Stability of Chlorophylls and Carotenoids in Sweet Potato Leaves during Microwave Cooking

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The effect of microwave cooking on the stability of chlorophylls and carotenoids in sweet potato leaves was studied. Each cooking treatment was conducted at 2450 MHz with an output power of 700 W for 0, 2, 4, or 8 min in duplicate. The various chlorophylls and carotenoids were analyzed by high-performance liquid chromatography with photodiode array detection. Results implied that in most cases the content of each pigment decreased along with the increase of heating time. Chlorophylls and the epoxy-containing carotenoids were the most susceptible to heat loss. Pigments formed during microwave cooking included chl *b* isomer, chl *a* isomer I, chl *a* isomer II, pheophytin *b*, pheophytin *a*, pyropheophytin *a*, *cis*-neochrome, and two lutein dehydration products, 3,4-didehydro-β,ε-caroten-3′-ol and 3′,4′-didehydro-β,β-caroten-3-ol.

INTRODUCTION

Sweet potato leaves (*Ipomoea* spp.), a green vegetable grown in Taiwan, are well-known for their protective effect against high blood pressure and cancer (Li and Liu, 1973). Since sweet potato leaves are also rich sources of chlorophylls and carotenoids (Tung et al., 1961), they have received considerable attention regarding their roles in human nutrition and health. The possible roles of carotenoids in the treatment of human disease such as skin cancer have been reported (Mathews-Roth, 1981, 1982, 1985). More recently, chlorophylls have attracted interest as phototherapeutic drugs (Sheer, 1991).

Due to the presence of a long chain of conjugated carbon–carbon double bonds, both chlorophylls and carotenoids are susceptible to light, oxygen, heat, and acid degradations. Jensen et al. (1982) studied the photoisomerization of *all-trans*-β-carotene in the presence of chlorophyll *a* and found that *all-trans*-β-carotene undergoes trans–cis isomerization under conditions where the triplet state is the most likely intermediate. The authors also suggested that the mechanism involved should be transfer of triplet energy from chlorophyll *a* to β-carotene and subsequent isomerization of triplet β-carotene. In another study dealing with carotene and chlorophyll bleaching by soybeans with and without seed lipoxygenase, Hildebrand and Hymowitz (1982) reported that lipoxygenase stimulates chlorophyll cooxidation more than it does carotene cooxidation. However, the cooxidation of carotene and chlorophyll relative to the peroxidation of linoleic acid is lower for purified lipoxygenase than for whole mature seed extracts.

The most important factors influencing the quantities of chlorophylls and carotenoids retained during heating are temperature and length of heat treatment. It has been well established that most chlorophylls are converted to pheophytins and pyropheophytins during processing of green vegetables (Schwartz et al., 1981; Khachik et al., 1986; Schwartz and Loreno, 1991). Since the bright green color of chlorophylls is more pleasing to the consumer than the olive-brown color of pheophytins and pyropheophytins, the selection of an appropriate heat treatment to preserve the green color of a vegetable is important. Schwartz et al. (1981) reported that blanching vegetables could induce the formation of chlorophylls *a′* and *b′*, the C_{10} epimers of chlorophylls *a* and *b*, respectively. In addition, the authors also reported that pyropheophytins were formed via C_{10} decarbomethoxylated derivatives of pheophytins during canning of vegetables. These results suggested that the extent of pyropheophytin formation was dependent upon the severity of the heat treatment.

Carotenoids can lose provitamin A activity because of formation of cis-isomers during heating (Khachik et al., 1986; Chen and Han, 1990; Chen, 1992). Khachik et al. (1986) found that about 60% of the total xanthophylls in Brussels sprouts was destroyed as a result of microwave cooking. The authors also found that there were no significant stereochemical changes in the ratio of the cis- to trans-isomers of neoxanthin, lutein epoxide, and lutein. However, in another study Chen (1992) reported that both *cis*-lutein and *cis*-β-carotene contents in garland chrysanthemum increased along with the increase of heating time when the microwave power was 180 W. Since these results are contradictory, the effect of microwave cooking on the formation of carotenoid and chlorophyll isomers in vegetables needs to be further investigated. The purpose of this study was to determine the effect of microwave cooking on the stability of chlorophylls and carotenoids in sweet potato leaves.

MATERIALS AND METHODS

Instrumentation. The HPLC instrument consisted of a SSI 222D pump (Scientific System Inc., State College, PA) with a Linear photodiode array detector (Linear Instrument, Reno, NV) and a phenomenex stainless steel column (25 cm × 4.6 mm i.d.) packed with Ultremex C_{18} 5-μm particle size (Torrance, CA). The data were stored and processed with an Axxiom 727 dual-channel chromatography data system (Axxiom Chromatography Inc., Calabasas, CA). A sensitivity of 0.01 AUFS was used. Spectrophotometric determinations were made with a Beckman DU-70 double-beam spectrophotometer (Irvine, CA). A solvent system of acetonitrile/methanol/chloroform/hexane (75:12.5:7.5:7.5 v/v/v/v) pumped at a flow rate of 1.0 mL min^{-1} was used (Chen and Chen, 1992). IR spectra of some carotenoids were determined using FT-IR (Nicolet 510 D).

