J. Food Sci. Nutr.
Vol. 6, No. 1, p. 1~5 (2001)

Identification of Carotenoid Oxidation Products in Pigment Extracts from *Star Ruby* Grapefruit Pulp at Different Temperatures with Exposure to Light

Soon-Mi Shim and Gun-Hee Kim†

Department of Foods and Nutrition, Duksung Women's University, Seoul 132-714, Korea

Abstract

Pigment extracts obtained from *Star Ruby* grapefruit pulp were stored at different temperatures (4.5°C, 23°C) and exposed to light. Many carotenoid oxidation products were formed due to light-exposure during storage periods. They were monitored by using HPLC with photodiode array detection and tentatively identified. Including (all-E)-lycopene and *trans*-β-carotene as predominant carotenoids in red grapefruit, 5Z-lycopene, 9Z-lycopene, 13Z-lycopene, and 15-*cis*-β-carotene were formed at 4.5°C, 23°C. Degradation of all-*trans* lycopene was more susceptible to light-exposure and temperature than that of all-*trans* β-carotene. The formation of lycopene cis isomers was favored under lighted condition. Respectively, (5Z)-lycopene was formed in greater amounts than other isomers at 23°C storage. The concentration of 15-*cis*-β-carotene was significantly increased during storage at 23°C.

Key words: *Star Ruby* grapefruit pulp, carotenoid oxidation, all-*trans* lycopene, all-*trans*-β-carotene

INTRODUCTION

There is evidence that β-carotene may have a protective effect against certain cancers (1) and lycopene has potentially beneficial biological activities (2-9) as well as twice the singlet oxygen quenching ability of β-carotene (10,11).

Since distinct biological functions (11,12) and different antiooxidant capacities (11,13,14) of *cis* and *trans* isomers have been reported, the presence of processing-induced *cis* isomeric carotenoids (15) has received considerable study (16-19). Several authors have reported contents (20) and oxidative degradation kinetics (11) of lycopene and β-carotene isomers in various model systems.

As carotenoids may isomerize or be degraded in light-permeable packaging or under additional light-exposure situations (21), interest has focused on the isomeric formation of carotenoid due to light-exposure. Significant effects of light-exposure on carotenoid stability and different degradation reaction kinetics between major carotenoids in aqueous model systems and in vegetable juices were reported (22,23).

Some red pigmented grapefruit products are packed in transparent packages and marketed under light exposure. However there is little study on the isomeric carotenoid during storage.

To understand the effect of light and temperature on the formation isomers of lycopene and β-carotene, this study was to quantity and tentatively identify carotenoid oxidation products in pigment extracts from *Star Ruby* grapefruit pulp.

MATERIALS AND METHODS

Sample preparation

Since grapefruit pigments are embedded in pulp, highly pigmented pulp was separated from red colored *Star Ruby* grapefruit using an ultra-filtration membrane (Pilot scale, Citrus Research Education Center, Univ. of Florida, Lake Alfred, FL. USA). Carotenoid pigments in the grapefruit pulp (9.3 °Brix) were extracted using solvents.

Carotenoid extraction

The extraction method for carotenoid followed that of Sadler and Davis (24). Extraction solvents used for carotenoid were EtOH : acetone : hexane (250:250:500). 100 mL of retentate was mixed with an aliquot of extraction solvents, which was homogenized for 1 min at speed 4 (Omni Mixer Homogenizer), then centrifuged for 5 min at 6500 rpm in a centrifuge (Model MP4R, International Equipment Company, Needham Heights, MA) at 4°C. The upper layer of sample containing the carotenoids was collected and pulp cake was extracted again for completing extraction carotenoid with the same procedure as previously described. All of the above procedure was done without exposure to light.

Carotenoid pigments were filtered through a 0.45 μm HV membrane filter (Fisher Scientific Co.) and flushed with nitrogen gas gently, closed, and left in the dark under refrigeration. And then the sample was poured into a separate funnel for removing oil.

