Carotenoid Esters in Vegetables and Fruits: A Screening with Emphasis on β-Cryptoxanthin Esters

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Carotenoids are found in food plants in free form or as fatty acid esters. Most studies have been carried out after saponification procedures, so the resulting data do not represent the native carotenoid composition of plant tissues. Therefore, nonsaponified extracts of 64 fruits and vegetables have been screened to determine the amount of carotenoid esters in food plants. Because one of the major problems in the quantitation of carotenoids is the availability of pure standard material, the total carotenoid ester content was calculated as lutein dimyristate equivalents. Lutein dimyristate was independently synthesized from lutein and myristoyl chloride. The highest ester concentrations were found in red chili (17.1 mg/100 g) and orange pepper (9.2 mg/100 g); most of the investigated fruits and vegetables showed concentrations up to 1.5 mg/100 g. Special attention was dedicated to β-cryptoxanthin esters. To enable an accurate detection of the β-cryptoxanthin ester content, β-cryptoxanthin was purified from papaya and used for synthesis of β-cryptoxanthin laurate, myristate, and palmitate, representing the major β-cryptoxanthin esters in food plants. The study proved tropical and subtropical fruits to be an additional source of β-cryptoxanthin esters in the human diet. The contents ranged from 8 μg/100 g β-cryptoxanthin laurate in Tunisian orange to 892 μg/100 g β-cryptoxanthin laurate in papaya.

Keywords: *Carotenoid; carotenoid esters; β-cryptoxanthin; lutein dimyristate*

INTRODUCTION

Recent studies have indicated the potential health benefits of a diet rich in carotenoids. Apart from their provitamin A capacity, animal and epidemiological studies showed carotenoids to be important under different aspects, for example, as antioxidants (*1*) and as preventing agents against cardiovascular diseases (*2*), age-related macular degeneration (*3*), and cataracts (*4*). Owing to the great interest in the role of carotenoids in human health, the carotenoid composition of foods has been analyzed extensively, whereby most papers focused on analysis and metabolism of β,β-carotene and lutein. Furthermore, authors have often disregarded the fact that in some plants mono- or dihydroxylated carotenoids (xanthophylls) occur esterified with various fatty acids. Because saponification is a commonly used step in carotenoid analysis, information concerning the natural binding form is lost due to this workup procedure. Consequently, results about carotenoid ester contents of fruits and vegetables are restricted to some reports that provide data on nonsaponified extracts of red pepper (*5*−*7*), squash products (*8*, *9*), tangerine concentrate (*10*), peaches (*11*), and marigold flowers (*12*, *13*). So far, no systematic attempt has been made to calculate the carotenoid ester content in various food plants, which are most abundant in the human diet.

Humans are unable to synthesize vitamin A de novo, so the vitamin is metabolically derived from oxidative cleavage of provitamin A carotenoids (β,ε-carotene, β,β-carotene, and β-cryptoxanthin) by 15,15′-dioxygenases (*14*, *15*). Due to its hydroxyl group, β-cryptoxanthin can be present as a mono acyl ester in food plants, being available for bioconversion to retinal in principle. Therefore, the occurrence of β-cryptoxanthin esters in several fruits has been exhaustively studied. The main β-cryptoxanthin esters occurring in plants (β-cryptoxanthin laurate, myristate, and palmitate) were synthesized according to procedures described previously (*8*, *9*, *12*), purified by preparative HPLC, and used as reference material for the identification and quantification of the respective carotenoid esters.

Taken together, our main purposes were to find out, on the one hand, which fruits and vegetables contain natural carotenoid esters at all and, on the other hand, which fruits and vegetables contain β-cryptoxanthin esters, offering an additional source of provitamin A carotenoids in the human diet.

MATERIALS AND METHODS

Samples. Fully ripe fruits and vegetables were obtained from local markets. Only the edible portions, without peels and seeds, were used. To obtain homogeneous samples, an appropriate amount (20−200 g) was minced for 1 min by an Ultra Turrax T 25 (Janke & Kunkel, Staufen, Germany) and processed immediately without deep-freezing. Wherever possible, the correct generic and species names as well as the countries of origin are given. Unfortunately, it was not possible to specify the variety in each case.