Materials. *trans*-β-Carotene, lutein (75% purity), chlorophyll *a* (chl *a*), and chlorophyll *b* (chl *b*) standards were purchased from Sigma (St. Louis, MO). Lutein was further purified using column chromatography as described by Chen et al. (1991). Pheophytin *a* and pheophytin *b* were prepared by adding a few drops of 0.1 N HCl to chl *a* and chl *b*, respectively. Pyropheophytin *a* was prepared using a method described by Schwartz et al. (1981). Lutein epoxide, neoxanthin, and violaxanthin standards were prepared from saponified water convolvulus extract by TLC (Chen et al., 1991). All HPLC grade solvents were

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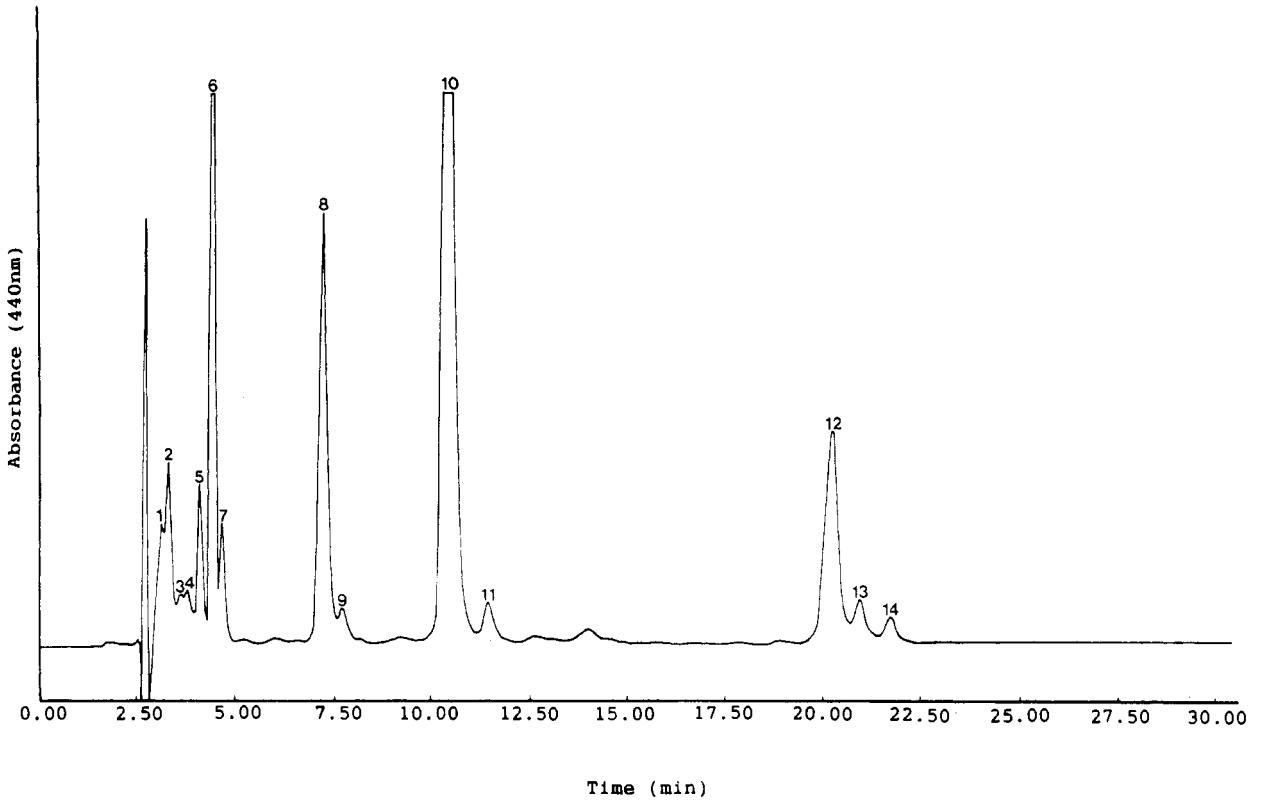

Figure 1. Chromatogram of unsaponified extract prepared from sweet potato leaves without cooking treatment. Chromatographic conditions are described in the text. Peaks: 1, 9-*cis*-neoxanthin; 2, violaxanthin; 3, violeoxanthin; 4, 13-*cis*-lutein epoxide; 5, lutein epoxide; 6, lutein; 7, 9-*cis*-lutein; 8, chl *b*; 9, chl *b*′; 10, chl *a*; 11, chl *a*′; 12, β-carotene; 13, 9-*cis*-β-carotene; 14, 13-*cis*-β-carotene.

purchased from Merck (Taiwan) Ltd. (Taipei, Taiwan) and filtered through a 0.2-μm membrane filter under vacuum prior to use. Sweet potato leaves were purchased fresh from a local supermarket. Approximately 1 kg of leaves was collected, and a 50-g subsample was randomly selected for cooking treatment.

