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Identification and Quantification of Zeaxanthin Esters in Plants Using Liquid Chromatography−Mass Spectrometry

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It has been suggested that lutein and zeaxanthin may decrease the risk for age-related macular degeneration. Surprisingly, oleoresins rich in zeaxanthin are not yet available on the market. Several authors have reported enhanced stability of esterified xanthophylls, so plants containing zeaxanthin esters were investigated to establish valuable sources for the production of durable oleoresins. Liquid chromatography−atmospheric pressure chemical ionization mass spectrometry [LC-(APCI)MS] was used to unequivocally identify zeaxanthin esters of a standard mixture and in several plant extracts. Zeaxanthin esters were quantified on the basis of their respective molecular masses using zeaxanthin for calibration; total zeaxanthin was determined after saponification of aliquots of the extracts. Thus, dried wolfberries (*Lycium barbarum*), Chinese lanterns (*Physalis alkekengi*), orange pepper (*Capsicum annuum*), and sea buckthorn (*Hippophae rhamnoides*) proved to be valuable zeaxanthin ester sources. The present LC-MS method allows for an even more detailed analysis of zeaxanthin esters than reported previously.

KEYWORDS: Zeaxanthin esters; LC-(APCI)MS; zeaxanthin oleoresin

INTRODUCTION

Zeaxanthin (β,β-carotene-3,3′-diol) and lutein (β,ϵ-carotene-3,3′-diol) are widely distributed in plants. Today it is accepted that a high intake of both xanthophylls protects against age-related macular degeneration (AMD) and age-related cataract formation (*1*, *2*), proving both xanthophylls to be ophthalmoprotective. The serum concentration of lutein and zeaxanthin may be influenced by dietary intake (*3*). This was confirmed by studies of Bernstein et al. (*4*), in which metabolites of zeaxanthin and lutein were isolated from human retina tissue.

As carotenoids are intensely colored, they are widely used as food colorants. Isolated as raw oleoresin by solvent extraction, they are available in large amounts while being relatively low-priced. With regard to the potential health benefits, zeaxanthin could serve as a multifunctional food additive. Surprisingly, no zeaxanthin oleoresin can be obtained commercially on an industrial scale. Thus, several plants containing high amounts of total zeaxanthin were investigated for their potential use as sources for the production of oleoresins. Because carotenoid esters are known to be more stable than free carotenoids (*5*), research was focused on plants containing native zeaxanthin esters. Evidence for an enhanced stability of xanthophyll esters in a lipoxygenase assay has been presented by Biacs et al. (*6*). Lam and But (*7*) postulated that the natural occurrence of esterified zeaxanthin contributes to the stability of the medicinal effect of wolfberries. In addition to these technological aspects, esterified zeaxanthin may be more bioavailable than the free form, as indicated by a recent study with rodents (*8*). It is known from human studies with lutein that ingestion of esterified lutein together with a high dietary fat content enhances bioavailability (*9*). On the other hand, the deposition rate in the egg yolk of hens was higher after the feeding of free xanthophylls (e.g., ref *10*). However, the fact that the expensive saponification step, which is prone to high losses, can be omitted emphasizes the advantages of the use of zeaxanthin ester-rich plants for potential oleoresin production.

Zeaxanthin concentrations of several plants were specified by different authors (e.g., refs *11*−*13*) and can be found in official databases (e.g., ref *14*). Humphries and Khachik (*15*) reported the total concentration of *all-trans*- and several *cis*-isomers of zeaxanthin in fruit after saponification of the extracts. Knowledge of the native zeaxanthin ester pattern is comparatively scarce. Basic work has been done by Wingerath and coworkers (*16*), who studied zeaxanthin esters in tangerine juice using MALDI mass spectrometry. Besides β-cryptoxanthin esters, zeaxanthin monopalmitate as well as five zeaxanthin diesters (dicaprate, laurate/myristate, dimyristate, myristate/palmitate, and dipalmitate) were identified. However, the total amount of zeaxanthin in tangerine juice concentrate was rather low (1.3 mg/100 g). In the following, the current knowledge of zeaxanthin esters in plants under investigation is summarized.

