

## Determination of Carotenoid Stereoisomers in Commercial Dietary Supplements by High-Performance Liquid Chromatography

ROBERT AMAN, SANDRA BAYHA, REINHOLD CARLE, AND ANDREAS SCHIEBER\*

Section Plant Foodstuff Technology, Institute of Food Technology, Hohenheim University,  
August-von-Hartmann-Strasse 3, D-70599 Stuttgart, Germany

A method for the determination of  $\beta$ -carotene, lutein, and zeaxanthin including their *cis*-isomers and  $\alpha$ -carotene in commercial dietary supplements by HPLC has been developed. The study comprises 11 oral dosage forms, including 9 soft gelatin capsules, 1 dragée, and 1 effervescent tablet formulation. The capsule content was extracted with an acetone–hexane mixture, and the gelatin shell was digested with papain to release carotenoids that had migrated into the coat. Sample preparation for tablets and dragées was carried out as described for the capsule content. Extraction recoveries exemplified for *all-trans*- $\beta$ -carotene and *all-trans*-lutein were  $95 \pm 5\%$  and  $93 \pm 2\%$ , and  $95 \pm 2\%$  and  $79 \pm 5\%$  after enzymatic treatment, respectively. Apart from *all-trans*- $\beta$ -carotene, its 9-*cis*- and 13-*cis*-isomers were detected in all samples, whereas no evidence for *cis*-isomerization of lutein and zeaxanthin could be obtained. Migration of carotenoids into the shells was only observed in the case of  $\beta$ -carotene. With the exception of one preparation, the  $\beta$ -carotene contents determined exceeded the dosage specified on the label by up to 48%, which results from stability overages necessary to compensate for losses during storage.

**KEYWORDS:** Dietary supplements;  $\beta$ -carotene;  $\alpha$ -carotene; lutein; zeaxanthin; *trans*–*cis*-isomers; migration

### INTRODUCTION

About 100 of the 600 naturally occurring carotenoids are nutritional constituents of the human diet. Although carotenes and xanthophylls are accumulated in human plasma and tissues equally well, only 20 carotenoids have been detected in human beings (1). The major carotenoids of human plasma are  $\beta$ -carotene, lycopene,  $\beta$ -cryptoxanthin, lutein,  $\alpha$ -carotene, and zeaxanthin (2). Apart from their function as provitamin A, carotenoids are excellent scavengers of singlet oxygen and other reactive oxygen species. Numerous epidemiological studies suggest an inverse correlation of carotene ingestion and degenerative diseases. In particular, protective effects against several types of cancer and cardiovascular and other diseases associated with aging have been described (3–8).

As a consequence of these studies the U.S. National Cancer Institute (NCI) implemented the so-called “5 A Day for Better Health” program in 1991 to increase the number of daily servings of fruits and vegetables to about 600–700 g. Although later surveys revealed an increased consumption of fruits and vegetables, intakes of dark green and cruciferous vegetables, which are considered particularly beneficial, still remained low (9). Therefore, dietary supplements and food fortification may

be an alternative route to the consumption of organic micro-nutrients that may provide health benefits (10). Functional foods and supplements represent a rapidly growing segment of the overall food market, owing to the loss of consumers’ confidence in the modern diet, the aging population, a growing trend toward self-medication, and an overall increase in health awareness and disease prevention among customers (11).

Although a number of carotenoids are obtained by synthesis, extraction from plant material is still in use. Natural carotenoids predominantly occur in their *all-trans* form. During technological treatment and processing, *all-trans*-carotenoids are partially converted into their *cis*-isomers. Nutritional consequences of these conversions are changes in bioavailability and physiological activity (12–15). In human beings and some animals *all-trans*- $\beta$ -carotene is more efficiently absorbed than its *cis*-isomers (16–19), while in the case of lycopene and astaxanthin the absorption of *cis*-isomers seems to be favored (20, 21). Therefore, the knowledge of carotene isomerization in dietary supplements is of essential importance.