Chemicals. Capsanthin, β-cryptoxanthin, lutein, and zeaxanthin were generously provided by Hoffmann-La Roche (Basel, Switzerland). A certified standard solution of violaxanthin ($c = 0.637$ mg/L) was purchased from VKI (Hørsholm, Denmark). Acetone, 2,6-di-*tert*-butyl-4-methylphenol (BHT), diethyl ether, ethyl acetate, *n*-hexane, light petroleum ether (40−60 °C), and methanol were purchased from Merck (Darmstadt, Germany). β-Apo-8′-carotenal, *tert*-butyl methyl ether, β,β-carotene, lauroyl chloride (98%), myristoyl chloride (99%),

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palmitoyl chloride (99%), stearoyl chloride (99%), and silica gel (70−230 mesh) were obtained from Fluka (Neu-Ulm, Germany). The solvents used were of analytical grade and were distilled before use. For HPLC analysis, ultrapure water from a Milli-Q 185 Plus apparatus (Millipore, Eschborn, Germany) was employed. Trivial names of carotenoids are used instead of the complex IUPAC nomenclature (*16*) throughout this text.

Preparation of Samples. Carotenoids were extracted by shaking the sample homogenate (2−10 g, depending on the color intensity of the respective sample) with methanol/ethyl acetate/light petroleum ether (1:1:1 v/v/v; 25 mL). Prior to extraction, an internal standard (10 mg of β-apo-8′-carotenal/100 mL of light petroleum/ethyl acetate; 1:1 v/v; 2 mL) was added to each sample. The upper phase was transferred to an Erlenmeyer flask. This extraction procedure was repeated thrice. The combined extracts were dried with anhydrous sodium sulfate (10 g), filtered through a small folded filter, and evaporated to dryness in vacuo at 30 °C. The residue was dissolved in methanol/*tert*-butyl methyl ether/BHT (1:1:0.01 v/v/v; 2 mL). After membrane filtration (0.45 μm), an aliquot of the solution was subjected to HPLC analysis. The whole procedure was performed in dim light and repeated thrice. Saponification of extracts was performed according to the method of Breithaupt (*17*).

Validation of the Extraction Method. The reproducibility of the method was investigated by spiking homogenized pear samples (5 g) with aliquots (1 and 2 mL) of an extract of red pepper [20 g of homogenate/20 mL final volume of methanol/*tert*-butyl methyl ether (1:1 v/v)]. The spiked samples were extracted and analyzed by HPLC as described under Preparation of Samples. The peak areas corresponding to carotenoid esters were summed and calculated as lutein dimyristate equivalents. The recoveries of carotenoid esters from spiked samples were 97 ± 2% (n = 5, spiked with 1 mL of paprika extract) and 96 ± 1% (n = 5, spiked with 2 mL of paprika extract) as determined by the peak areas corresponding to carotenoid esters. Pears were chosen for this purpose as they do not contain any carotenoid esters. Thus, incorrect results due to coeluting carotenoids are avoided. Additionally, the HPLC peak areas of the internal standard (ISTD), β-apo-8′-carotenal, were monitored to determine possible carotenoid losses during the workup procedure. The recovery of β-apo-8′-carotenal from extractions of various samples accounted for >96% as determined by HPLC.

Isolation of β-Cryptoxanthin. The total carotenoids of fresh papayas (700 g) were isolated by repeated extraction with diethyl ether/light petroleum ether (1:1 v/v) until the extracts were colorless. The extracts were combined, dried over anhydrous sodium sulfate, and filtered. The solvent was evaporated under reduced pressure, and the residue was dissolved in diethyl ether (200 mL). For saponification, methanolic potassium hydroxide (30% w/v; 30 mL) was added and the solution stored overnight at room temperature. To remove excess of alkali, the solution was washed three times with water (100 mL each), dried over anhydrous sodium sulfate, filtered, and evaporated to dryness. The resulting residue was dissolved in *n*-hexane (20 mL) and subjected to preparative open column chromatography (glass column, 400 × 20 mm) on silica gel (10 g) in aliquots of 5 mL, employing light petroleum ether and acetone as mobile phases. The first band, obtained by elution with light petroleum ether, consists mainly of β,β-carotene. The second band (light petroleum ether/acetone, 9:1 v/v) was shown to consist of a single component, which was identified as β-cryptoxanthin by comparison of the HPLC retention time and absorption spectra with those of authentic reference material (yield = 2.0 mg). Further polar carotenoids, remaining on the column, were discarded with the silica gel.