Red Pepper (*Capsicum annuum* L.). Capsanthin esters occurring in red pepper have been studied extensively (e.g., refs *17*−*19*). Less information is available concerning native zeaxanthin esters, occurring as minor constituents. Biacs and Daood

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(*20*) reported the occurrence of two zeaxanthin diesters but did not identify them. In a recent study, we used liquid chromatography−atmospheric pressure chemical ionization mass spectrometry [LC-(APCI)MS] to identify carotenoid esters in paprika and found zeaxanthin diesters (laurate/myristate, dimyristate, myristate/palmitate, and dipalmitate) in fruits from local markets (*19*). However, individual zeaxanthin esters were not quantified.

Orange Pepper and Orange Thai Chilies (*Capsicum annuum* L.). Sommerburg et al. (*11*) investigated the carotenoid composition of several foods and reported that the highest molar percentage of zeaxanthin is found in orange pepper, followed by egg yolk, corn, and orange juice. However, the zeaxanthin ester pattern was not investigated. Davies et al. (*21*) described the carotenoid pattern of some color varieties of ornamental pepper after saponification. They found zeaxanthin to be one of the parent xanthophylls of orange and red fruits. However, the detailed zeaxanthin ester pattern is still unknown.

Wolfberries (*Lycium barbarum*). Wolfberries (Gou Qi Zi, *Fructus lycii*) are commonly used as traditional Chinese food and herbal medicine for the therapy of a number of disorders, including visual problems (*7*). The red color of the berries is mainly caused by zeaxanthin dipalmitate, the characteristic carotenoid in *Lycium* varieties (*22*, *23*). Direct analyses of native extracts of *Lycium* had been performed by Li et al. (*24*). Wolfberries have been used as a dietary source of zeaxanthin in several feeding studies (*8*, *25*). Recently, physiological effects of zeaxanthin dipalmitate from *Lycium chinense* were studied with rats as model organisms (*26*). *L. chinense* was further described by Khachik in a patent as a plant source for zeaxanthin from saponified extracts (*27*). However, total carotenoid esters have not been characterized using LC-(APCI)MS yet.

Sea Buckthorn Berries (*Hippophae rhamnoides* L.). Sea buckthorn fruits are rich in lipids, carotenoids, vitamin C, and several other micronutrients. Plenty of research has been done on the different constituents. As an example, Pintea et al. (*28*) studied intensively the lipid fraction of carotenolipoproteins. Novruzov (*29*) investigated the carotenoids of varieties grown in Azerbaidjan and found α- and β,β-carotene, lycopene, and zeaxanthin. One of the main native carotenoids has been described as zeaxanthin dipalmitate (*30*, *31*). Further zeaxanthin esters were not given in the literature. Although processes for the production of food dyes from sea buckthorn are published (*31*, *32*), the oleoresin is not available on the market. In the present study, two different varieties originating from Germany and Romania were investigated.

Chinese Lanterns (*Physalis alkekengi*). In contrast to the fruit of *Physalis peruviana* L. (cape gooseberry, goldenberry), *P. alkekengi* is mainly used as an ornamental plant. The bitter components present in their green parts may irritate the human intestinal tract; therefore, the berries are normally not consumed. The carotenoid composition of the fresh red cups was previously studied by Booth (*33*), who found zeaxanthin dipalmitate as the main component.

Persimmon (*Diospyros kaki*). Daood et al. (*34*) investigated the carotenoids, sugars, and organic acids from kaki. Because zeaxanthin diesters were not separated by chromatography, individual peak assignment could not be achieved. However, the area percentage corresponding to zeaxanthin diesters amounted to 16%, proving the high zeaxanthin concentration. Detailed data concerning zeaxanthin diesters are not available.

Zucchini Blossoms (*Cucurbita pepo* L.). The occurrence of zeaxanthin in flower petals has been known for a long time (*35*, *36*). The best documented example is marigold (*Tagetes erecta* L.), which is commercially used for the production of highly concentrated lutein oleoresins comprising 4−5% zeaxanthin (*37*). As far as we know, the carotenoid ester pattern of zucchini blossoms has not been studied yet. Thus, we investigated extracts of zucchini blossoms as one example of an intensely yellow flowering plant with potential use for zeaxanthin oleoresin production.

Maoka et al. (*38*) described the occurrence of free zeaxanthin in plant seeds, and we have additionally investigated an assortment of seeds [ginkgo (*Ginkgo biloba*), bittersweet (*Celastrus orbiculatus*), squash (*Cucurbita moschata*; butternut bush, calabaza), and corn (*Zea mays*)].