Dietary supplements are commonly marketed as oral dosage formulations such as dragées, tablets, capsules, or powders which are very similar to pharmaceutical products. Due to their lipophilic character carotenoids are mostly applied in an oily carrier enclosed in soft gelatin capsules providing sufficient protection from light and oxygen. Despite a great number of methods reported for the determination of carotenoids in food

\* To whom correspondence should be addressed. Phone: +49-(0)711-459-3125. Fax: +49-(0)711-459-4110. E-mail: schieber@uni-hohenheim.de.

(22), quantitative studies on the carotenoid stereoisomer profile and content uniformity of dietary supplements and functional foods are very limited. Our own investigations of commercial vitaminized drinks (23, 24) and studies reported by others (25) have revealed considerable discrepancy between determined carotenoid contents and those specified on the label. For the consumer the problem arises that the amounts of carotenoids ingested via food supplements cannot be exactly calculated. In the view of adverse effects observed after intake of high doses of isolated  $\beta$ -carotene (26, 27), however, accurate quantification of carotenoids in dietary supplements is a prerequisite. In the case of nutraceuticals based on gelatin capsules, migration of carotenoids into the shells also needs to be taken into consideration since it may affect both the extraction yield and quantification. Therefore, the objective of the present study was to establish a method for the extraction and HPLC determination of carotenoid stereoisomers from commercial dietary supplement formulations.

## MATERIALS AND METHODS

**Materials.** All chemicals used were purchased from VWR (Darmstadt, Germany) and were of reagent grade. HPLC solvents were of gradient grade. Ultrapure water was used for HPLC analysis and sample preparation. *all-trans*- $\beta$ -Carotene was obtained from Sigma-Aldrich (Taufkirchen, Germany). *all-trans*- $\alpha$ -Carotene was isolated from a carotenoid mixture from carrots (the ratio of  $\beta$ - to  $\alpha$ -carotene is approximately 2:1) purchased from Sigma-Aldrich by preparative chromatography. *all-trans*-Lutein and *all-trans*-zeaxanthin were supplied by Hoffmann-La Roche (Basel, Switzerland). Eleven commercial dietary supplements were obtained from local drugstores. Papain was purchased from VWR.

**Isolation of *all-trans*- $\alpha$ -Carotene by Preparative Chromatography.** The separation was carried out on a 250 mm  $\times$  10 mm i.d. semipreparative 5  $\mu$ m C<sub>30</sub> reversed-phase column (YMC, Wilmington, MA). The HPLC system (Bischoff, Leonberg, Germany) consisted of an LC-CaDI 22-14 system controller, two 2250 solvent delivery HPLC compact pump modules, and an SPD-10AVp UV/vis detector (Shimadzu, Kyoto, Japan). All data were processed by the McDACq32 Control software (version 2.0) (Bischoff, Leonberg, Germany). The chromatographic runs were monitored at 445 nm.

The carotenoid mixture (5 mg) was weighed into a flask, dissolved in a few drops of toluene, and made up to a volume of 5 mL with 2-propanol. Aliquots of 500  $\mu$ L of the solution were used for HPLC. After injection of the solution *all-trans*- $\alpha$ -carotene was eluted isocratically with methanol/*tert*-butyl methyl ether (MTBE)/water (62:35:3, v/v) at ambient temperature for 30 min. The flow rate was set at 4 mL/min.

**Sample Preparation.** The commercial preparations investigated comprised nine soft gelatin capsule formulations, one dragée, and one effervescent tablet.

Carotenoids dissolved in the excipient (vegetable oil) of the capsules and in the gelatin shells were determined separately. Capsules were cut with a scalpel. To avoid degradation and isomerization, amber glassware was used and processing was developed under dim light conditions. The carotenoids were extracted with a mixture of acetone and hexane (1:1, v/v) containing butylated hydroxytoluene (BHT) (62.5 mg/100 mL) and butylated hydroxyanisole (BHA) (62.5 mg/100 mL) as antioxidants, and the solution was made up to a volume of 200 mL. Aliquots of 4–10 mL depending on the carotenoid content and 50 mL of the extraction mixture were transferred into an amber glass separatory funnel. The solution was washed with 50 mL of sodium chloride solution (10 g/100 mL) and twice with 50 mL of water to remove acetone. The aqueous phase was reextracted with ethyl acetate until it was colorless. The pooled organic phases were dried with sodium sulfate (2 g) and evaporated in vacuo ( $T < 30^\circ\text{C}$ ). The residue was dissolved in 2-propanol and made up to a volume of 10–25 mL depending on the carotenoid content.