Cooking Treatment. Microwave cooking treatment was conducted in duplicate. Fifty grams of fresh leaves was placed in a heat-resistant bag to prevent moisture loss and cooked in the microwave oven for 0, 2, 4, and 8 min. The microwave oven was operated at 2450 MHz with an output power of 700 W; a turntable was used to obtain uniform cooking.

Extraction of Carotenoids and Chlorophylls. Each raw and cooked sample from 50 g of leaves was mixed with 5 g of $MgCO_3$ and 100 mL of extractant (hexane/acetone/absolute alcohol/toluene, 10/7/6/7), and the mixture was blended for 3 min. The extract was vacuum filtered through a Büchner funnel fitted with a No. 1 filter paper, and the filtrate was collected in a suction flask prefilled with 100 mL of 10% Na_2SO_4. The residue was reextracted with 100 mL of extractant and 100 mL of hexane to remove all of the pigments. The crude extract in the suction flask was poured into a separatory funnel to form two layers. The upper layer containing chlorophylls and carotenoids was collected and evaporated to 50 mL under vacuum using a rotary evaporator. Portions of the crude extract (0.5 mL) were purified using a silica cartridge (Sep-Pak, Waters Associates, Boston, MA) and eluted with 5 mL of hexane/acetone/methanol (70/20/10 v/v/v). The eluate was evaporated to dryness under vacuum and dissolved in 3 mL of chloroform. Then the solution was filtered through a 0.2-μm membrane filter and stored at −30 °C until use.

Separation of Carotenoids and Chlorophylls by HPLC. A quaternary solvent system of acetonitrile/methanol/chloroform/hexane (75/12.5/7.5/7.5 v/v/v/v) pumped at a flow rate of 1 mL/min was used (Chen and Chen, 1992). The eluate was monitored at 440 nm with a sensitivity of 0.01 AUFS. The various carotenoids and chlorophylls were identified by comparing retention times of separated peaks with reference standards and cochromatography with added standards. Also, the individual HPLC peaks of 3–10 runs were selectively collected and pooled, and the solvents were removed under vacuum. Each pigment was dissolved in an appropriate solvent for spectra analysis (Davies, 1976; Bauernfeind, 1981). An epoxide test was conducted to identify the presence of the epoxy-containing carotenoids such as neoxanthin, violaxanthin, and lutein epoxide (Davis, 1976).

Identification of Carotenoids by IR Spectra. Some carotenoids were also identified by IR spectra (Moss and Weedon, 1976). Approximately 20 injections of eluates were collected, the solvent was removed under vacuum, and the residue was dissolved in chloroform. The solution was spread on a KBr disk as a thin film for IR analysis. The scanning range was between 400 and 4000 cm^{-1}.

Quantification of Carotenoids and Chlorophylls by HPLC. The various carotenoids and chlorophylls were quantified using an external calibration method. The calibration curves for each pigment were obtained by area measurement of reference compounds at four concentrations. Pigments prepared for the calibration curve included β-carotene, lutein, lutein epoxide, neoxanthin, violaxanthin, chl *a*, chl *b*, pheophytin *a*, pheophytin *b*, and pyropheophytin *a*. The calibration curves gave good linearity (r^2 = 0.99–1.00), and the relative standard deviations were less than 3%. *cis*-Neoxanthin was calculated as neoxanthin and violeoxanthin as violaxanthin equivalents, *cis*-β-carotene was calculated as β-carotene equivalent, and chl *a*′ and chl *a* isomer were calculated as chl *a* and chl *b*′ and chl *b* isomer as chl *b* equivalents. Chl *a*, chl *a*′, chl *a* isomer, pheophytin *a*, and pyropheophytin *a* were quantified using 660 nm, while chl *b*, chl *b*′, chl *b* isomer, and pheophytin *b* were quantified using 645 nm.