MATERIALS AND METHODS

Chemicals. Light petroleum (boiling fraction 40−60 °C), methanol, ethyl acetate, and acetone were purchased from Merck (Darmstadt, Germany); *tert*-butyl methyl ether, *n*-hexane, pyridine (99.8%, over molecular sieve), lauroyl chloride, myristoyl chloride, palmitoyl chloride, and stearoyl chloride (purity of all acyl chlorides = 99%) were from Sigma-Aldrich (Taufkirchen, Germany); and silica gel (0.063−0.2 mm) was from J. T. Baker (Griesheim, Germany). All solvents were distilled before use. High-purity water was prepared with a Milli-Q 185 Plus water purification system (Millipore, Eschborn, Germany). Zeaxanthin as reference substance was generously provided by Roche Vitamins (Basel, Switzerland).

Plant Samples. Fresh red and orange peppers (*Ca. annuum* L.), orange Thai chili (*Ca. annuum* L.), zucchini blossoms (*Cu. pepo* L.), persimmon (*D. kaki* L.), and kernels of fresh sweet corn (*Z. mays* L.), which were purchased attached to the cob, were obtained from retail shops. Heat-dried wolfberries (*L. barbarum* L.) originating from China were kindly provided by Rich Nature Nutraceutical Labs, Lynnwood, WA (http://www.richnature.com). Fresh Chinese lanterns (*P. alkekengi*) together with their red ripe husks (not dried) were obtained from florist R. Mergenthaler, Stuttgart, Germany. Freshly deep frozen sea buckthorn berries (*H. rhamnoides* L.) from Germany and Romania were kindly provided by Bayernwald Früchteverwertung GmbH, Hengersberg, Germany. Fresh seeds from ginkgo (*G. biloba* L.), a squash variety (butternut bush; *Cu. moschata*), and bittersweet (*Ce. orbiculatus* Thunb.) were obtained from B&T World Seeds, Olonzac, France (http://www.b-and-t-world-seeds.com).

Zeaxanthin Ester Synthesis. Zeaxanthin was isolated from orange peppers (*Ca. annuum* L.). Fruits were cut into small pieces, portions were homogenized with an Ultra Turrax T 25 (Janke & Kunkel, Staufen, Germany), and the homogenate was extracted immediately with methanol/ethyl acetate/light petroleum (1:1:1 v/v/v) until the solid residue was colorless. Combined extracts were saponified with methanolic KOH (30%, w/v) in diethyl ether overnight. Zeaxanthin was isolated using open column chromatography on silica gel. For elution, a mixture of light petroleum/acetone 94:6 (v/v) was applied. The concentration of the resulting solution was calculated on the basis of an ϵ value of 144.5×10^3 [L/(mol·cm)] in ethanol at 450 nm (*39*) and a molecular mass of 568.9 g/mol. Subsequently, zeaxanthin mono- and diesters were synthesized as described earlier (*40*). In brief, zeaxanthin (0.5−1 mg) was dissolved in pyridine (2 mL), and the respective acyl chloride (400 μL) was added dropwise over a period of 3 h. The reaction solution was transferred into a separatory funnel, washed with water twice, dried over anhydrous sodium sulfate, and evaporated to dryness. The residue was redissolved in ethanol (5 mL) and kept at −20 °C. To separate *all-trans*-monoesters from diesters as well as from *cis*-isomers, semipreparative HPLC on C30 material was employed using isocratic solvent mixtures consisting of methanol and TBME. For laurate and myristate esters, a mixture of 60:40 (v/v) was used; for palmitate and stearate esters, 45:55 (v/v) was applied. Directly after isolation, the combined fractions of multiple separations were evaporated to dryness under dim light, redissolved in ethanol, and stored at −20 °C.

Preparation of Samples. *Extraction of Carotenoids.* Portions were cut into small pieces and homogenized for 10−15 s. Samples of Chinese lanterns (fruits, 3 g; husks, 0.5 g), orange and red peppers (5 g each), orange Thai chili (2 g), sea buckthorn (10 g), wolfberry (2 g), persimmon (5 g), kernels of sweet corn (10 g), and zucchini blossoms

