and the calibration curves for both were obtained by plotting area against concentration. The calibration curves gave good linearity for both (r^2 0.9893 for all-trans-β-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 and Chl a isomers I and II 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 then the spectra were normalized and overlaid to see if there is any difference in curve shape. The purity of each peak can be assessed to be 100% if no difference in curve shape is observed. Duplicate analyses were conducted and mean values were determined. The rate constants of degradation of total amounts of Chl a and β-carotene were also determined using a method described by Chen *et al.* (1994).

RESULTS AND DISCUSSION

HPLC separation of all-trans-β-carotene and its cis isomers

Many HPLC methods have been developed to separate all-trans-β-carotene and its cis isomers using a polymeric C_{18} column (Chen and Chen, 1993, 1994; Chen *et al.*, 1994, 1995). However, using these methods in this study can only result in partial resolution of all-trans-β-carotene and its four cis isomers, mainly because the column-to-column variability was greater for a polymeric column than for a monomeric column (Epler *et al.*, 1992; Chen *et al.*, 1995). Thus, a new method has to be developed. After various studies we found that a ternary solvent system of methanol-acetonitrile-tetrahydrofuran (57:42:1, v/v/v), with flow rate at 1.0 ml/min, sensitivity at 0.02 AUFS and detection wavelength at 450 nm, was able to resolve all-trans-β-carotene and its four cis isomers, 9-cis-, 13-cis-, 15-cis-, and 13,15-di-cis-β-carotene. Figure 1 shows the HPLC chromatogram of all-trans-β-carotene and its four cis isomers during illumination in the presence of Chl a and methyl stearate for 3 h. The separation was complete within 12 min and the capacity factor (k′) for all-trans-, 9-cis-, 13-cis-, 15-cis- and 13,15-di-cis-β-carotene was 4.2, 4.8, 5.1, 3.1 and 2.6, respectively. It has been well established that the k′ values should be controlled between 2 and 10 so that an ideal separation can be achieved (Dolan, 1990). The purity of each peak was assessed to be close to 100% as no difference in curve shape was observed.

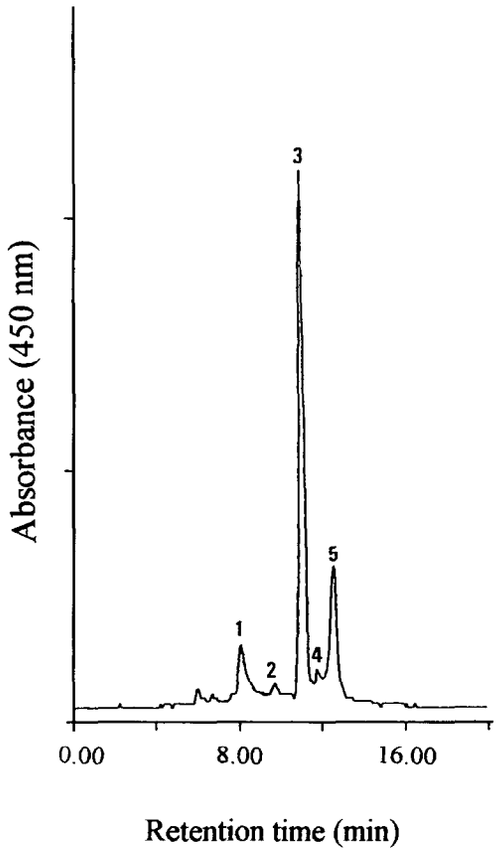

Fig. 1. HPLC chromatogram of all-trans-β-carotene and its isomers in the presence of Chl a and methyl stearate during illumination for 3 h. Chromatographic conditions described in text. Peaks: 1 = 13,15-di-cis-β-carotene. 2 = 15-cis-β-carotene, 3 = all-trans-β-carotene, 4 = 9-cis-β-carotene, 5 = 13-cis-β-carotene.

HPLC separation of Chl a and its isomers

A ternary solvent system of methanol-acetonitrile-water (94:5:1, v/v/v) was developed to separate Chl a and its three isomers, with flow rate at 0.8 ml/min, sensitivity at 0.02 AUFS and detection wavelength at 660 nm. Figure 2 shows the HPLC chromatogram of Chl a and its isomers during illumination in the presence of all-trans-β-carotene and methyl linoleate for 3 h. The separation was complete within 13 min, and the k′ values for Chl a, Chl a′, Chl a isomer I, and Chl a isomer II were 1.5, 2.2 1.0 and 1.3, respectively. Chl a isomers I and II are probably oxidation products formed during illumination because the spectra of both were identical to those of Chl a and Chl a′.