RESULTS AND DISCUSSION

Figure 1 shows the HPLC chromatogram of carotenoids and chlorophylls in fresh sweet potato leaves. A total of 14 peaks was resolved within 22 min by employing a quaternary solvent system of acetonitrile/methanol/chloroform/hexane (75/12.5/12.5/7.5 v/v/v/v). This solvent system was chosen on the basis of a previous study by Chen and Chen (1992), who used the same solvent system to resolve 14 peaks in water convolvulus within 22 min. However, in that study two minor peaks were not identified. In this study each peak was identified by

Table I. Identification Data of Carotenoids and Chlorophylls in Fresh and Cooked Sweet Potato Leaves by HPLC

pigment	visible spectra[a]			epoxide test	
	λ_{max} found, nm	solvent	λ_{max} reported,[b] nm	hypsochromic shift	color
9-*cis*-neochrome[c]	397, 420, 448	eluant[d]	–[e]	–	–
	–	ethanol	–	–	–
9-*cis*-neoxanthin	413, 436, 464	eluant	–	396, 418, 449	green
	413, 436, 465	ethanol	–	396, 418, 448	green
violaxanthin	420, 443, 471	eluant	–	382, 405, 432	blue
	417, 440, 469	ethanol	417, 440, 469	380, 403, 431	blue
violeoxanthin	(416), 440, 469	eluant	–	(380), 402, 432	blue
	–	ethanol	(415), 436, 464	–	–
13-*cis*-lutein 5,6-epoxide	419, 440, 470	eluant	–	402, 423, 452	green
	–	ethanol	–	–	–
lutein 5,6-epoxide	421, 443, 473	eluant	–	403, 426, 456	green
	419, 440, 469	ethanol	420, 442, 471	402, 423, 453	green
lutein	423, 445, 474	eluant	–	–	–
	421, 444, 473	ethanol	422, 445, 474	–	–
9-*cis*-lutein	421, 442, 471	eluant	–	–	–
	418, 440, 470	ethanol	–	–	–
3,4-didehydro-β,ϵ-caroten-3′-ol[c]	426, 448, 476	eluant	–	–	–
	424, 447, 476	ethanol	–	–	–
3′,4′-didehydro-β,β-caroten-3-ol[c]	429, 451, 480	eluant	–	–	–
	426, 450, 479	ethanol	–	–	–
chlorophyll *b*	458, 648	eluant	–	–	–
	453, 644	acetone	453, 645	–	–
chlorophyll *b*′	458, 648	eluant	–	–	–
	453, 644	acetone	453, 645	–	–
chlorophyll *b* isomer[c]	459, 648	eluant	–	–	–
	453, 644	acetone	–	–	–
chlorophyll *a*	428, 663	eluant	–	–	–
	430, 662	acetone	431, 662	–	–
chlorophyll *a*′	430, 663	eluant	–	–	–
	430, 663	acetone	431, 662	–	–
chlorophyll *a* isomer I[c]	430, 662	eluant	–	–	–
	428, 661	acetone	–	–	–
chlorophyll *a* isomer II[c]	430, 662	eluant	–	–	–
	428, 661	acetone	–	–	–
pheophytin *b*[c]	429, 650	eluant	–	–	–
	428, 650	acetone	430, 652	–	–
pheophytin *a*[c]	408, 663	eluant	–	–	–
	408, 664	acetone	409, 664	–	–
β-carotene	(430), 456, 485	eluant	–	–	–
	(424), 448, 476	hexane	(425), 450, 477	–	–
9-*cis*-β-carotene	(426), 451, 481	eluant	–	–	–
	–	hexane	–	–	–
13-*cis*-β-carotene	(421), 447, 478	eluant	–	–	–
	–	hexane	–	–	–
pyropheophytin *a*[c]	409, 664	eluant	–	–	–
	409, 664	acetone	–	–	–

[a] Values in parentheses represent shoulders on spectra curves. [b] Reported values of absorption spectra are from four references: Davis (1976); Bauernfeind (1981); Khachik et al. (1986); and Chen and Chen (1992). [c] Pigment formed during microwave cooking. [d] Eluant = acetonitrile/methanol/chloroform/hexane (75/12.5/7.5/7.5 v/v/v/v). [e] Data not available.