Synthesis of Xanthophyll Fatty Acid Esters. β-Cryptoxanthin laurate, myristate, palmitate, and stearate as well as lutein dimyristate were prepared from isolated β-cryptoxanthin or pure lutein, respectively, and the appropriate fatty acid chlorides according to published procedures (*9*, *12*). For purification, the crude products were dissolved in the mobile phase and 2 mL of each preparation was applied in multiple injections to semipreparative HPLC (retention times of the respective β-cryptoxanthin esters: laurate, 11 min; myristate, 17 min; palmitate, 23 min; stearate, 30 min; retention time of lutein dimyristate, 31 min). The resulting fractions were combined and evaporated to dryness, and the residue was redissolved in light petroleum ether (β-cryptoxanthin ester) or ethanol (lutein dimyristate). The concentrations were determined spectrophotometrically using the molar extinction coefficients of β-cryptoxanthin [ϵ_{mol} = 131900; 449 nm; light petroleum ether (*18*)] and lutein [ϵ_{mol} = 144800; 445 nm; ethanol (*18*)]. Quantitative determinations based upon ϵ_{mol} reveal correct results because esterification does not change the shape of the respective absorption spectrum (*19*). The absorption maxima of the β-cryptoxanthin esters and lutein dimyristate were identical to the data cited in the literature (*9*, *12*), and the spectra showed no additional cis-peaks at 340 nm.

The identity of the substances was checked by liquid chromatography−mass spectrometry (LC-MS) analysis in the atmospheric pressure chemical ionization ($APcI^+$) mode according to a procedure described previously (*20*). The molecular ion signal of each β-cryptoxanthin ester appears as a quasi-molecular ion $[M + H]^+$ with m/z 735 (laurate), m/z 763 (myristate), or m/z 791 (palmitate). In each case, m/z 535, generated by loss of the respective fatty acid, was detected. Lutein dimyristate was characterized by its $[M + H]^+$ signal at m/z 989 and two typical fragment ions at m/z 761 [$M + H^+ - CH_3(CH_2)_{12}COOH$] and m/z 533 [$M + H^+ - [CH_3(CH_2)_{12}COOH]_2$].

Identification of Carotenoids by HPLC. Carotenoids were identified by comparing their specific retention times and absorption spectra with those of reference standards. The purity of the peaks was confirmed spectrophotometrically. Diode array scans at the slopes and the apex of the peaks were identical. For qualitative identification of capsanthin, lutein, and zeaxanthin, methanolic standard solutions (c = 10 mg/L) were applied. For preparing a β,β-carotene standard solution, light petroleum ether/ethyl acetate (1:1 v/v) was used instead of methanol. β,ϵ-Carotene and lycopene were identified by means of their absorption maxima given by Britton et al. (*18*) (β,ϵ-carotene: λ_{max} 423/444/473 nm; lycopene: λ_{max} 446/472/503 nm). In native extracts of dark green vegetables (e.g., spinach and zucchini) zeaxanthin coeluted with chlorophyll *a* during HPLC analysis. In these cases zeaxanthin was identified after elimination of chlorophyll by the saponification step.

Quantification of Carotenoid Esters by HPLC. Because reference standards for carotenoid esters are not available commercially, the total amount of carotenoid esters was calculated as lutein dimyristate equivalents in micrograms per 100 g of edible part. For this purpose, only the areas corresponding to peaks that disappear after alkaline treatment were summed. Triplicate samples were analyzed in each case, and the mean value was determined. For calibration of lutein dimyristate, aliquots of the solution used for spectrophotometrical determination of the concentration were evaporated to dryness, redissolved in 2 mL of methanol/*tert*-butyl methyl ether/BHT (1:1:0.01 v/v/v), further diluted to the required concentrations (0.5−20.0 mg/L), and subjected immediately to HPLC analysis. For calculation of lutein dimyristate equivalents, a calibration curve was created by plotting the peak area versus the concentration. The limits of quantitation (LOQ) and determination (LOD) of lutein dimyristate equivalents (micrograms per 100 g) were calculated from this calibration graph according to the recommendations of the Deutsche Forschungsgemeinschaft (*21*) and were based on the use of 2 g of sample/2 mL final volume: LOQ, 62 μg/100 g; LOD, 40 μg/100 g.

For quantification of β-cryptoxanthin esters, aliquots of the solutions used for spectrophotometrical determination were treated as described above. Calibration for each β-cryptoxanthin ester was performed in the range of 0.1−2.5 mg/L. LOQ/LOD values of the respective β-cryptoxanthin esters are very similar, so β-cryptoxanthin laurate was used as a model compound for calculation. LOQ/LOD values (*21*) were deter-