Percentage changes of all-trans-β-carotene and its cis isomers during illumination in the presence of Chl a and fatty acid esters

Table 1 shows the percentage changes of all-trans-β-carotene and its cis isomers during illumination in the presence of Chl a and methyl stearate for 3 h. The amount of all-trans-β-carotene decreased by 8% after 1h illumination, and approached equilibrium after prolonged exposure to light for 3 h. No significant percentage change was observed for 15-cis-β-carotene. In contrast, the amounts of 13-cis-β-carotene and 13,15-di-cis-β-carotene increased by 14.8% and 2.6% respectively, after 3 h illumination. 9-cis-β-carotene decreased by 3.4%. The degradation of total amount of β-carotene during illumination in the presence of Chl a and methyl stearate fits the first-order model because a linear correlation ($r^2 = 0.9363$) was observed for the plot of logarithm of the total β-carotene concentration versus time, and the rate constant was 0.21 (h^{-1}).

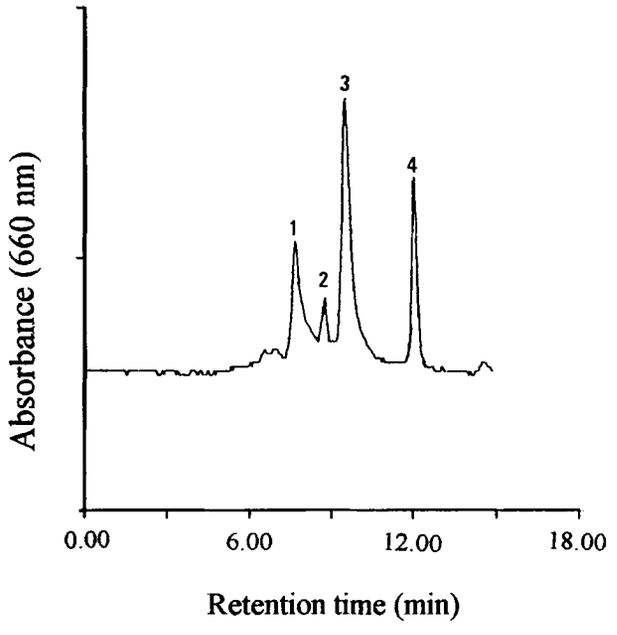

Fig. 2. HPLC chromatogram of Chl a and its isomers in the presence of β-carotene and methyl linoleate during illumination for 3 h. Chromatographic conditions described in text. Peaks I = Chl a isomer I, 1 = Chl a isomer II, 3 = Chl a, 4 = Chl a′.

The percentage changes of all-trans-β-carotene and its cis isomers during illumination in the presence of Chl a and methyl oleate for 3 h are also shown in Table 1. The amounts of all-trans-β-carotene and 9-cis-β-carotene decreased by 9.2% and 3.5%, respectively, after 3h illumination, while the amounts of 13-cis-β-carotene and 13,15-di-cis-β-carotene increased by 8.6% and 2.3%. 15-cis-β-carotene, increased by 2.2% after 30 min illumination, and decreased by 0.4% thereafter. The degradation of total amount of β-carotene during illumination in the presence of Chl a and methyl oleate fits the first-order model because a linear correlation ($r^2 = 0.9423$) was observed for the plot of logarithm of the total β-carotene concentration versus time, and the rate constant was 0.19 (h^{-1}).

The percentage changes of all-trans-β-carotene and its cis isomers during illumination in the presence of Chl a and methyl linoleate for 3 h are also shown in Table 1. The amounts of all-trans-β-carotene and 9-cis-β-carotene decreased by 3.5% and 2.4%, respectively, after 3 h illumination, while the amounts of 13-cis-β-carotene and 13,15-di-cis-β-carotene increased by 3.5% and 3.3%. 15-cis-β-carotene, remained unchanged in the initial illumination period and then decreased sharply after prolonged exposure to light for 3 h. The degradation of total amount of β-carotene fits the first-order model durin illumination in the presence of Chl a and methyl linoleate because a linear correlation ($r^2 = 0.9168$) was observed for the plot of logarithm of the total β-carotene concentration versus time. and the rate constant was 0.14 (h^{-1})

By comparison of the results described above, it can be found that, in the presence of Chl a and fatty acid esters, 13-cis-β-carotene was formed in highest amount during illumination, followed by 13, 15-di-cis-β-carotene and 15-cis-β-carotene. The formation of 13, 15-di-cis-β-carotene may be due to conversion of 15-cis-β-carotene as reported by Chen *et al.* (1994, 1995) and Chen and Huang (1997). In the presence of Chl a, the rate constant for degradation of total amount of β-carotene in methyl stearate was higher than that in methyl oleate or in methyl linoleate. This may be explained as follows: during illumination Chl a can absorb energy and transfer it to triplet oxygen to form a more reactive singlet oxygen, which then reacts with β-carotene or fatty acid esters to form hydroperoxides and free radicals (Foote *et al.*, 1968) (Fig. 3). It has been well established that the formation of free radicals and hydroperoxides during illumination can facilitate destruction of Chl a and β-carotene (Endo *et al.*, 1984; Usuki *et al.*, 1984). In the three fatty acid esters used in this study. methyl linoleate is more susceptible to formation of hydroperoxides and free radicals than methyl oleate or methyl stearate. Thus, in the presence of methyl linoleate, more destruction of