comparison of retention time of the unknown with reference standards and by cochromatography with added standards. Some unknowns were also identified by comparing absorption spectra with reference values reported in the literature (Davis, 1976; Bauernfeind, 1981; Khachik et al., 1986; Chen and Chen, 1992) and by appropriate chemical reaction. The epoxy-containing carotenoids were preliminarily identified as lutein epoxide, violaxanthin, and neoxanthin on the basis of their absorption spectra, which were characterized by hypsochromic shifts of 17, 38, and 17 nm upon acidification with 0.1 N HCl and color changes from yellow to green, blue, and green, respectively (Table I). Further identification of cis carotenoids were based on a hypsochromic shift of 2–5 nm, a reduction in fine structure, and a hypochromic effect on absorbance in comparison to the absorption spectra of the parent trans compounds (Goodwin, 1980). The central cis-isomers of carotenoids were identified by a strong peak present in the UV region at about 340 nm (Goodwin, 1980; Khachik et al., 1986). Peak 1 was identified as 9-*cis*-neoxanthin on the basis of absorption spectra [eluant, λ_{max} = 413, 436, 464 nm; ethanol, λ_{max} = 413, 436, 465 nm (Table I)]. A hypsochromic shift of 2 nm occurred in the comparison with the absorption spectra of *trans*-neoxanthin in ethanol (Davis, 1976; Bauernfeind, 1981). The absence of a strong cis peak in the UV region and the exclusion of sterically hindered isomers (7-cis, 7′-cis, 11-cis, 11′-cis) indicated the presence of 9-*cis*-neoxanthin (Khachik et al., 1986). The occurrence of natural neoxanthin (9-cis) in chloroplast has been well established (Goodwin, 1980; Khachik et al., 1986). Khachik et al. (1986) also reported that the presence of *trans*-neoxanthin in green vegetables may be an artifact due to extraction or chromatography. Nevertheless, *trans*-neoxanthin was not detected in fresh sweet potato leaves. Peaks 2 and 3 were identified as violaxanthin and violeoxanthin, respectively. The absence of a strong peak in the UV region and the presence of a shoulder at 416 nm (Davis, 1976) confirmed the identity of violeoxanthin (9-*cis*-violaxanthin) (Table I). Peaks 4 and 5 were identified as *cis*-lutein epoxide and lutein epoxide, respectively, on the basis of absorption spectra and epoxide test (Table I). The presence of a strong peak in the UV region indicated the presence of 13- or 13′-*cis*-lutein epoxide. Peaks 6 and 7

Table II. Effects of Microwave Cooking with High Output Power (700 W) on the Chlorophyll and Carotenoid Contents[a] in Sweet Potato Leaves

pigment	heating time			
	0 min	2 min	4 min	8 min
9-*cis*-neochrome				4.18
9-*cis*-neoxanthin	69.24	50.11	17.49	
violaxanthin	100.56	41.00	13.64	
violeoxanthin	25.94	27.33	13.99	
13-*cis*-lutein 5,6-epoxide	28.35	22.78	13.29	6.92
lutein 5,6-epoxide	69.51	54.66	34.28	19.72
lutein	209.66	145.76	122.44	92.32
9-*cis*-lutein	36.85	33.76	28.56	
3,4-didehydro-β,ϵ-caroten-3′-ol				10.70
3′,4′-didehydro-β,β-caroten-3-ol				10.49
chlorophyll *b*	260.84	163.98	90.96	6.92
chlorophyll *b*′	29.03	54.66	30.09	2.10
chlorophyll *b* isomer				25.18
chlorophyll *a*	891.11	364.40	267.62	41.96
chlorophyll *a*′	60.42	182.20	118.94	12.59
chlorophyll *a* isomer I		19.59	7.35	
chlorophyll *a* isomer II			6.65	31.47
pheophytin *b*				21.52
pheophytin *a*		16.95	36.01	23.58
β-carotene	152.37	113.88	72.41	44.06
9-*cis*-β-carotene	30.00	22.78	10.49	28.33
13-*cis*-β-carotene	20.18	10.02	8.25	6.29
pyropheophytin *a*				16.79

[a] Average of duplicate analyses in micrograms per gram.

were identified as lutein and *cis*-lutein, respectively. *cis*-Lutein may be designated 9-*cis*-lutein because of the absence of a strong peak in the UV region. Peaks 8, 9, 10, and 11 were assigned as chl *b*, chl *b*′, chl *a*, and chl *a*′, respectively. Chl *b*′ and chl *a*′ were identified on the basis of absorption spectra (Table I) and retention behavior on HPLC chromatogram as reported by Schwartz et al. (1981) and Khachik et al. (1986). Peaks 13 and 14 were identified as 9-*cis*-β-carotene and 13-*cis*-β-carotene on the basis of absorption spectra (Table I) and the ratios of absorbance of the cis peak to the absorbance of the middle main absorption peak, which amounted to 0.12 and 0.33, respectively (Quackenbush, 1987; Saleh and Tan, 1991).