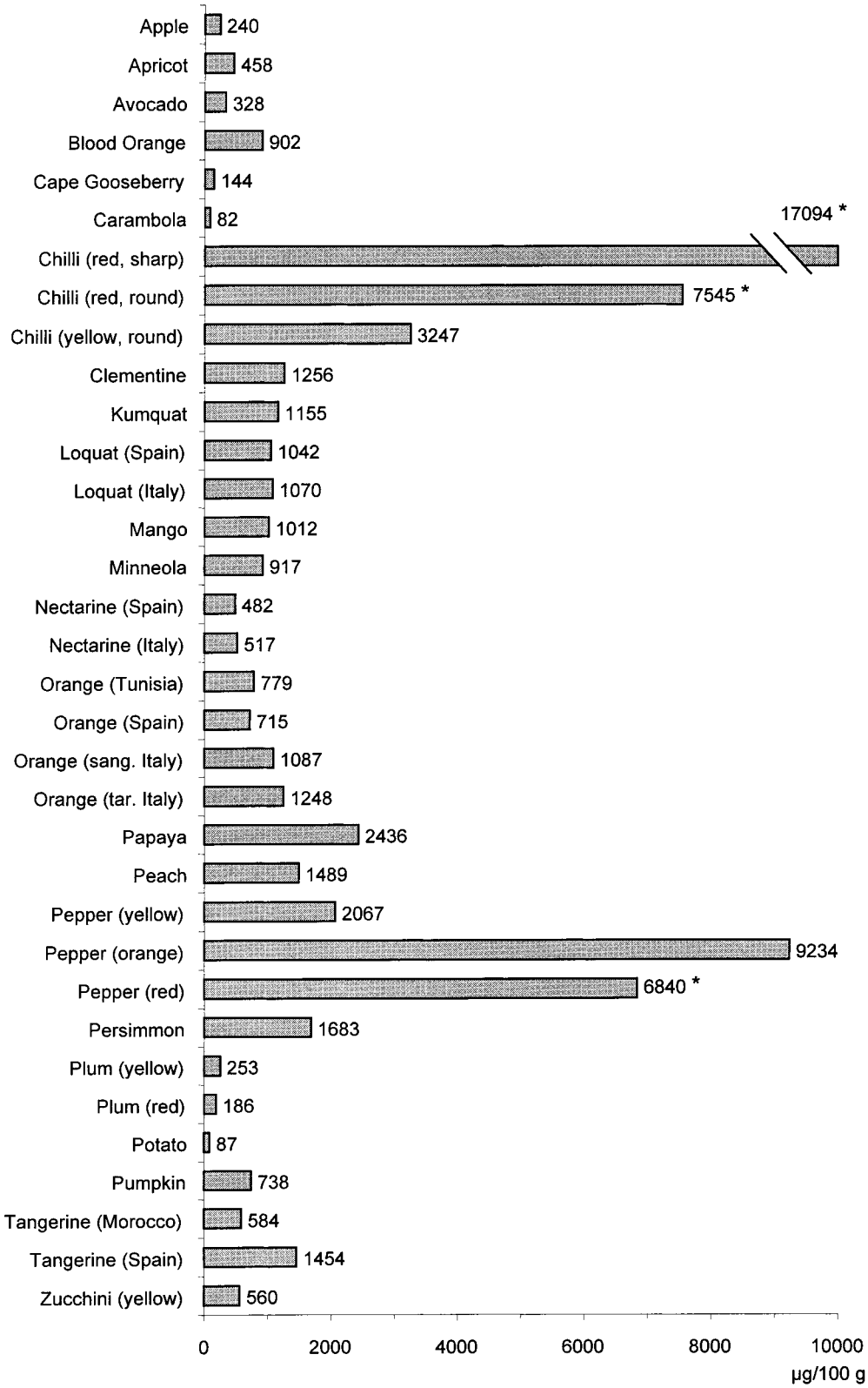

Figure 1. Fruits and vegetables, containing carotenoid esters, calculated as lutein dimyristate equivalents (μg/100 g). Due to the spectral properties of capsanthin and capsorubin, the carotenoid ester content of fruits marked with an asterisk (*) is underestimated by this method to ~16%.

mined as follows (referring to the use of 2 g of sample/2 mL final volume): LOQ, 8 μg/100 g; LOD, 5 μg/100 g.

Apparatus. High-performance liquid chromatography (HPLC) was performed on an HP1050 HPLC system which comprised an autosampler, a gradient pump, a diode array detector (DAD) module (Hewlett-Packard, Waldbronn, Germany), and an external column thermoregulator (Mistral, Spark, The Netherlands). UV absorbance of the carotenoids was recorded at 450 nm. A YMC analytical column (YMC Europe, Schermbeck, Germany) with C30-reversed phase material (250 × 4.6 mm, 5 μm) including a precolumn (Nucleosil C18, 10 × 4.6 mm, 5 μm, Bischoff, Leonberg, Germany) was used and kept at 35 °C. The mobile phase was described previously (*17*); the injection volume was 20 μL.