The extracted gelatin shells were transferred into an amber glass separatory funnel. After addition of 10 mL of water and papain (the

ratio of shell to papain is 1:1, w/w) the shells were incubated at ambient temperature for 1 h. The resulting digest was treated with 50 mL of sodium chloride solution (10 g/100 mL) and extracted with 50 mL of the extraction mixture. The aqueous phase was discarded, and the organic phase was washed twice with 50 mL of water. After the organic phase was dried with sodium sulfate (2 g), the extract was evaporated in vacuo ( $T < 30^\circ\text{C}$ ). The residue was dissolved in 2-propanol and made up to a volume of 5–50 mL depending on the carotenoid content.

Tablets and dragées were gently ground in a mortar. An aliquot was dissolved in 50 mL of sodium chloride solution (10 g/100 mL) in a separatory funnel. Further steps of sample preparation corresponded to those described for the capsules. Sample preparation was performed in triplicate, and HPLC analyses were carried out in duplicate.

**Recovery Studies.** For stability and recovery *all-trans*- $\beta$ -carotene and *all-trans*-lutein were selected as representatives of carotenes and xanthophylls. Studies were assessed for the extraction of capsule contents and after enzymatic digestion of carotene-free soft gelatin capsules. For this purpose, aliquots of 1 mL of prepared carotenoid solutions (0.5 mg of carotenoid/25 mL of hexane) were extracted as described above. All determinations were performed in triplicate.

**Separation of Carotenoid Stereoisomers by HPLC.** HPLC analyses were performed on a model 2690 Waters separation module equipped with an autosampler injector, a model Jetstream 2 plus Waters column oven, and a model 2996 Waters UV/vis photodiode array detector controlled by a Millennium 32 (version 3.20) workstation (Waters, Milford, MA). The separation of carotenoid stereoisomers was carried out using a 250 mm  $\times$  4.6 mm i.d., 5  $\mu$ m, analytical scale C<sub>30</sub> reversed-phase column (YMC) at a column temperature of 20  $^\circ\text{C}$  and a flow rate of 1 mL/min. For the separation of  $\beta$ -carotene isomers and  $\alpha$ -carotene, eluent A consisted of methanol/MTBE/water (81:15:4, v/v/v), and eluent B of MTBE/methanol/water (90:6:4, v/v/v). A linear gradient from 100% A to 44% A within 50 min was used. Lutein and zeaxanthin stereoisomers were separated within 80 min using a linear gradient from 100% A (methanol/MTBE/water, 92:4:4, v/v/v) to 12% B (MTBE/methanol/water, 90:6:4, v/v/v). The injection volume was 20  $\mu$ L. The carotenoids were detected at the wavelengths of maximum absorption in the mobile phase: *all-trans*- $\alpha$ -carotene, 445 nm; *all-trans*- $\beta$ -carotene, 452 nm; 9-*cis*- $\beta$ -carotene, 445 nm; 13-*cis*- $\beta$ -carotene, 445 nm; *all-trans*-lutein, 445 nm; 9-*cis*-lutein, 438 nm; 9'-*cis*-lutein, 438 nm; 13-*cis*-lutein, 438 nm; 13'-*cis*-lutein, 438 nm; *all-trans*-zeaxanthin, 450 nm; 9-*cis*-zeaxanthin, 445 nm; 13-*cis*-zeaxanthin, 445 nm.