Storage test

Pigment extracts were equally divided (14 mL) into 15 mL glass test tubes. The glass test tubes were laid in the light chamber at 4.5°C and 23°C. A second set of samples was covered with aluminum foil to prevent light exposure and stored at the same temperature. A custom built light chamber was maintained at a constant light intensity of 4150K with cool white fluorescent light (Gretag Macbeth C.W.F). Samples

†Corresponding author. E-mail : ghkim@center.duksung.ac.kr
Phone: 81-2-901-8496, Fax: 81-2-901-8372

were pulled and analyzed every day for 7 days.

Chromatography

HPLC with photodiode array detection using C_{30} is generally accepted as a modern method of choice to separate, identify, quantify, and comparably resolve *cis*, *trans* lycopene isomers (20) and *cis*, *trans* β-carotene (25,26).

High-performance liquid chromatography with a Waters 996 photodiode array detector (Waters Corp., Milford, MA) was equipped with a Waters 600E system controller (Waters Corp., Milford, MA) and model 717plus auto sampler (Waters Corp., Milford, MA). The standard of lycopene and β-carotene was obtained from Sigma Chemical Co. (St. Louis, MO). C_{30} column (YMC CarotenoidTM) with mobil phase was ACN : MeOH (75% : 25%) and flow rate 1.5 mL/min. After filtering through a Nylon 0.45 μm filter (Fisher Scientific Co.), sample injection volumes of 10 μL were analyzed. Duplicated injections were conducted. PDA data acquisition, processing, and reporting with the PDA software requires use of the base Millennium software. Chromatographic software was used to obtain and store absorption spectra from 300 to 600 nm. Chromatograms at 450 nm were used to detect spectra of peaks.

Statistical analysis

Each peak was quantified using absolute calibration curves, all test results are the average of duplicated samples. All statistical analyses including means, standard deviations of the means, analysis of variance (ANOVA), and Paired t-test were performed using Sigma stat 2.0 from SPSS, Inc. (Chicago, IL) with significance at $p<0.05$. Plots for evaluation of the data were done by using Microsoft Excel computer software.

RESULTS AND DISCUSSION

Chromatographic separation and carotenoid spectral identification

The chromatogram of pigment extracts from *Star Ruby* grapefruit is shown in Fig. 1. Identification of major peaks and other peaks are presented in Table 1 based on retention time, spectral and chromatographic information. According to reports (11,17,27) the predominant peak eluted at approximately 20.3 min is lycopene with an absorption maximum at 472~473 nm and the other major peak at 8.2 min is β-carotene with absorption maximum at 450~452 nm.

Since relative absorption of *trans*-lycopene (peak 12, Table 1) and reaction rate matched well with those of Schierle et al. (20), it can be regarded as (all-E)-lycopene. Peaks (6-11, 14-16) appear to be *cis* isomers of lycopene because its spectral maxima are shifted to slightly shorter wavelengths (Table 1). The formation of a *cis* peak typically appears at 330~350 nm (20,28). The *cis* isomers of lycopene were tentatively identified by previous reports (20,29). Comparing to relative absorption of '*cis*-peak' (Table 1, shown in square brackets), peak 8, 9 can be identified as 13Z-lycopene (0.58), peak 10, 11 as 9Z-lycopene (0.12) and peak 13 as 5Z-lycopene (0.07).

The second isomer (peak 2, Table 1) was tentatively identified as 15-*cis*-β-carotene on the basis of the following. First, 15-*cis*-β-carotene was eluted prior to all *trans*-β-carotene (30). Second, Q ratio can be defined as the absorbance ratio of the *cis* peak to the middle main absorption peak (31). Third, the absorption wavelength of *cis* isomers corresponded with those reported by Chen et al. (32).