Table 1. Fruits and Vegetables that Contain Carotenoid Esters: Taxonomic Names and Main Parent Carotenoids (β,β-Carotene is Present in all Samples but is not Listed)

no.	common name	taxonomic name[a]	main parent xanthophylls identified[b]
1	apple (Gala)	*Malus domestica* Borkh.	V
2	apricot (France)	*Prunus armeniaca* L.	Cr, L
3	avocado	*Persea americana* Mill.	L
4	blood orange	*Citrus sinensis* (L.) Osbeck	Cr, L, V
5	cape gooseberry	*Physalis peruviana* L.	L
6	carambola	*Averrhoa carambola* L.	L[c]
7	chili (red, sharp)	*Capsicum frutescens* L.	Ca, Cr, Z
8	chili (red, round)	*Capsicum frutescens* L.	Ca, Cr, Z
9	chili (yellow, round)	*Capsicum frutescens* L.	L, V
10	clementine (Fortuna, Spain)	*Citrus reticulata* Blanco	Cr
11	kumquat (Israel)	*Fortunella margarita* (Lour.) Swingle	Cr, L, V
12	loquat (Spain)	*Eriobotrya japonica* Lindl.	Cr
13	loquat (Italy)	*Eriobotrya japonica* Lindl.	Cr
14	mango	*Mangifera indica* L.	V
15	minneola (Turkey)	—[d]	Cr
16	nectarine (Spain)	*Prunus persica* Batsch var. *nucipersica* (L.) C. K. Schneid.	Cr, L, V, Z
17	nectarine (Italy)	*Prunus persica* Batsch var. *nucipersica* (L.) C. K. Schneid.	Cr, L, V, Z
18	orange (Maltaise, Tunisia)	*Citrus sinensis* Pers.	Cr, L, V
19	orange (Navel, Spain)	*Citrus sinensis* Pers.	Cr, L, V
20	orange (Sanguinello, Italy)	*Citrus sinensis* Pers.	Cr, L, V
21	orange (Tarocco, Italy)	*Citrus sinensis* Pers.	Cr, L, V
22	papaya	*Carica papaya* L.	Cr, V[e]
23	peach	*Prunus persica* (L.) Batsch	Cr, V
24	pepper (yellow)	*Capsicum annuum* L. Grossum Grp.	L, V, Z
25	pepper (orange)	*Capsicum annuum* L. Grossum Grp.	Cr, Z
26	pepper (red)	*Capsicum annuum* L. Grossum Grp.	Ca, V, Z
27	persimmon	*Diospyros kaki* L. f.	Cr, Z
28	plum (yellow, Spain)	*Prunus domestica* L.	Cr, L
29	plum (red, Spain)	*Prunus domestica* L.	Cr, L
30	potato (Siglinde)	*Solanum tuberosum* L.	L, V
31	pumpkin	*Cucurbita pepo* L.	L, V, Z
32	tangerine (Fortune, Morocco)	*Citrus reticulata* Blanco	Cr, L
33	tangerine (Solach, Spain)	*Citrus reticulata* Blanco	Cr, L
34	zucchini (yellow)	*Cucurbita pepo* L.	L[b]

[a] The taxonomic names were specified according to the International Standards of Nomenclature (*24*). Grp. designates a cultivar group. [b] Abbreviations: Ca, capsanthin; Cr, β-cryptoxanthin; L, lutein; V, violaxanthin; Z, zeaxanthin. [c] The fruits contain only trace amounts of β,β-carotene. [d] Minneola, which belong to the tangelo group, are a product of cross-breeding *Citrus* × *aurantium* and possess no kernels. The correct taxonomic name is unfortunately not known to the authors. [e] The fruits contain the carotene lycopene.

The system for semipreparative HPLC consisted of a Kronlab (Sinsheim, Germany) HD 2−200 pump combined with a Kronlab SpectraFlow 500 variable-wavelength detector (detection wavelength = 450 nm) and a YMC semipreparative column (250 × 20 mm, C30, 5 μm) including a YMC guard column (10 × 20 mm, C18, 5 μm). The injection volume was 2.0 mL and the flow rate 20 mL/min. A mixture of *tert*-butyl methyl ether, methanol, and water (55:41:4 v/v/v) was used as isocratic eluent.

UV−vis spectroscopic analyses were performed in quartz cuvettes (1 cm) using a scanning Perkin-Elmer (Überlingen, Germany) Lambda 2 spectrophotometer.