**Identification and Quantification of Carotenoids.** Standards of *cis*-isomers were obtained by iodine-catalyzed photoisomerization of *all-trans*- $\beta$ -carotene, *all-trans*-lutein, and *all-trans*-zeaxanthin (28) and were recovered by preparative HPLC. The separation of  $\beta$ -carotene isomers was carried out as described for the isolation of *all-trans*- $\alpha$ -carotene. Major xanthophyll stereoisomers were isolated isocratically with acetone/water (89:11, v/v) at a flow rate of 4 mL/min. Predominant chromatographic peaks were identified by LC-MS (APCI<sup>+</sup> mode) and HPLC-NMR spectroscopy (1D and 2D NMR spectra were recorded in the stopped-flow mode). Carotenoids extracted from dietary supplements were identified by comparison of their retention times and absorption spectra with those of authentic standards. Concentrations were based on linear calibration curves. Concentrations of stock solutions were determined spectrophotometrically on the basis of published data (29). Specific absorption coefficients and wavelength maxima used were as follows: *all-trans*- $\alpha$ -carotene, 2710 at 445 nm (hexane); *all-trans*- $\beta$ -carotene, 2592 at 450 nm (hexane); *all-trans*-lutein, 2550 at 445 nm (ethanol); *all-trans*-zeaxanthin, 2540 at 450 nm (ethanol). Concentrations of *cis*-isomers were calculated using the calibration curves of the corresponding *all-trans*-carotenoids.

## RESULTS AND DISCUSSION

**Samples.** Eleven commercial dietary supplements were investigated for their carotenoid profile. The carotenoid preparations comprised nine soft gelatin capsule formulations (Table 1, preparations 1–9) containing a vegetable oil as excipient. Two preparations (preparations 10 and 11) without any gelatin cover were an effervescent tablet and a dragée, covered with

**Table 1.** Specified and Determined Amounts (Milligrams per Capsule, Dragée, and Effervescent Tablet) of *all-trans*- $\alpha$ -Carotene and  $\beta$ -Carotene Stereoisomers in Commercial Dietary Supplements (A, Content; B, Shell)

preparation	<i>all-trans</i> - $\alpha$ -carotene		<i>all-trans</i> - $\beta$ -carotene		13- <i>cis</i> - $\beta$ -carotene		9- <i>cis</i> - $\beta$ -carotene		total $\beta$ -carotene, A + B	specified amount
	A	B	A	B	A	B	A	B		
1	0.12	— <sup>a</sup>	26.14	—	1.13	—	0.62	—	27.89	25.00
2	—	—	22.53	0.02	0.72	—	0.45	—	23.72	18.00
3	1.02	—	9.21	0.13	0.71	0.01	1.35	—	11.41	10.00
4	1.24	—	7.65	—	1.04	—	2.63	—	11.32	9.98
5	8.95	—	7.11	—	0.87	—	2.82	—	10.80	8.60
6	—	—	6.98	—	0.24	—	0.18	—	7.40	6.00
7	0.35	—	4.65	—	0.50	—	0.80	—	5.95	5.00
8	0.41	—	3.85	—	0.31	—	0.35	—	4.51	3.30
9	—	—	0.36	0.57	0.16	0.02	0.10	0.01	1.22	2.00
10	0.03	<i>b</i>	4.01	<i>b</i>	0.72	<i>b</i>	0.13	<i>b</i>	4.86	4.80
11	0.05	<i>b</i>	0.24	<i>b</i>	0.05	<i>b</i>	0.08	<i>b</i>	0.37	0.25

<sup>a</sup> Dash indicates not detectable (<0.001 mg). <sup>b</sup> Product 10 was an effervescent tablet; product 11 was a dragée.

shellac, respectively. According to the labeled specification, the predominant excipients of the dragée were calcium hydrogen phosphate, sucrose, vitamin C, magnesium oxide, calcium carbonate, potassium chloride, and cellulose, and the matrix of the effervescent tablet mainly consisted of citric acid, calcium carbonate, sorbitol, and dextrose. Whereas isolated carotenoids were obviously used as supplements to preparations 1, 2, 6, 7, 9, and 10, in the case of preparations 3, 4, 5, 8, and 11, more complex carotenoid mixtures originating from plant extracts were used. The amount of  $\beta$ -carotene was specified on the label of all products investigated (**Table 1**), ranging from 0.25 mg (preparation 11) to 25.0 mg (preparation 1). In contrast,  $\alpha$ -carotene was exclusively labeled on preparation 5, whereas lutein was specified in preparations 9 and 11. For zeaxanthin no specification was provided. Although the shelf lives of preparations 6 and 8 had already expired by 26 and 36 months, respectively, both supplements were included in this study to obtain information about the long-term stability of carotenoids.