Table 1. Percentage changes of all-trans-β-carotene and its cis isomers in the presence of Chl a and fatty acid esters during illumination for varied lengths of time[a]

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
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
10	4.4	1.0	83.0	0.2	11.4	4.1	3.1	81.0	0.6	11.2	5.2	1.2	81.5	1.7	10.4
30	5.1	1.9	80.0	0.9	12.1	4.0	3.5	81.0	1.4	10.1	5.6	1.3	80.3	1.5	11.3
60	5.1	0.9	76.0	0.8	17.2	3.6	2.9	80.0	0.6	12.9	5.7	0.7	79.8	1.5	12.3
120	5.7	0.6	70.8	0.7	22.2	5.4	3.2	75.0	0.3	16.1	6.4	0.5	80.8	1.8	10.5
180	5.8	0.5	70.8	0.7	22.2	5.5	3.1	74.8	0.6	16.0	6.5	0.4	80.5	1.7	10.9

[a]Average of duplicate analyses.

Chl a should be observed, and less singlet oxygen was available to react with β-carotene, which in turn results in the lowest degradation rate. As the three fatty acid esters can also compete with Chl a or β-carotene for formation of hydroperoxides, this would make the reaction between Chl a and β-carotene less intense. Also, it has been reported that unsaturated fatty acids, such as linoleic acid, are more susceptible to reacting with free radical than β-carotene (Arya *et al.*, 1979; Carnevale *et al.*, 1979). Hence, the reaction between β-carotene and free radical would be less intense in the presence of methyl linoleate, and a lower degradation rate of β-carotene is thus observed. In addition to degradation, β-carotene may be oxidized by autooxidation and photosensitized oxidation under light. As the reaction rate for the latter was found to be 1450 times higher than the former (Nawar, 1985), the oxidation rate of β-carotene will be greater under light storage in the presence of a sensitizer. Moreover, it has been demonstrated that the reaction between β-carotene and singlet oxygen would rather go by physical quenching than chemical quenching. i.e. it would rather undergo isomerization than degradation (Young and Brewer, 1978; Krinsky, 1979). This would explain why β-carotene is able to quench singlet oxygen during photosensitized oxidation. For the relationship between Chl a and β-carotene during illumination, Chl a may also transfer energy to β-carotene and result in isomerization of triplet β-carotene (Jensen *et al.*, 1982; O'Neil and Schwartz, 1995). It is also possible that the high energy triplet β-carotene can be convered to low energy singlet β-carotene by vibrational degradation or other radiationless transitions (Fig. 3).

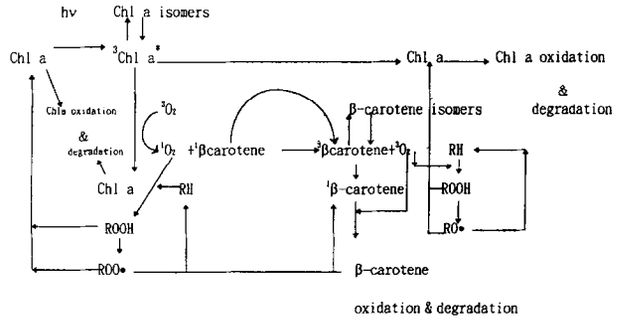

Fig. 3. Postulated relationship between Chl a and β-carotene in the presence of fatty acid esters during illumination at 10°C for 3 h. RH: fatty acid; ROOH: hydroperoxide; $^3O_2$: triplet oxygen; $^1O_2$: singlet oxygen; ^{3}Chl a*: excited triplet Chl a.

Percentage changes of Chl a and its isomers during illumination in the presence of all-trans-β-carotene and fatty acid esters

Table 2 shows the percentage changes of Chl a and its isomers during illumination in the presence of all-trans-β-carotene and fatty acid esters for 3 h. The amount of Chl a decreased with increasing illumination time, and approached equilibrium, after exposure time reached 60 min. In contrast, the amount of Chl a isomer I increased with increasing exposure time, and the increased percentage was 31.6% after prolonged illumination for 3 h. For Chl a′, it increased by 9.3% after 30 min illumination and showed significant decrease thereafter. A similar trend was observed for Chl a isomer II with an increase by 22.9% after 60 min illumination, which showed no significant decrease thereafter. The degradation of total amount of Chl a fits the first-order model because a linear correlation ($r^2 = 0.9765$) was observed for the plot of the logarithm of total Chl a concentration versus time, and the rate constant was 0.34 (h^{-1}).