RESULTS AND DISCUSSION

Carotenoid Esters in Fruits and Vegetables. Advances in isolation and chromatographic separation methodologies proved carotenoid esters to be much more widely distributed in nature than previously expected. They occur not only in different yellow-red paprika varieties but also in several fruits and vegetables, being important components of the daily human diet. Because these food plants contain a wide range of carotenoid esters, it is not feasible to synthesize all derivatives in a preparative scale. Therefore, the total amounts of carotenoid esters have been calculated as lutein dimyristate equivalents. Lutein dimyristate was synthesized independently, using lutein and myristoyl chloride. Because most xanthophyll esters in fruits and vegetables appear as diesters and lutein, β-cryptoxanthin, and violaxanthin as well as zeaxanthin exhibit similar absorption spectra and extinction coefficients, the results are suitable to compare the carotenoid ester contents of several food plants. For carotenoids having spectral properties different from those of lutein, for example, capsanthin, which shows a bathochromic maximum shift of ∼30 nm relative to the detection wavelength (450 nm), the true ester concentration is higher than the values calculated by the method described here. Consequently, this leads to an underestimation of the total carotenoid ester content in the case of fruits comprising high amounts of capsanthin esters (red chili and red pepper). To investigate this fact, we analyzed a solution containing capsanthin and lutein (10 μmol/L each) by HPLC and compared the resulting peak areas (data not shown). In this way the underestimation of capsanthin relative to lutein was calculated to be 16%. However, for the purpose of this study, this error is considered to be acceptable. These xanthophylls are unique components of red pepper and red chili, so this problem is restricted to these fruits.

The results obtained are shown in Figure 1, and the taxonomic names are listed in Table 1. β,β-Carotene was present in all extracts, and it is not explicitly listed in Table 1. The highest ester concentrations have been determined in red sharp chili (17.1 mg/100 g) and orange pepper (9.2 mg/100 g), whereas red pepper, yellow chili, and yellow pepper contained lesser carotenoid ester amounts (6.8, 3.2, and 2.1 mg/100 g, respectively). The very high ester content of red chili is

Table 2. Fruits, Vegetables, and Mushrooms Containing no Carotenoid Esters and Main Carotenoids Detectable (*β,β*-Carotene is Present in all Samples but is not Listed)

no.	common name	taxonomic name[a]	main carotenoid(s) identified[b]
35	aubergine	*Solanum melongena* L.	L
36	banana	*Musa* × *paradisiaca* L.	αC, L
37	broccoli	*Brassica oleracea* L. convar. *botrytis* var. *italica* P.	L, V
38	Brussels sprouts	*Brassica oleracea* L. Gemmifera Grp.	L, V,[c]
39	cactus fig	*Opuntia ficus-indica* (L.) Mill.	L
40	carrot	*Daucus carota* L. ssp. *sativus* (Hoffm.) Schübl. et G. Martens	αC
41	chanterelles	*Cantharellus cibarius* Fr.	−
42	Chinese gooseberry (New Zealand)	*Actinidia deliciosa* (A. Chev.) C. F. Liang et A. R. Ferguson	L
43	corn	*Zea mays* L.	L, Z[c]
44	cress	*Lepidium sativum* L.	L, V
45	cucumber	*Cucumis sativus* L.	L
46	dandelion	*Taraxacum officinale* F. H. Wigg.	Cr, L, V, Z[d]
47	fennel (plant)	*Foeniculum vulgare* Mill.	L, V
48	gooseberry (green)	*Ribes uva-crispa* L.	L
49	granadille (Colombia)	*Passiflora edulis* Sims	Z
50	grapefruit (pink)	*Citrus paradisi* Macfad.	Ly
51	kale	*Brassica oleracea* L. Sabellica Grp.	L, V
52	lemon	*Citrus limon* (L.) Burm.	Cr, L[c]
53	melon	*Cucumis melo* L.	−[c]
54	net melon	*Cucumis melo* L. Reticulatus Grp.	L[c]
55	parsley	*Petroselinum crispum* (Mill.) Nym. var. *crispum*	L, V, Z[d]
56	pear	*Pyrus communis* L.	L[c]
57	pepper (green)	*Capsicum annuum* L. Grossum Grp.	L, Z
58	quince	*Cydonia oblonga* Mill.	−[c]
59	spinach	*Spinacia oleracea* L.	L, V, Z[d]
60	squash	*Cucurbita maxima* Duchesne	αC, L, V
61	strawberry (Spain)	*Fragaria x ananassa* (Duchesne) Gudés	L
62	tomato, red (Italy)	*Lycopersicon esculentum* Mill.	L, Ly
63	tomato, yellow (Italy)	*Lycopersicon esculentum* Mill.	L[e]
63	watermelon	*Citrullus lanatus* (Thunb.) Matsum.	L, Ly
64	zucchini (green)	*Cucurbita pepo* L.	L, V, Z[d]