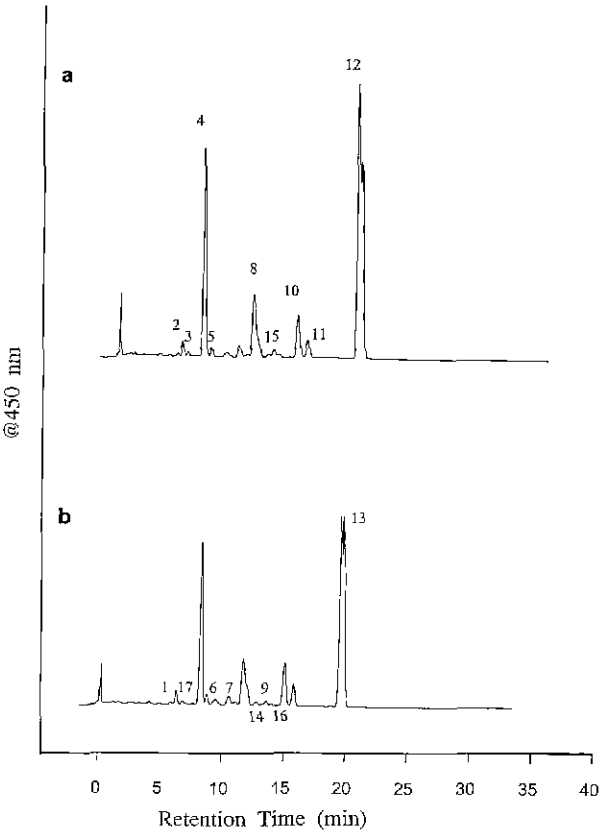

Fig. 1. HPLC chromatogram of carotenoid oxidation products in pigment extracts under exposed to light during 7 days. a: stored at 4.5°C, b: stored at 23°C. Peak identification (Table 1) described in the text

The peak at 7.654 min (peak 17, Table 1) appears to be β-carotene, because of its close spectral match with result (33).

Stability of all-*trans*-lycopene and its *cis* isomers in pigment extracts during storage

Since lycopene gradually decreased with increasing storage time under all conditions, it implies that (all-E)-lycopene was converted to lycopene isomers during storage. (all-E)-lycopene stored under light at 23°C showed significant degradation. A first-order plot of overall photodegradation of (all-E)-lycopene is presented (Fig. 2). The rate of change percent in (all-E)-lycopene was 6.5±0.0695 day-1 and it was unstable in light and temperature. We observed the same trends as Pesek and Warthesen (21) who reported that the reaction of lycopene photodegradation was dependent on the light intensity and temperature. Table 2 shows the effect of light-exposure at different temperatures on a change in the ratio of lycopene isomers in pigment extracts. In the sample stored at 4.5°C, as major isomers, (13Z) and (9Z)-lycopene were slowly formed

Table 1. Spectral information from chromatographic peaks shown in carotenoid oxidation products

HPLC peak[1)]	RT., min	Pigment	Spectra maxima[2)] of the HPLC PDA (nm) and relative absorption of '*cis*-peak'[3)]					Carotenoid[4)]
1	3.491	β-carotene *cis* isomer		333 3	422.0	442.8	457.2	
2	6.626	β-carotene *cis* isomer	(Q=1.9[5)])	333.3	420.0	447.6	471.7	15-*cis*-β-carotene[6)]
3	6.999	β-carotene cis isomer		350.1	425.0	447.6	476.6	
4	8.241	β-carotene (*trans*)			430.0	452.4	481.4	*trans*-β-carotene
5	8.455	β-carotene isomer			430.0	452 4	481.4	
6	10.838	Lycopene *cis* isomer	[0.33]	361.7	442.3	471.7	500.3	(xZ)-Lycopene
7	10.973	Lycopene *cis* isomer	[0.30]	361.7	442.3	471.7	500.3	(xZ)-Lycopene
8	11.924	Lycopene *cis* isomer	[0.57]	361.7	442.3	466.9	495.9	(13Z)-Lycopene
9	12.096	Lycopene *cis* isomer	[0.58]	361.7	442.3	466.9	495.9	(13Z)-Lycopene
10	15.406	Lycopene *cis* isomer	[0.12]	358.1	442.3	466.9	500.3	(9Z)-Lycopene
11	16.269	Lycopene *cis* isomer	[0.12]	359.1	442.3	466.9	500.3	(9Z)-Lycopene
12	20.305	Lycopene (*trans*)	[0.06]		447.6	471.7	505.6	(all-E)-Lycopene
13	20.499	Lycopene isomer	[0.07]		447.6	471.7	505.6	(5Z)-Lycopene
14	13.555	Lycopene *cis* isomer	[0 33]	359 1	438.0	462.1	495 9	(xZ)-Lycopene
15	13.592	Lycopene *cis* isomer	[0.40]	359.1	442.3	462.1	495.9	(xZ)-Lycopene
16	13.635	Lycopene *cis* isomer	[0.36]	359.1	442.3	462.1	495.9	(xZ)-Lycopene
17	7.654	Zeta carotene			380.3	404.8	428.3	