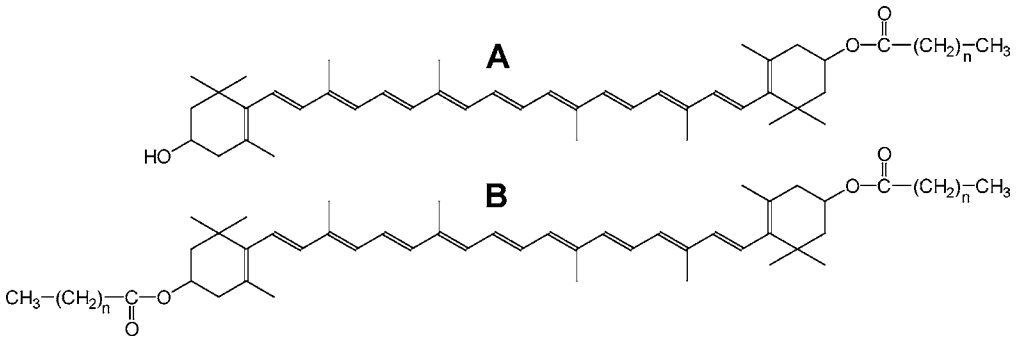

Figure 1. Structures of zeaxanthin (Z) monoesters (**A**) [Z laurate (**1**; $n = 10$), Z myristate (**2**; $n = 12$), Z palmitate (**3**; $n = 14$), Z stearate (**4**; $n = 16$)] and homogeneous zeaxanthin diesters (**B**) [Z dilaurate (**5**; $n = 10$), Z dimyristate (**6**; $n = 12$), Z dipalmitate (**7**; $n = 14$), Z distearate (**8**; $n = 16$)].

(10 g) were extracted three times with a mixture of methanol/ethyl acetate/light petroleum (1:1:1 v/v/v; 50 mL each). Seeds from ginkgo, butternut bush, and bittersweet were ground and extracted likewise (2 g each). The supernatants were collected and filtered, dried with anhydrous sodium sulfate, and evaporated to dryness. The residues were dissolved in TBME/methanol (1:1, v/v; 10 mL) and subjected to HPLC/DAD or LC-(APCI)MS analyses.

Saponification. For saponification, an aliquot of the crude extract (5−10 mL) was evaporated to dryness under reduced pressure and redissolved in diethyl ether (100 mL). After the addition of methanolic KOH (30%, w/v; 5 mL), the flask was stored overnight. The reaction mixture was transferred into a separatory funnel, washed with water three times, dried with anhydrous sodium sulfate, evaporated to dryness under reduced pressure, and subjected to HPLC analysis (final volume = 5−10 mL, according to the volume used for saponification).

High-Performance Liquid Chromatography (HPLC) and LC-(APCI)MS. *Apparatus and Conditions.* The HPLC consists of an HP 1100 modular system (Hewlett-Packard GmbH, Waldbronn, Germany) with a diode array detector (DAD, 450 nm). For separation, a 250 × 4.6 mm i.d., 5 μm, YMC analytical column (YMC Europe, Schermbeck, Germany) with C30-reversed phase material including a 10 × 4.0 mm i.d. precolumn was used and kept at 35 °C. LC-(APCI)MS was performed on an HP 1100 modular HPLC system, coupled to a Micromass (Manchester, U.K.) VG platform II quadrupole mass spectrometer. The MS parameters and the mobile phases have been detailed (*40*). Mass spectra of zeaxanthin esters were acquired with a scan range of *m*/*z* 400−1200. Data were processed with MassLynx 3.2 software.

Calibration. Calibration of zeaxanthin was performed in the range of 0.3−15 mg/L using zeaxanthin obtained from orange pepper (see above). A calibration curve was created by plotting the peak area (DAD, 450 nm) versus the concentration. For the calculation of zeaxanthin esters, the same graph was applied, specifying the zeaxanthin concentration in micromoles per liter. Concentrations of zeaxanthin esters were calculated according to the respective molecular masses.

Detection Limit of Zeaxanthin Diesters [LC-(APCI)MS]. For estimating the detection limit of zeaxanthin diesters, zeaxanthin dipalmitate was employed as a model compound. On the basis of the intensity of the fragment ion at *m*/*z* 1045.9 ($[M + H]^+$), a signal-to-noise ratio of 3:1, and an injection volume of 20 μL, the detection limit was estimated to be 0.4 μg/mL.