**Methodology.** In contrast to functional foods, dietary supplements are very similar to pharmaceutical products. Therefore, routine methods used in food analysis could not be applied for the extraction of carotenoids from soft gelatin capsules since potential migration of analytes into the shells had to be considered. As a consequence, after extraction of the fatty excipient, gelatin capsules were digested with papain for complete release of the active ingredients. In a preliminary study Pronase N was also found to be a suitable proteolytic enzyme; however, due to lower costs of papain, the latter was given preference in the present study. With both enzyme preparations complete degradation of the gelatin matrix was accomplished within 1 h.

Reproducibility of the extraction methods was found to be very good. For *all-trans*- $\beta$ -carotene and *all-trans*-lutein recoveries of  $95 \pm 5\%$  and  $93 \pm 2\%$  were determined when extracted from the capsule contents. After carotenoid-free gelatin capsules were spiked with *all-trans*- $\beta$ -carotene and *all-trans*-lutein and enzymatic degradation with papain, recoveries amounting to  $95 \pm 2\%$  and  $79 \pm 5\%$  were found, respectively. The lower recovery of lutein may be attributed to its higher lability compared with those of other carotenoids (30, 31). However, since migration of lutein into the cover was not observed in any of the samples investigated, further optimization of the extraction parameters was not considered necessary.

**Application of  $\alpha$ -Carotene and  $\beta$ -Carotene in Dietary Supplements.** From **Table 1** it becomes evident that *all-trans*- $\beta$ -carotene was detected in all preparations, as could be expected from the label. In contrast, *all-trans*- $\alpha$ -carotene was found in

eight supplements, although at very low levels in preparations 10 and 11. The three preparations (2, 6, and 9) which were devoid of  $\alpha$ -carotene contained carotenoids obviously added as isolated compounds.

With the exception of product 9, overages of  $\beta$ -carotene ranging from 1% to 48% were generally found in the dietary supplements investigated. Even after the date of expiration, preparations 6 and 8 showed  $\beta$ -carotene contents exceeding the specified dosage by 23% and 36%. Since the declaration of the vitamin content has to be guaranteed during the whole shelf life of dietary supplements, stability overages were added to compensate for losses of the active ingredients during storage. The standardization of plant extracts may pose another problem, giving rise to discrepancies between analyzed and specified carotenoid contents. For a more accurate calculation of carotenoid dosages that are ingested via supplements, admissible deviations from the product specification should be fixed. The Association of German Chemists recommended that a deviation of  $\pm 30\%$  for vitamin A should be tolerated. Overages of 50% of the specified content, however, should not be accepted (32).

While  $\alpha$ -carotene was not detected in any of the shells, migration of  $\beta$ -carotene was observed in three soft gelatin formulations (2, 3, and 9). The extent of migration was obviously not dependent on the  $\beta$ -carotene content since the highest amount was found in the shell of product 9 having a very low carotenoid content, whereas no migration was observed for sample 1. Also storage time apparently did not affect migration of  $\beta$ -carotene since none of the shells contained this carotenoid after expiration of their shelf lives (preparations 6 and 8).

Carotenoids to be used as food ingredients can be obtained either by synthesis or by extraction of plant material. At present, only eight carotenoids are synthesized on an industrial scale, among them  $\beta$ -carotene, lycopene, and zeaxanthin (33). In contrast to the stereochemically pure carotenoids, the active ingredients recovered from plants may also contain *cis*-isomers. From **Table 1** it can be seen that 13-*cis*- and 9-*cis*- $\beta$ -carotene were present in all supplements. Carotene applied in dietary supplements often originates from microalgae, e.g., *Dunaliella salina* containing *all-trans*- $\beta$ -carotene and 9-*cis*- $\beta$ -carotene at a ratio of about 60% to 40% (34). Samples containing extracts from *D. salina* (preparations 4 and 11), as specified on the label, showed an increased amount of 9-*cis*- $\beta$ -carotene (approximately 23%) compared to other preparations (2–13%). Relatively large amounts of *cis*-isomers of  $\beta$ -carotene found in preparation 5 may also originate from palm oil extracts, which are known to contain up to approximately 40% *cis*-isomers of  $\beta$ -carotene (35).