[1)]Peak numbers as in chromatogram shown in Fig. 1. [2)]Main maxima are underlined
[3)]Relative absorption of '*cis*-peak' shown in square brackets means absorption at the subsidiary peak (at approx. 360 nm) divided by the absorption at maxima nm.
[4)]*cis* isomers of lycopene were tentatively identified by previous reports (20,29).
[5)]Reported values of Q ratio are from Tsukida et al. (31). [6)]Reported values of visible spectra are from Chen et al. (32).

Table 2. The effect of light-exposure at different temperatures on a change in the ratio of lycopene isomers in pigment extracts from *Star Ruby* grapefruit pulp

Storage condition	The change in the ratio of lycopene isomer (% of total lycopene)				
	Storage time (day)	13Z	9Z	5Z	xZ
4.5°C dark	0	1.6	0.0	0.0	1.0
	1	3.1	0.9	0.0	1.2
	2	3.3	1.1	0.0	1.2
	3	3.9	1.5	0 0	0.4
	5	3.5	1.4	0.0	1 3
	6	3.0	1.0	0.0	1.7
	7	3.0	0.9	0.0	1.7
4.5°C light	0	1.6	0.0	0.0	1.0
	1	3.0	1.2	0.0	1.2
	2	3.7	2.0	0.0	1.5
	3	3.4	2.4	1.3	1.6
	5	3.1	3.1	3.3	1.4
	6	2.4	3.1	3.6	1.8
	7	2.6	3.3	4.2	1.8
23°C dark	0	3.2	1.6	0.0	1.0
	1	3.0	1.6	0.0	0.9
	2	8.9	3.0	4.7	1.2
	3	14.8	4.1	7.7	2.8
	5	16 8	6.1	17.0	2.5
	6	18.1	6.2	19.0	3.3
	7	18.1	7.9	21.1	3.4
23°C light	0	3.6	1.3	0.0	1.5
	1	4.3	1.2	0.0	0.4
	2	11.1	5.3	11.8	2.5
	3	13.2	7.0	16.9	3.0
	5	14.1	8.8	22.9	2.7
	6	14.2	9.0	26.5	2.9
	7	14.7	10.4	27.5	3.1

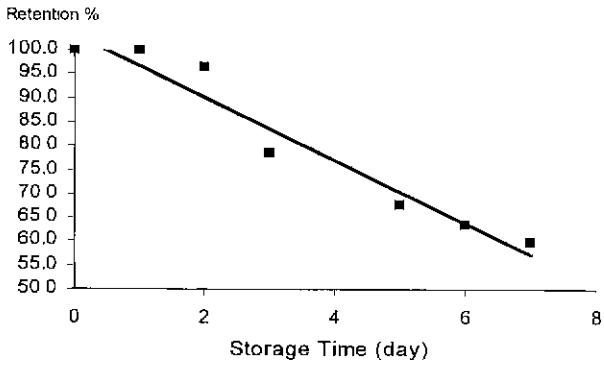

Fig. 2. First-order plot of degradation of total (all-E)-lycopene in pigment extracts stored at 23°C under light condition (r^2=0.95).

during storage time. In the case of the dark samples, with increasing (xZ)-lycopene, (13Z) and (9Z) slightly declined. However (9Z)-lycopene gradually increased under light at the same temperature. It implied that (13Z)-lycopene was converted to (xZ)-lycopene and (9Z)-lycopene was somewhat more sensitive to light-exposure than (13Z)-lycopene at the same temperature. As it is shown in Table 2, we could observe similar results in the sample stored at 23°C. Under lighted storage, an increase in (9Z)-lycopene and (5Z)-lycopene was favored. Respectively, the (5Z)-lycopene was formed in greater amounts than other isomers under light-exposure at 23°C. Schierle et al. (20) observed the effect of heat on the ratio of lycopene isomers in aqueous and oily dispersions of tomato paste. Heat treatment clearly increased the percentage of all the (Z)-isomers. In this study the same results were found in that the amount of all the (Z) isomers was accelerated by increasing duration of light-exposure and temperature. Many studies have also reported an increment of the (Z)-isomers of lycopene

due to increasing temperatures in different treatments of tomato and fruit juice (17,34-36).