RESULTS AND DISCUSSION

Method Performance. To evaluate the applicability of the HPLC method for separation of zeaxanthin esters, eight zeaxanthin monoesters and homogeneous diesters (C12, C14, C16, and C18; **1**−**8**) were synthesized and separated on a C30 column. **Figure 1** illustrates the structures of these compounds. **Figure 2** shows the resulting chromatogram and demonstrates the applicability of the method for the analysis of zeaxanthin esters. Multiple analyses and the addition of synthesized standard compounds proved the stability of xanthophyll esters during extraction. Small peaks correspond to *cis*-isomers, incompletely

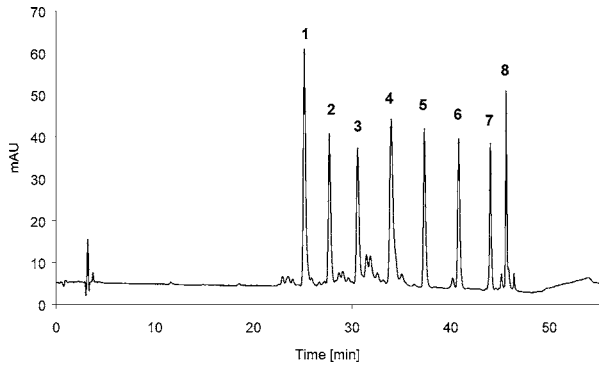

Figure 2. HPLC chromatogram (DAD, 450 nm) of a mixture of zeaxanthin mono- and diesters. Peak assignment (Z = zeaxanthin): Z laurate (**1**), Z myristate (**2**), Z palmitate (**3**), Z stearate (**4**), Z dilaurate (**5**), Z dimyristate (**6**), Z dipalmitate (**7**), and Z distearate (**8**).

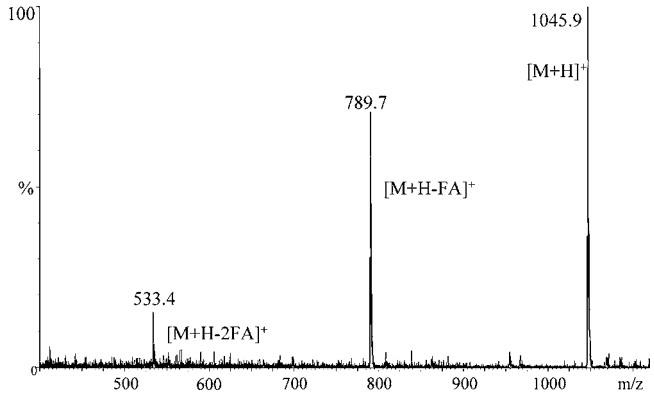

Figure 3. Mass spectrum (APCI, positive mode) of zeaxanthin dipalmitate (corresponding to **7**, **Figure 2**) as an example of the fragmentation pattern of a homogeneous zeaxanthin diester.

removed by semipreparative HPLC. For unequivocal identification, the standard mixture and all extracts obtained from plants were analyzed using LC-(APCI)MS in the positive mode. In each case, the quasimolecular ion ($[M + H]^+$) was clearly detectable. The fragmentation pathway was dominated by the loss of one ($[M + H - FA]^+$) or two ($[M + H - 2FA]^+$) fatty acids. In the case of zeaxanthin monoesters, this led to the formation of *m*/*z* 551.4, whereas diesters formed *m*/*z* 533.4, the "backbone" of zeaxanthin. An exemplary mass spectrum obtained from zeaxanthin dipalmitate (**7**) is given in **Figure 3**, and the data set used for identification of **1**−**8** is presented in **Table 1**. The described fragmentation pathway not only allows for the identification of homogeneous esters but also allows for the assignment of mixed esters, which likewise occur in the analyzed plant extracts. Thus, all carotenoid esters described

Table 1. LC-(APCI)MS Data of Synthesized Zeaxanthin Mono- and Diesters (FA = Fatty Acid; Z = Zeaxanthin)

	m/z [a]		
compound	$[M + H]^+$	$[M + H - FA]^+$	$[M + H - 2FA]^+$
C12 – Z (**1**)	751.6 (100%)	551.4 (43%)	
C14 – Z (**2**)	779.6 (100%)	551.4 (43%)	
C16 – Z (**3**)	807.7 (100%)	551.4 (45%)	
C18 – Z (**4**)	835.7 (100%)	551.4 (51%)	
C12/C12 – Z (**5**)	933.8 (100%)	733.6 (92%)	533.4 (36%)
C14/C14 – Z (**6**)	989.8 (100%)	761.6 (81%)	533.4 (21%)
C16/C16 – Z (**7**)	1045.9 (100%)	789.7 (61%)	533.4 (13%)
C18/C18 – Z (**8**)	1101.9 (97%)	817.7 (100%)	533.4 (35%)

[a] The fragment ions (exact masses) and signal intensities (in parentheses) are given. The assignment of **1**–**8** corresponds to the peak numbering in **Figure 2**.

in this study were identified via their molecular ions and the resulting fragment ions by LC-(APCI)MS.