Besides naturally occurring *cis*-stereoisomers, extraction and formulation of carotenoids may cause *trans*–*cis*-isomerization. Our previous investigations on the presence of  $\beta$ -carotene stereoisomers in vitaminized drinks had shown relatively high amounts (up to 30.8%) of *cis*-isomers in drinks exclusively supplemented with synthetic  $\beta$ -carotene (23). The observation of enhanced  $\beta$ -carotene isomerization after hot dissolution of  $\beta$ -carotene has recently been confirmed (36). Carotenoid *cis*-isomers have also been demonstrated to occur in fresh green vegetables, and their proportion may be increased by processing, especially by canning (unpublished results). In the case of dietary supplements, *cis*-isomers which were detected in all products investigated may be due to technological preparation of capsules.

**Application of Lutein and Zeaxanthin in Dietary Supplements.** Among the xanthophylls, lutein was specified in two dietary supplements, whereas zeaxanthin was not included in their specifications. According to the label, products 9 and 11 should contain 2 and 0.25 mg of lutein per capsule or dragée, respectively. However, *all-trans*-lutein could only be detected in product 9 (3.11 mg), accompanied by a low level of *all-trans*-zeaxanthin (0.16 mg). Both xanthophylls were also found in sample 4 (0.23 and 0.08 mg per capsule), although not specified on the label. No evidence for the presence of *cis*-isomers of lutein and zeaxanthin was obtained. Since no lutein was detected in sample 11, it is concluded that it has been supplemented in its esterified form. Lutein used as a food and feed ingredient is often recovered from the petals of *Tagetes erecta* composed primarily of lutein esters (37). Since differing bioavailability has been reported for free and esterified xanthophylls (38), a more accurate specification would be desirable.

In the present study an efficient method for the extraction and determination of carotenoid stereoisomers in commercial dietary supplements has been established. Although only small amounts of carotenoids have been shown to migrate into the gelatin shells, enzymatic digestion of the gelatin capsules is strongly recommended to ensure complete extraction and accurate quantification, especially when carotenoids are present in low quantities, as shown for preparation 9. In view of the rapidly growing market of dietary supplements, the method presented here may find application both in the food and pharmaceutical industries and by food inspection authorities.