[a] The taxonomic names were specified according to the International Standards of Nomenclature (*24*). Grp. designates a cultivar group. [b] Abbreviations: αC, *β,ϵ*-carotene; Ca, capsanthin; Cr, *β*-cryptoxanthin; L, lutein; Ly, lycopene; V, violaxanthin; Z, zeaxanthin. [c] These fruits contain only trace amounts of *β,β*-carotene. [d] The fruits contain only trace amounts of zeaxanthin, determined after removal of chlorophyll by saponification of the extracts. [e] Fruits of the pale yellow-orange tomato genotype ["high-*β* mutant" (*25*)] contained no lycopene.

due to a concentrating effect, caused by drying of the ripe fruits after harvest. All other fruits and vegetables showed concentrations up to 1.7 mg/100 g (persimmon) with the exception of papaya, which comprised a remarkably high ester concentration of 2.4 mg/100 g. Citrus fruits (clementine, kumquat, minneola, nectarine, orange, and tangerine), some tropical fruits (loquat, mango, and peach), pumpkin, and yellow zucchini have medium ester contents (0.5−1.5 mg/100 g), whereas apple, apricot, avocado, cape gooseberry, carambola, plum, and potato were poor sources (<0.5 mg/100 g). Papaya, pink grapefruit, tomato, and watermelon contained additionally substantial amounts of the carotene lycopene, whereas banana, carrots, and squash comprised *β,ϵ*-carotene, both unambiguously identified by comparison of the respective UV spectra with the data given by Britton et al. (*18*). Remarkable variations of the total carotenoid ester content were realized when different cultivars and various countries of origin were considered. This can be demonstrated by comparing the carotenoid ester concentration of several oranges (Figure 1). The highest carotenoid ester amount was found in the cultivar Tarocco, a semi-blood orange grown in Italy (1.2 mg/100 g; Table 1, no. 21); the lowest concentration was determined in the cultivar Navel from Spain (0.7 mg/100 g; Table 1, no. 19). This shows clearly the difficulty in predicting the carotenoid ester content of one species if the cultivar and provenance are not known.

Parent Xanthophylls of Native Carotenoid Esters. Xanthophyll esters disappear completely after saponification, which results in a corresponding increase of the respective xanthophyll peak. That way, the main parent xanthophylls can be identified by comparison with reference standards. Table 1 lists capsanthin, *β*-cryptoxanthin, lutein, violaxanthin, and zeaxanthin as parent xanthophylls of food plant carotenoid esters. In contrast, Table 2 shows free xanthophylls in fruits and vegetables, which comprise no carotenoid esters at all. Additionally, the hydrocarbons *β,ϵ*-carotene, *β,β*-carotene, and lycopene were screened in this study. Interestingly, there are numerous yellow-orange fruits that contain no carotenoid esters (e.g., banana, cactus fig, grenadille, pink grapefruit, pear, quince, and strawberry). Likewise, fruits with high fat or high carotenoid contents (e.g., carrot and corn) do not necessarily contain carotenoid esters. This points out that a prediction free of doubts concerning the absence of xanthophyll esters can be made only in the case of dark green color fruits and vegetables (e.g., broccoli, Brussels sprouts, fennel, parsley, and spinach).

Amounts of *β*-Cryptoxanthin Esters in Several Fruits. Special attention was dedicated to *β*-cryptoxanthin esters that act as vitamin A precursors. Figure 2 shows typical HPLC chromatograms of nonsaponified and saponified extracts of tangerine. Tangerines (*Citrus reticulata*), the smallest species in the economically important family of citrus fruits, contain high amounts of *β*-cryptoxanthin esters. This is impressively demonstrated by the increase of the *β*-cryptoxanthin peak detected after saponification of an tangerine extract (see Figure 2B). Several small peaks eluting just in front of the *β*-cryptoxanthin peak were identified by their UV spectra as *cis*-isomers of carotenoids. Due to their low

Table 3. Concentration of Free β-Cryptoxanthin and β-Cryptoxanthin Esters of Selected Fruits and Vegetables