Zeaxanthin Esters in Selected Plants. Table 2 summarizes the zeaxanthin ester patterns as well as the concentration of free (native) and total (after saponification) zeaxanthin of all plants investigated in this study. Additionally, the area percentages of zeaxanthin compared to total carotenoids, calculated from representative chromatograms of saponified samples, are given [Z/C (%)]. Although ϵ values of carotenoids and UV−vis maxima are different, this value allows for estimation of the proportion of zeaxanthin compared to total carotenoids present in the respective extract.

Zeaxanthin esters other than those listed in **Table 2** were not detected. In the case of monoesters, no derivatives comprising fatty acids with chain lengths shorter than C12 or longer than C18 were found. In none of the investigated plants were unsaturated fatty acids detectable in the zeaxanthin ester fraction. With the exception of zeaxanthin dilaurate (**5**), which was found only in orange Thai chili, and zeaxanthin distearate (**8**), which was present only in zucchini blossoms, other homogeneous diesters (**6**, **7**) were widely distributed. With the occurrence of mixed carotenoid esters, only fatty acids differing in two methylene groups were present (e.g., **12** and **13**, **Table 2**). This is in accordance with previous results of our group (*13*). With the exception of persimmon extracts, all samples contained zeaxanthin dipalmitate (**7**), although in varying concentrations. Thus, the predominant fatty acid used in the biosynthesis of zeaxanthin esters is palmitic acid.

The predominant xanthophyll of orange pepper was free zeaxanthin (**11**, **Figure 4A**), accompanied by small amounts of free lutein (**10**) and antheraxanthin (3,3′-dihydroxy-5,6-epoxy-β,β-carotene; **9**). Zeaxanthin mono- and diesters formed minor compounds. To our knowledge, this is the first presentation of the complete xanthophyll pattern of commonly consumed orange pepper. In contrast to orange pepper, orange Thai chilies and red pepper comprised only minute amounts of free zeaxanthin, whereas the major part occurred esterified (see **Table 2**). The xanthophyll ester pattern of Thai chilies was extremely complex. We did not focus in this research on identification of the remaining xanthophyll esters. The xanthophyll pattern of wolfberries differed significantly from those of all other plants under investigation. In accordance with previous results (*7*), zeaxanthin dipalmitate (**7**) was by far the predominant carotenoid (Z/C = 89%, **Table 2**). Furthermore, minor quantities of β-cryptoxanthin palmitate were present (data not shown). Two different varieties of sea buckthorn, originating from Germany and Romania, were examined. Only the Romanian variety additionally contained a mixed zeaxanthin ester (**13**) in minor amounts (**Table 2**). The zeaxanthin ester patterns of Chinese lanterns berries and husks were identical. **Figure 4B** shows a chromatogram obtained from a native husk extract. As in wolfberry extracts, zeaxanthin dipalmitate (**7**) was the main xanthophyll. Persimmon contained only minute amounts of total zeaxanthin (Z/C = 5%). As discussed earlier (*13*), the typical xanthophyll is β-cryptoxanthin. Extracts of zucchini blossoms showed the most complex xanthophyll pattern of all plants under investigation; zeaxanthin esters formed only minor components (Z/C = 19%). In fresh kernels of sweet corn as well as in seeds from ginkgo, zeaxanthin was present in the free form, yet no zeaxanthin esters were detected. However, zeaxanthin concentrations were low in both cases (kernels of sweet corn, 0.14 mg/100 g; ginkgo, 0.35 mg/100 g). Neither in bittersweet nor in butternut bush seeds, a variety of squash, was zeaxanthin present.