## LITERATURE CITED

- (1) Britton, G.; Liaaen-Jensen, S.; Pfander, H. P. Synthesis from a different perspective: How nature does it. In *Carotenoids. Vol. 3: Biosynthesis and Metabolism*; Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhäuser Verlag: Basel, Switzerland, 1998; pp 1–12.
- (2) Riso, P.; Porrini, M. Determination of carotenoids in vegetable foods and plasma. *Int. J. Vitam. Nutr. Res.* **1997**, *67*, 47–54.
- (3) Handelman, G. J. The evolving role of carotenoids in human biochemistry. *Nutrition* **2001**, *17*, 818–822.
- (4) Schünemann, H. J.; McCann, S.; Grant, B. J. B.; Trevisan, M.; Muti, P.; Freudenheim, J. L. Lung function in relation to intake of carotenoids and other antioxidant vitamins in a population-based study. *Am. J. Epidemiol.* **2002**, *155*, 463–471.
- (5) Michaud, D. S.; Feskanich, D.; Rimm, E. B.; Colditz, G. A.; Speizer, F. E.; Willett, W. C.; Giovannucci, E. Intake of specific carotenoids and risk of lung cancer in 2 prospective US cohorts. *Am. J. Clin. Nutr.* **2000**, *72*, 990–997.
- (6) Slattery, M. L.; Benson, J.; Curtin, K.; Ma, K. N.; Schaeffer, D.; Potter, J. D. Carotenoids and colon cancer. *Am. J. Clin. Nutr.* **2000**, *71*, 575–582.
- (7) Nishino, H. Cancer prevention by natural carotenoids. *J. Cell. Biochem.* **1997**, *27*, 86–91.
- (8) Landrum, J. T.; Bone, R. A.; Kilburn, M. D. The macular pigment: A possible role in protection from age-related macular degeneration. *Adv. Pharmacol.* **1997**, *38*, 5537–556.
- (9) Johnston, C. S.; Taylor, C. A.; Hampl, J. S. More Americans are eating “5 a day” but intakes of dark green and cruciferous vegetables remain low. *J. Nutr.* **2000**, *130*, 3063–3067.
- (10) Richer, S.; Stiles, W.; Statkute, L.; Pulido, J.; Frankowski, J.; Rudy, D.; Pei, K.; Tsipursky, M.; Nyland, J. Double-masked, placebo-controlled, randomized trial of lutein and antioxidant supplementation in the intervention of atrophic age-related macular degeneration: The veterans LAST study (Lutein Antioxidant Supplementation Trial). *Optometry* **2004**, *75*, 216–229.
- (11) Ransley, J. K. The rise and rise of food and nutritional supplements—an overview of the market. In *Food and Nutritional Supplements*; Ransley, J. K., Donnelly, J. K., Read, N. W., Eds.; Springer-Verlag: Berlin, Germany, 2001; pp 1–16.
- (12) Castenmiller, J. J. M.; West, C. E. Bioavailability and bioconversion of carotenoids. *Annu. Rev. Nutr.* **1998**, *18*, 19–38.
- (13) Böhm, V.; Puspitasari-Nienaber, N. L.; Ferruzzi, M. G.; Schwartz, S. J. Trolox equivalent antioxidant capacity of different geometrical isomers of  $\alpha$ -carotene,  $\beta$ -carotene, lycopene, and zeaxanthin. *J. Agric. Food Chem.* **2002**, *50*, 221–226.
- (14) Werman, M. J.; Mokady, S.; Ben-Amotz, A. Bioavailability of the isomer mixture of phytoene and phytofluene-rich alga *Dunaliella bardawil* in rat plasma and tissues. *J. Nutr. Biochem.* **2002**, *13*, 585–591.
- (15) Olson, J. A. Bioavailability of carotenoids. *Arch. Latinoam. Nutr.* **1999**, *49*, 21S–25S.
- (16) Parker, R. S. Absorption, metabolism, and transport of carotenoids. *FASEB J.* **1996**, *10*, 542–551.
- (17) Jensen, C. D.; Howes, T. W.; Spiller, G. A.; Pattison, T. S.; Whittam, J. H.; Scala, J. Observations on the effects of ingesting *cis*- and *trans*-beta-carotene isomers on human serum concentrations. *Nutr. Rep. Int.* **1987**, *35*, 413–422.
- (18) Erdmann, J. W.; Thatcher, A. J.; Hofmann, N. E.; Lederman, J. D.; Block, S. S.; Lee, C. M.; Mokady, S. All-*trans*  $\beta$ -carotene is absorbed preferentially to 9-*cis*  $\beta$ -carotene, but the latter accumulates in the tissues of domestic ferrets (*Mustela putorius furo*). *J. Nutr.* **1998**, *128*, 2009–2013.
- (19) Deming, D. M.; Teixeira, S. R.; Erdman, J. W. All-*trans*  $\beta$ -carotene appears to be more bioavailable than 9-*cis* or 13-*cis*  $\beta$ -carotene in gerbils given single oral doses of each isomer. *J. Nutr.* **2002**, *132*, 2700–2708.
- (20) Stahl, W.; Sies, H. Uptake of lycopene and its geometrical isomers is greater from heat-processed than from unprocessed tomato juice in humans. *J. Nutr.* **1992**, *122*, 2161–2166.
- (21) Osterlie, M.; Bjerkeng, B.; Liaaen-Jensen, S. Plasma appearance and distribution of astaxanthin *E/Z* and *R/S* isomers in plasma lipoproteins of men after single dose administration of astaxanthin. *J. Nutr. Biochem.* **2000**, *11*, 482–490.
- (22) Su, Q.; Rowley, K. G.; Balazs, N. D. H. Carotenoids: Separation methods applicable to biological samples. *J. Chromatogr., B* **2002**, *781*, 393–418.
- (23) Marx, M.; Schieber, A.; Carle, R. Quantitative determination of carotene stereoisomers in carrot juices and vitamin supplemented (ATBC) drinks. *Food Chem.* **2000**, *70*, 403–408.
- (24) Schieber, A.; Marx, M.; Carle, R. Simultaneous determination of carotenes and tocopherols in ATBC drinks by high-performance liquid chromatography. *Food Chem.* **2002**, *76*, 357–362.
- (25) Barua, A. B.; Olson, J. A. Reversed-phase gradient high-performance liquid chromatographic procedure for simultaneous analysis of very polar to nonpolar retinoids, carotenoids and tocopherols in animal and plant samples. *J. Chromatogr., B* **1998**, *707*, 69–79.
- (26) Omenn, G. S.; Goodman, G. E.; Thornquist, M. D.; Balmes, J.; Cullen, M. R.; Glass, A.; Keogh, J. P.; Meyskens, F. L.; Valanis, B.; Williams, J. H.; Barnhart, S.; Hammar, S. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. *N. Engl. J. Med.* **1996**, *334*, 1150–1155.