Stability of all-trans-β-carotene and its cis isomers in pigment extracts during storage

Quantitative losses of all-*trans*-β-carotene were 8% in the dark samples and 12% in light-exposed samples after 7 days storage at 4.5°C (Fig. 3). Light-exposed samples appeared to retain less β-carotene than dark samples and the difference was significant ($p<0.05$) under this condition. Contrary to our expectation, degradation rates of all-*trans*-β-carotene at 23°C during storage were erratic (It was not presented).

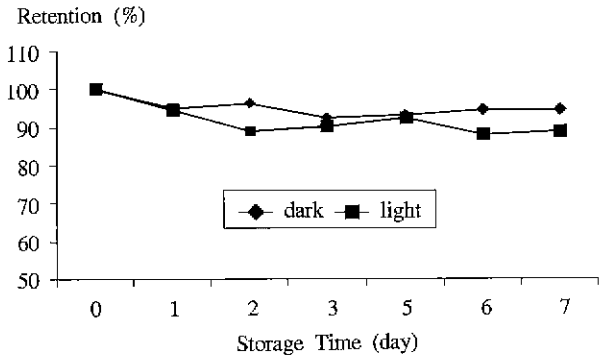

Fig. 3. Quantitative loss of *trans*-β-carotene in pigment extracts stored at 4.5°C during 7 days.

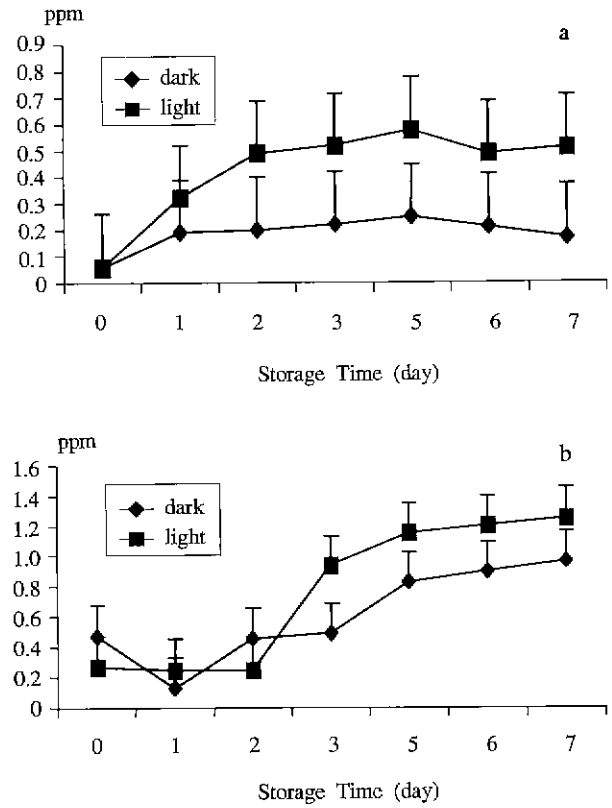

Fig. 4. The concentration change of 15-*cis*-β-carotene in pigment extracts during 7 days. a: stored at 4.5°C, b : stored at 23°C.

It has been well reported that numerous *cis* isomers are theoretically possible and two major *cis* isomers (9, 13-*cis*-β-carotene) are generally formed from all-*trans* in food systems (37,38). Interestingly, we only found 15-*cis*-β-carotene and the concentration change of 15-*cis*-β-carotene formation during storage was described in Fig. 4. Chen and Tang (39) reported that only with a minor increase at 23°C under lighted condition, there were no significant changes ($p>0.05$) in the concentrations of 15-*cis*-β-carotene at 4.5°C. However we observed that the concentration change of 15-*cis*-β-carotene formation in light-exposed samples stored at 23°C was significantly increased ($p<0.05$). It is reasonable to assume that other major β-carotene isomers (9, 13-β-carotene) in carrot pulp waste (39) formed in relatively greater amounts than 15- *cis*-β -carotene. It can be concluded that the formation of 15-*cis* isomers was susceptible to light exposure and high temperature.