Table II shows the effect of microwave heating on the carotenoid and chlorophyll contents in sweet potato leaves when the output power is 700 W. In most cases the carotenoid and chlorophyll contents decreased along with the increase of heating time. However, when the heating time was 2 min, the contents of violeoxanthin, chl *b*′, chl *a*′, chl *a* isomer I, and pheophytin *a* increased significantly (Table II). This result implied that chl *a* was gradually converted to chl *a*′, chl *a* isomer I, and pheophytin *a*, while chl *b* was converted to chl *b*′ during the cooking process. It has been well documented that the liberation of organic acid from vegetable during cooking results in the conversion of chl *a* to pheophytin *a* (Schwartz and Lorenzo, 1990). Interestingly, the formation of pheophytin *b* from chl *b* was not observed. It has been well established that chl *a* is more susceptible to heat loss than chl *b*, and the conversion rate of chl *a* to pheophytin *a* should be 2–6 times higher than that of chl *b* to pheophytin *b* (Schwartz and Von Elbe, 1983; Canjura et al., 1991). Chls *a*′ and *b*′, the C_{10} epimers of chls *a* and *b*, increased most during cooking. The formation of chl *a*′ and chl *b*′ from chl *a* and chl *b* can proceed at room temperature, and the reaction rate is even faster when vegetables are blanched (Schwartz et al., 1981; Von Elbe et al., 1986; Schwartz and Lorenzo, 1990). The absorption spectrum of chl *a* isomer is identical to that of chl *a*, and it is probably an oxidation product of chl *a* during cooking. The slight increase of violeoxanthin content does not necessarily mean that the isomerization of violaxanthin occurred during the initial cooking process. This is because that variation in violeoxanthin content may exist among samples, which in turn can cause experimental errors. This phenomenon has been observed by several workers (Khachik et al., 1986; Chen, 1992).

As the heating time increased to 4 min, the losses of most pigments continued to increase (Table II). Chl *a* isomer II and pheophytin *b* began to form, while pheophytin *a* content continued to increase. The absorption spectrum of chl *a* isomer II is identical to that of chl *a*, and hence it is probably another oxidation product of chl *a*. The significant loss of chl *a* isomer I indicated that chl *a* isomer II was probably formed from chl *a* isomer I. Interestingly, both chl *a*′ and chl *b*′ contents began to decrease. This result implied that the destruction rate of chl *a*′ and chl *b*′ was greater than the formation rate of chl *a*′ and chl *b*′ when the heating time reached 4 min.

As the heating time increased to 8 min, the losses of most pigments reached plateaus (Figure 2; Table II). In contrast, both chl *b* isomer and chl *a* isomer II contents increased to the maximum. Also, *cis*-neoxanthin, violaxanthin, violeoxanthin, *cis*-lutein, and chl *a* isomer I were completely destroyed. Instead, *cis*-neochrome, pyropheophytin *a*, and two lutein dehydration products, 3,4-didehydro-β,ϵ-caroten-3′-ol and 3′,4′-didehydro-β,β-caroten-3-ol, were formed. Pyropheophytin *a* was identified on the basis of absorption spectra (Table I) and retention behavior on HPLC chromatogram as reported by Schwartz et al. (1981). Fraser and Frank (1985) found that pheophytin *a* and pyropheophytin *a* could be distinguished on the basis of the absorbance ratio at 665 and 650 nm. The formation of pyropheophytin *a* from pheophytin *a* during canning of vegetables has been observed by Schwartz et al. (1981). The authors also found that blanching vegetables did not result in the formation of pyropheophytin *a*. Obviously, pyropheophytin *a* can only be formed under severe heating condition. Interestingly, in this study pheophytin *b* did not result in the formation of pyropheophytin *b*. This is probably due to the low concentration of pheophytin *b* present in heated sweet potato leaves. Two lutein dehydration products, 3,4-didehydro-β,ϵ-caroten-3′-ol and 3′,4′-didehydro-β,β-caroten-3-ol, were identified on the basis of absorption spectra (Table I) and IR spectra (Figures 3 and 4). The absorption spectra of these lutein derivatives were similar to that of lutein, except that bathochromic shifts of 3 and 6 nm, respectively, occurred. The increased absorption wavelength apparently results from the increased number of conjugated carbon–carbon double bonds after dehydration of lutein, which amounted to 11 and 12 for 3,4-didehydro-β,ϵ-caroten-3′-ol and 3′,4′-didehydro-β,β-caroten-3-ol, respectively (Figure 5). The former was formed through cleavage of the hydrogen atom on the third carbon and the hydroxy group on the fourth carbon of the β ring, while the latter was formed through cleavage of the hydroxy group on the third carbon and the hydrogen atom on the sixth carbon of the ϵ ring. It has been established that the increased absorption wavelength should be around 5 nm if one more conjugated double bond was added within the ring (Moss and Weedon, 1976). The IR spectra of both showed that a strong absorption band was located at 3400 cm^{-1}, which indicated the presence of a hydroxy group. The weak absorption band located at 1640 cm^{-1} indicated the presence of a long chain of conjugated carbon–carbon double bonds. As for the absorption band located between 2800 and 3200 cm^{-1}, it represented aliphatic hydrocarbon. From the above discussion these two lutein derivatives