- (27) The Alpha-Tocopherol and Beta Carotene Cancer Prevention Group. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. *N. Engl. J. Med.* **1994**, *330*, 1029–1035.
- (28) Zechmeister, L. *Cis-trans Isomeric Carotenoids, Vitamins A and Arylpolyenes*; Springer-Verlag: Vienna, Austria, 1962; pp 51–55.
- (29) Britton, G. UV/visible spectroscopy. In *Carotenoids. Vol. 1B: Spectroscopy*; Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhäuser Verlag: Basel, Switzerland, 1995; pp 13–62.
- (30) Kimura, M.; Rodriguez-Amaya, D. B.; Godoy, H. T. Assessment of the saponification step in the quantitative determination of carotenoids and provitamin A. *Food Chem.* **1990**, *35*, 187–195.
- (31) Rodriguez-Amaya, D. B. *A Guide to Carotenoid Analysis in Foods*; ILSI Press: Washington, DC, 2001; p 20.
- (32) Anonymous. Empfehlungen zu Toleranzen für Nährstoffschwankungen bei der Nährwertkennzeichnung. *Lebensmittelchemie* **1999**, *53*, 161.
- (33) Meyer, K. Carotinoide—Bedeutung und technische Synthesen. *Chem. Unserer Zeit* **2002**, *3*, 178–192.
- (34) Orset, S.; Leach, G. C.; Morais, R.; Young, A. J. Spray-drying of the microalga *Dunaliella salina*: Effects on  $\beta$ -carotene and isomer composition. *J. Agric. Food Chem.* **1999**, *47*, 4782–4790.
- (35) Darnako, D.; Cheryan, M.; Jerrel, J.; Perkins, E. G. Simultaneous HPLC analysis of palm carotenoids and tocopherols using a C-30 column and a photodiode array detector. *J. Liq. Chromatogr. Relat. Technol.* **2000**, *23*, 1873–1885.
- (36) Marx, M.; Stuparic, M.; Schieber, A.; Carle, R. Effects of thermal processing on *trans-cis* isomerization of  $\beta$ -carotene in carrot juices and carotene-containing preparations. *Food Chem.* **2003**, *83*, 609–617.
- (37) Rivas, J. D. L. Reversed phase high-performance liquid chromatographic separation of lutein and lutein fatty acid esters from marigold flower petal powder. *J. Chromatogr.* **1989**, *464*, 442–447.
- (38) Bowen, P. E.; Herbst-Espinosa, S. M.; Hussain, E. A.; Stacewicz-Sapuntzakis, M. Esterification does not impair lutein bioavailability in humans. *J. Nutr.* **2002**, *132*, 3668–3673.

---

Received for review May 3, 2004. Revised manuscript received August 2, 2004. Accepted August 6, 2004. This study was supported by a grant from the Landesstiftung Baden-Württemberg gGmbH, Germany (Project P-LS-E/FDPA1-21).

JF049304L