From the results of this study, the predominant reactions are isomerization of all-*trans*-lycopene to various lycopene *cis* isomers under light-exposure. Representatively, the formation of (5Z)-lycopene was the most labile than other lycopene *cis* isomers under lighted conditions and stored at high temperature. *trans*-lycopene degraded more rapidly than *trans*-β-carotene by light exposure and temperature.

REFERENCES

1. O' Neil, C.A., Schwartz, S.J. and Catignani, G.L. : Cmparison of liquid chromatographic methods for the determination of *cis-trans* isomers of β-carotene. *J. Assoc. Off. Anal. Chem.*, **74**, 36 (1991)
2. Clinton, S.K. : Lycopene : Chemistry, biology, and implication for human health and disease. *Nutr. Rev.*, **56**, 35 (1998)
3. Diaz, M.N., Frei, B., Vita, J.A. and Keaney, J.F : Antioxidants and atheroscierotic heart disease. *New Eng. J. Med.*, **337**, 408 (1997)
4. Gester, H. : The potential role of lycopene for human health. *J. Am. Col. Nutr.*, **16**, 109 (1997)
5 Khachik, F., Beccher, G.R. and Smith, J.C. Jr. : Lutein, lycopene, and their oxidative metabolites in chemoprevention of cancer. *J. Cell Biochem. Suppl.*, **22**, 236 (1995)
6. Nguyen, M.L. and Schwartz, S.J. . Lycopene : Chemical and biological properties. *Food Tech.*, **53**, 38 (1999)
7. Ojima, F., Sakamoto, H., Ishiguro, Y. and Terao, J. : Consumtion of carotenoids in photosensitized oxidation of human plasma and plasma low-density lipoprotein. *Free Radio. Biol. Med*, **45**, 377 (1993)
8 Ribayo-Mercado, J.D., Garmyn, M., Gilchrest, B.A. and Russei, R.M. : Skin lycopene is destroyed preferentially over β-carotene during ultraviolet irradiation in humans. *J. Nutr.*, **125**, 1854 (1995)
9. Weisburger, J.H. : Evaluation of the evidence on the role of tomato products in disease prevention. *Proc. Soc. Exp. Biol. Med.*, **218**, 140 (1998)
10. DiMascio, P., Kaiser, S. and Sies, H. : Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Arch. Biochem. Biophys.*, **274**, 532 (1989)
11. Henry, L.K , Catignani, G.L. and Schwartz, S.J. : Oxidative degradation kinetics of lycopene, lutein and 9cis and all-trans β-carotene. *JAOCS*, **75**, 823 (1998)
12. Stahl, W. and Sies, H. : Physical quenching of singlet oxygen and cis-trans isomerization of carotenoids. *Ann. N.Y. Acad. Sci.*, **691**, 10 (1993)