		β-cryptoxanthin, μg/100g			
no.	common name	free	laurate[a]	myristate[a]	palmitate[a]
4	blood orange	27 ± 2	12 ± 1	22 ± 1	61 ± 1
7	chili (red, sharp)	894 ± 80	267 ± 17	415 ± 20	259 ± 4
8	chili (red, round)	69 ± 2	18 ± 1	154 ± 1	21 ± 2
10	clementine (Spain)	38 ± 3	234 ± 11	317 ± 5	64 ± 2
11	kumquat (Israel)	27 ± 2	66 ± 4	80 ± 7	27 ± 2
12	loquat (Spain)	10 ± 1	247 ± 16	98 ± 7	289 ± 22
13	loquat (Italy)	10 ± 1	242 ± 8	103 ± 4	276 ± 10
15	minneola (Turkey)	45 ± 3	140 ± 2	182 ± 1	80 ± 1
16	nectarine (Spain)	26 ± 1	24 ± 3	35 ± 2	59 ± 2
17	nectarine (Italy)	17 ± 1	23 ± 3	75 ± 3	29 ± 1
18	orange (Tunisia)	49 ± 1	8 ± 1	24 ± 1	55 ± 2
19	orange (Spain)	28 ± 1	13 ± 1	33 ± 1	19 ± 1
20	orange (Italy)	43 ± 4	16 ± 1	42 ± 3	65 ± 1
21	orange (Italy)	40 ± 4	27 ± 2	58 ± 5	35 ± 3
22	papaya	143 ± 2	892 ± 26	103 ± 2	86 ± 2
23	peach	91 ± 1	210 ± 8	66 ± 2	127 ± 4
25	pepper (orange)	238 ± 2	70 ± 4	183 ± 1	37 ± 3
27	persimmon	55 ± 4	519 ± 23	46 ± 2	83 ± 4
32	tangerine (Morocco)	99 ± 4	99 ± 4	152 ± 2	43 ± 2
33	tangerine (Spain)	46 ± 1	245 ± 13	357 ± 13	130 ± 5

[a] The given values represent means ± standard deviations of three independent determinations.

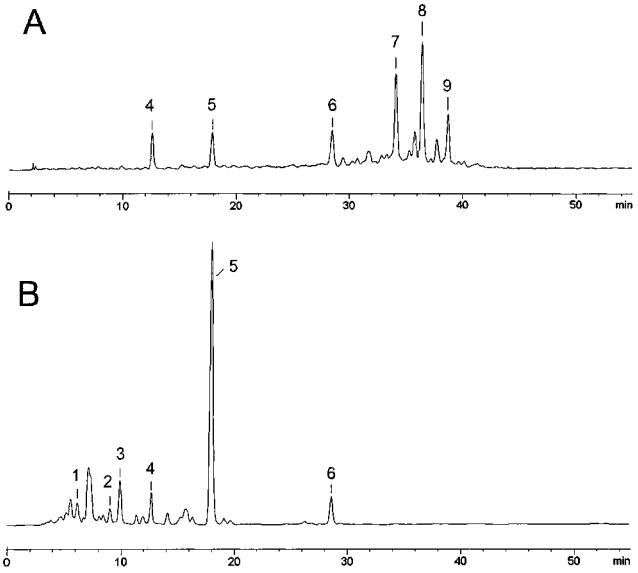

Figure 2. Typical HPLC chromatograms (DAD, 450 nm) of a nonsaponified (A) and a saponified (B) extract of tangerine (for conditions, see Materials and Methods). Both chromatograms are in the same scale. Peak assignment: 1, violaxanthin; 2, lutein; 3, zeaxanthin; 4, β-apo-8′-carotenal (ISTD); 5, β-cryptoxanthin. 6, β,β-carotene; 7, β-cryptoxanthin laurate; 8, β-cryptoxanthin myristate; 9, β-cryptoxanthin palmitate.

concentration, we did not attempt to identify the respective parent carotenoids by evaluation of the UV data. Although these peaks were small, incorrect quantitative results may be obtained if data from chemically saponified extracts are used to monitor the total carotenoid content of fruits and vegetables.

Peak assignment of β-cryptoxanthin esters was validated by LC-MS analysis in the $APcI^+$ mode (see *Materials and Methods*). That way, β-cryptoxanthin was purified from papaya and used for synthesis of β-cryptoxanthin laurate, myristate, and palmitate. The quantitative results obtained by HPLC with external calibration are presented in Table 3. Besides free β-cryptoxanthin (10−894 μg/100 g), β-cryptoxanthin laurate, myristate, and palmitate were present in various amounts (listed in Table 3) in all plant extracts. The contents ranged from 8−892 μg/100 g β-cryptoxanthin laurate to 22−415 μg/100 g β-cryptoxanthin myristate and 19−289 μg/100 g β-cryptoxanthin palmitate. The highest β-cryptoxanthin ester amounts were found in papaya and persimmon, in which β-cryptoxanthin laurate clearly dominates the ester spectrum (892 and 519 μg/100 g, respectively). The comparison of different provenances demonstrates that the β-cryptoxanthin ester spectrum is not constant in each case (e.g., orange and tangerine). This may be explained by differences in fruit maturity and environmental production conditions, which are generally not known. Additionally, in some fruits the esterification degree of carotenoids increases during ripening (*23*). Furthermore, these process may continue after harvest, resulting in varying β-cryptoxanthin ester amounts at different times of storage. For this reasons it is rather difficult to estimate the β-cryptoxanthin ester amounts present in the daily diet of consumers.

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