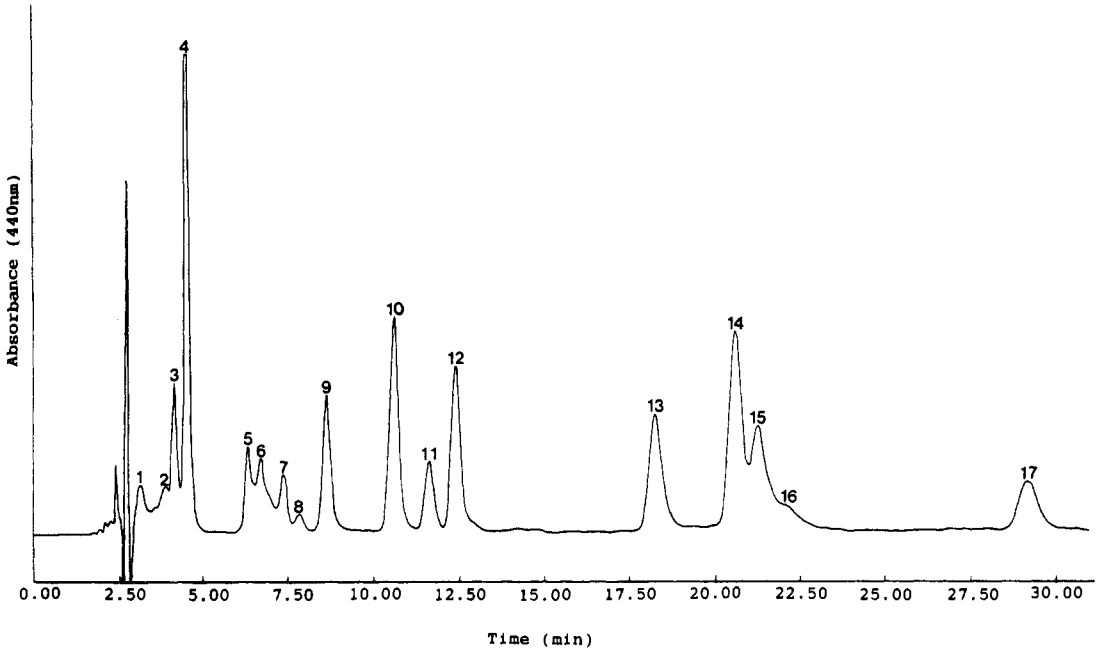

Figure 2. Chromatogram of unsaponified extract prepared from sweet potato leaves after microwave cooking (700 W) for 8 min. Chromatographic conditions are described in the text. Peaks: 1, 9-*cis*-neochrome; 2, 13-*cis*-lutein epoxide; 3, lutein epoxide; 4, lutein; 5, 3,4-didehydro-β,ϵ-caroten-3′-ol; 6, 3′,4′-didehydro-β,β-caroten-3-ol; 7, chl *b*; 8, chl *b*′; 9, chl *b* isomer; 10, chl *a*; 11 chl *a*′; 12, chl *a* isomer II; 13, pheophytin *b*; 14, β-carotene; 15, 9-*cis*-β-carotene; 16, 13-*cis*-β-carotene; 17, pyropheophytin *a*.

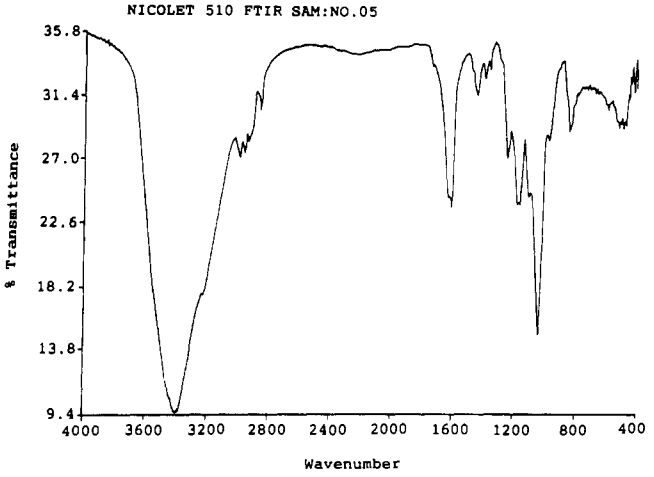

Figure 3. Infrared spectra of 3,4-didehydro-β,ϵ-caroten-3′-ol.