The percentage changes of Chl a and its isomers during illumination in the presence of all-trans-β-carotene and methyl oleate for 3 h are also shown in Table 2. The amount of Chl a decreased by 36.7% while the amount of Chl a′ increased by 8.7% after 3 h illumination. A similar trend was observed for Chl a isomer I with an increase of 20.1% after exposure time reached 3 h. Chl a isomer II, increased by 1.9% after 30 min illumination, and then further decreased by 6.0% after prolonged exposure to light for 3 h. The degradation of total amount of Chl a fits the first-order model because a linear correlation ($r^2 = 0.9867$) was observed for the plot of the logarithm of total Chl a concentration versus time, and the rate constant was 0.42 (h^{-1}).

Table 2. Percentage changes of Chl a and its isomers in the presence of β-carotene and fatty acid esters during illumination for varied lengths of time[a]

Time (min)	Methyl stearate				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′
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
10	19.4	13.2	58.9	8.5	6.4	9.3	82.9	1.4	2.4	6.3	77.9	13.4
30	38.6	24.1	28.0	9.3	12.6	10.1	75.0	2.3	5.6	9.4	72.0	13.0
60	35.4	31.1	25.1	8.4	18.4	8.4	70.1	3.1	10.4	12.1	63.1	14.4
120	40.5	28.5	25.0	6.0	29.5	7.5	60.0	3.0	12.5	5.8	59.0	22.7
180	40.7	29.1	25.0	5.2	29.2	16.1	46.0	8.7	11.7	7.9	59.0	21.4

[a]Average of duplicate analyses

The percentage changes of Chl a and its isomers during illumination in the presence of all-trans-β-carotene and methyl linoleate for 3 h are also shown in Table 2. The amount of Chl a decreased by 23.7% while the amount of Chl a′ increased by 21.4% after 3 h illumination. Chl a isomer I showed decrease in the initial period of illumination, and then further increased to 11.7% after 3 h exposure to light. In contrast, the amount of Chl a isomer II increased by 3.9% after 1 h illumination, and then further decreased by 1.5% after exposure time reached 3 h. The degradation of total amount of Chl a fits the first-order model because a linear correlation ($r^2 = 0.9707$)was observed for the plot of the logarithm of total Chl a concentration versus time, and the rate constant was 0.63 (h^{-1}).

By comparison of the results shown above, it can be found that, in the presence of β-carotene, Chl a′ was formed in higher amount in methyl linoleate than in methyl oleate or methyl stearate. In contrast, both Chl a isomers I and II were formed in greater amount in methyl stearate than in methyl oleate or methyl linoleate. This is probably because methyl linoleate can compete with Chl a for singlet oxygen, and thus less singlet oxygen is available for formation of Chl a′. In contrast, more singlet oxygen is available for formation of Chl a isomers I and II in the presence of methyl stearate. In the presence of β-carotene the degradation rate of total amount of Chl a during illumination was found to be highest in methyl linoleate, followed by in methyl oleate and in methyl stearate. This is probably because methyl linoleate is more susceptible to formation of hydroperoxides and free radicals than methyl oleate or methyl stearate, which in turn can facilitate the degradation rate of Chl a. In addition, Chl a itself may also absorb energy and this results in degradation and isomerization. Endo *et al.* (1984) pointed out that the degradation of total amount of Chl a in the presence of methyl linoleate during illumination for 24 h fits the first-order model. In this study both the isomerization and degradation of Chl a were observed, probably because during illumination at 10°C both light and heat energies could be provided by the fluorescent tube. It has been reported that the fluorescent tube (40 W) constitutes about 2% visible light, 18% fluorescent light, 26% heat by radiation and 54% heat by conduction (Cheng, 1987). However, in another study Chen and Huang (1997) reported that no isomerization of Chl a was observed when illuminated at −5.4°C. Apparently this difference can be attributed to the low illumination temperature (−5.4°C) used in that study, which may dissipate heat to a considerable extent, and hence only degradation of Chl a was observed. Since most energy is emitted from the fluorescent tube by heat conduction, the amount of Chl a′ can be formed in proportional to the increase of illumination time. This result implies that heat energy plays an imporant role for formation of chl a′ during illumination. Many researchers have also observed the formation of Chl a′ from Chl a during blanching or microwave cooking of vegetables (Schwartz *et al.*, 1981; Von Elbe *et al.*, 1986; Schwartz and Lorenzo, 1991; Chen and Chen, 1993). From the preceding results it may be concluded that, in the presence of fatty acid esters, Chl a is more susceptible to isomerization and degradation than β-carotene during illumination at 10°C. Further research is necessary to elucidate the conversion mechanism between Chl a and its isomers during illumination.

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