13. Jimenez, C. and Pick, U. : Differential reactivity of β-carotene isomers from *Dunaliella bardawil* toward oxygen radicals. *Plant Physiol.*, **101**, 385 (1993)
14. Levin, G. and Mokady, S. : Antioxidant activity of 9-*cis* compared to all-*trans* carotene *in vitro*. *Free Radicals Biol. Med.*, **17**, 77 (1994)
15. Fennema, O.R. : *Food Chemistry*. Marcel Dekker, INC., New York (1996)
16. Cole, E.R. and Kapur, N.S The stability of lycopene. I. Degradation of oxygen *J. Sci. Food Agric.*, **8**, 360 (1957)
17. Lovric, T., Sablek, Z. and Boskovic, M. : *Cis-trans* isomerization of lycopene and color stability of foam-mat dried tomato powder during storage. *J. Sci. Food Agric.*, **21**, 641 (1970)
18. Tsukida, K., Saiki, K. and Sugiura, M. : Structural elucidation of the main *cis*-β-carotenes. *J. Nutr. Sci. Vitaminol.*, **27**, 551 (1981)
19. Minguez-Mospuera, M.I. and Jaren-Galan, M. : Kinetics of the decolouring of carotenoid pigments. *J. Sci. Food Agric.*, **67**, 153 (1995)
20 Schierle, J., Bretzel, W , Bühler, I., Faccin, N., Hess, D., Steiner, K. and Schuep, W. Content and isomeric ratio of lycopene in food and human blood plasma. *Food Chemistry*, **59**, 459 (1997)
21. Pesek, C.A. and Warthesen, J J. : Kinetic model for photoisomerization and concomitant photodegradation of β-carotenes. *J. Agric. Food Chem.*, **38**, 1313 (1990)
22. Pesek, C A. and Warthesen, J.J. : Photodegradation of carotenoids in a vegetables juices system. *J. Food Sci.*, **52**, 744 (1987)
23. Pesek, C.A. and Warthesen, J.J. : Characterization of the photodegradation of β-carotene in aqueous model systems. *J. Food Sci.*, **53**, 1517 (1988)
24. Sadler, G. and Davis, J. · Rapid extraction of lycopene and β-carotene from reconstituted tomato paste and pink grapefruit homogenates. *J. Food Sci.*, **55**, 1460 (1990)
25. Emenhiser, C., Simunovic, N., Sander, L.C. and Schwartz, S.J. Separation of geometric isomers in biological extracts using a polymeric C_{30} column in reversed-phase liquid chromatography *J. Agric. Food Chem.*, **44**, 3887 (1996)
26. Rousseff, R., Raley. L. and Hofsommer, H.J. : Application of diode array detection with a C_{30} reversed phase column for the separation and identification of saponified orange juice carotenoids. *J. Agric. Food Chem.*, **44**, 2176 (1996)
27. Deuel, J.J. : *The lipids*. Interscience, London, Vol. I (1951)
28. Khachik, F., Beecher, G.R. and Lusby, W.R. : Separation, identification and qualification of the major carotenoids in extracts of apricot, peaches, cantaloupe, and pink grapefruit by liquid chromatography. *J. Agric. Food Chem.*, **37**, 1465 (1989)
29. Hengartner, U , Bernhard, K., Mayer, K., Englert, G. and Glinz, E. : Synthesis, isolation and NMS-spectroscopic characteriztion of fourteen (Z)-isomers of lycopene and of some acetylenic didehydro-and tetrahydrolycopenes. *Helv. Chim. Acta*, **75**, 1848 (1992)
30. Chen, B.H., Chen, T M. and Chen, J.T Kinetic model for studying the isomerization of α-carotene and β-carotene during heating illumination. *J. Agric. Food Chem.*, **42**, 2397 (1994)
31. Tsukida, K., Takii, K. and Kayama, Y. : Separation and determination of *cis/trans*-β-carotenes by high-performance liquid chromatography. *J Chromatogr.*, **31**, 103 (1982)
32. Chen, B.H., Peng, H.Y. and Chen, H.E. : Change of carotenoids, color, and vitamin A contents during processing of carrot juice. *J. Agric. Food Chem.*, **43**, 1912 (1995)
33. Rousseff, R., Sadler, G.D., Putnam, T.J. and Davis, J.E. : Determination of β-carotene and other hydrocarbon carotenoids in Red Grapefruit cultivars. *J. Agric. Food Chem.*, **40**, 47 (1992)
34. Boskovic, M.A. . Fate of lycopene in dehydrated tomato products : carotenoid isomerization in a food system. *J. Food Sci.*, **44**, 84 (1979)
35. Godoy, H T. and Rodriguez-Amaya, D.B. : Fate of carotneoids of papaya (*Carica papaya*) during processing and storage. *Cienc. Technol. Aliment.*, **11**, 210 (1991)
36. Kamasawud, P. and Crouz, J.C. : 1. Mechanism of formation of volatile compounds by thermal degradation of carotenoids in aqueous medium. 2. Lycopene degradation. *J. Agric. Food Chem.*, **38**, 1238 (1990)
37. Chandler, L.A. and Schwartz, S.J. : HPLC separation of *cis-trans* carotene isomers in fresh and processed fruits and vegetables. *J. Food Sci.*, **52**, 669 (1987)
38. Sweeney, J.P. and Marsh, A.C. : Effect of processing on provitamin A in vegetables. *J. Am. Diet. Assoc.*, **59**, 238 (1971)
39. Chen, B H. and Tang, Y.C. : Processing and stability of carotenoid powder from carrot pulp waste. *J. Agric. Food Chem.*, **46**, 2312 (1998)

(Received November 9, 2000)