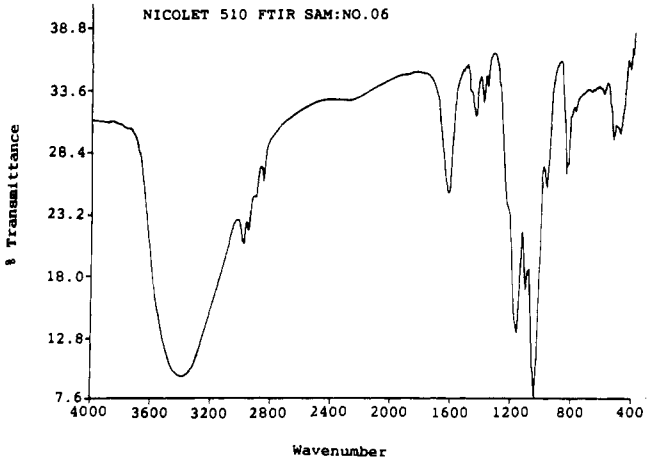

Figure 4. Infrared spectra of 3′,4′-didehydro-β,β-caroten-3-ol.

can be identified as 3,4-didehydro-β,ϵ-caroten-3′-ol and 3′,4′-didehydro-β,β-caroten-3-ol.

By comparison of data in Table II, it can be found that no isomerization of lutein and β-carotene to their stereo-

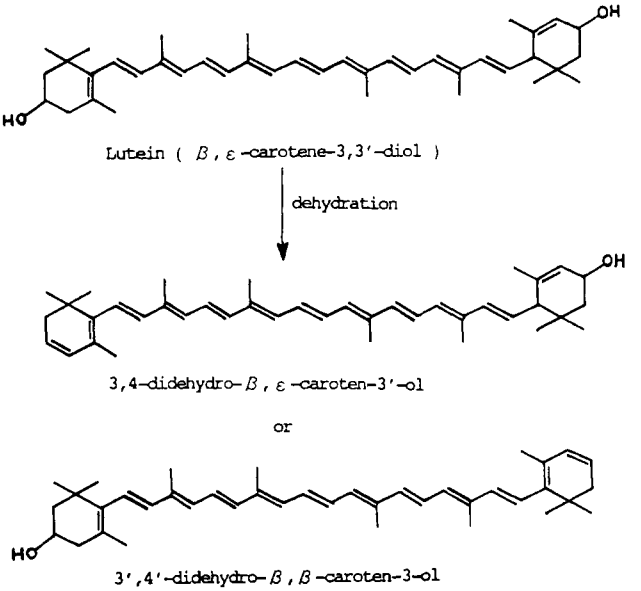

Figure 5. Structures of 3,4-didehydro-β,ϵ-caroten-3′-ol and 3′,4′-didehydro-β,β-caroten-3-ol.

isomers was observed as a result of microwave cooking. This is in agreement with a report by Khachik et al. (1986), who found that *trans*-β-carotene and its 15,15′-cis-isomer were not greatly affected by the application of heat. However, in another study Chen (1992) reported that significant isomerization of *trans*-lutein and *trans*-β-carotene to their geometrical isomers occurred during microwave cooking of vegetables. This contradictory result may be due to the presence of chlorophylls interfering with isomerization of carotenoids in vegetables during cooking, because only carotenoid was studied in the experiment by Chen (1992). It is also possible that the carotenoid isomers formed during heating are not completely eluted from the column by the solvent system employed in this study. In contrast to the result described above, the isomerization of chl *a* to chl *a*′ and of chl *b* to chl *b*′ did occur during the initial cooking process.

Nevertheless, both chl *a'* and chl *b'* were gradually destroyed when the heating time reached 4 min and above. Of all the carotenoids, *cis*-neoxanthin, violaxanthin, and violeoxanthin were the most unstable because they were completely destroyed when the heating time reached 8 min. This result demonstrated that the epoxy-containing carotenoids were more susceptible to heat loss than other carotenoids. This phenomenon has been observed by many authors (Khachik et al., 1986; Chen, 1992). In addition, *cis*-lutein was found to be more susceptible to heat loss than *cis*-β-carotene because of complete destruction of the former under microwave cooking at 700 W for 8 min. The formation of *cis*-neochrome from *cis*-neoxanthin was also observed. It has been reported that the rearrangement of 5,6-epoxide to 5,8-epoxide could occur during cooking of vegetables (Minguez-Mosquera et al., 1989). However, in this study the formation of luteoxanthin or auroxanthin from violaxanthin and that of lutein 5,8-epoxide from lutein 5,6-epoxide were not observed. This is probably because these epoxy-containing carotenoids were intermediate products formed in minor amounts during cooking, which were then destroyed in the subsequent cooking process.

In conclusion, the isomerization of chlorophylls and the rearrangement of carotenoid 5,6-epoxide to 5,8-epoxide do occur during microwave cooking of vegetables. Of all the pigments investigated, the epoxy-containing carotenoids and chlorophylls are the most susceptible to heat loss, followed by lutein and β-carotene. Two lutein dehydration products, 3,4-didehydro-β,ε-caroten-3'-ol and 3',4'-didehydro-β,β-caroten-3-ol, can be formed when vegetables are microwave-cooked at 700 W for 8 min. Further research is necessary to determine if the presence of chlorophylls can interfere with the isomerization of carotenoids during microwave cooking of vegetables.

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