Occurrence of Antheraxanthin. In samples with high concentrations of zeaxanthin esters (e.g., wolfberry), acylated antheraxanthin was found as a minor component. Antheraxanthin esters were identified on the basis of the UV−vis spectrum, with maxima at 421/443/473 nm (*39*) and the corresponding ester masses. With the exception of orange and red pepper extracts, antheraxanthin dipalmitate (**15**) (*m/z* 1061.9 $[M + H]^+$; *m/z* 1043.9 $[M + H - H_2O]^+$; *m/z* 805.6 $[M + H - FA]^+$; *m/z* 549.4 $[M + H - 2FA]^+$) was unequivocally identified in all samples. As an example, the occurrence of **15** in an extract of Chinese lantern husks is shown in **Figure 4B**. Free antheraxanthin (**9**) was found in small amounts only in orange pepper extracts (**Figure 4A**). The occurrence of antheraxanthin in zeaxanthin-rich plants can be explained by the precursor function of zeaxanthin in the enzymatic pathway of the xanthophyll biosynthesis (*41*). It is noteworthy that the second epoxidation step, which leads to symmetric violaxanthin, obviously does not occur in the plants under investigation, as violaxanthin was not present in saponified extracts.

Concentration of Total Zeaxanthin. To determine the total amount of zeaxanthin and to evaluate a potential commercial use as zeaxanthin oleoresin sources, aliquots of all sample extracts were saponified using methanolic KOH. The total zeaxanthin concentrations ranged from 0.04 (persimmon) to 82.4 (wolfberry) mg/100 g (**Table 2**). The lower concentrations obtained in some cases after saponification (e.g., red pepper) may actually be a result of small losses or degradation reactions during this workup step or may be a consequence of the presence of *cis*-isomers of zeaxanthin esters, which were not taken into account. The high concentration of zeaxanthin in wolfberries is a result of the drying process, as the loss of water amounts to 86% (personal communication, Rich Nature Nutraceutical Labs). The original zeaxanthin concentration of fresh berries therefore was ∼12 mg/100 g. Surprisingly, the zeaxanthin concentration of the dried berries was ∼40 times that previously reported (average = 2.4 mg/100 g) (*7*). This huge discrepancy may be explained by a special drying process or by the variety used in this study.

To estimate the commercial use of plants as zeaxanthin source, the total lutein concentration of marigold flower petals is used for comparison. Piccaglia and Grandi (*42*) investigated 10 samples obtained from *T. erecta* and *T. patula* petals and found a mean lutein concentration of 170.6 mg/100 g (range = 16.8−569.9 mg/100 g). Neither of the plants under investigation showed a zeaxanthin concentration as high as that of marigold species. However, if an oleoresin composed of zeaxanthin as the main carotenoid is required, wolfberries (Z/C = 89%), Chinese lanterns (fruit, Z/C = 69%; husk, 58%), orange pepper (Z/C = 44%), and sea buckthorn (Z/C = 31−39%) may be of

Table 2. Concentrations of Zeaxanthin Ester as Well as Free and Total Zeaxanthin (Milligrams per 100 g) (after Saponification) in Different Plants (Z = Zeaxanthin)[a]

compound	orange pepper	red pepper	orange Thai chili	sea buckthorn (Germany)	sea buckthorn (Romania)
C 12 – Z (**1**)	0.25 ± 0.04				
C 14 – Z (**2**)	0.69 ± 0.05	6.43 ± 0.72			
C 16 – Z (**3**)	0.28 ± 0.03				
C12/C12 – Z (**5**)			0.28 ± 0.03		
C14/C14 – Z (**6**)	0.38 ± 0.03	5.42 ± 0.49	1.68 ± 0.14		
C16/C16 – Z (**7**)	0.18 ± 0.02	1.67 ± 0.11	0.31 ± 0.02	5.69 ± 0.06	4.02 ± 0.03
C12/C14 – Z (**12**)	0.24 ± 0.03		1.32 ± 0.10		
C14/C16 – Z (**13**)	0.35 ± 0.03	4.50 ± 0.39	1.00 ± 0.07		0.68 ± 0.03
free Z (**11**)	1.86 ± 0.25	1.26 ± 0.14	0.04 ± 0.01	0.25 ± 0.03	0.26 ± 0.01
total Z	3.03 ± 0.23	16.75 ± 2.04	2.57 ± 0.24	3.34 ± 0.06	2.34 ± 0.01
Z/C[b] (%)	44	15	16	39	31

compound	Chinese lantern (fruit)	Chinese lantern (husk)	wolfberry[c]	zucchini blossom	persimmon
C 16 – Z (**3**)	1.24 ± 0.13	10.07 ± 2.06			
C14/C14 – Z (**6**)				0.66 ± 0.07	0.05 ± 0.01
C16/C16 – Z (**7**)	7.81 ± 0.31	98.28 ± 14.90	160.95 ± 0.44	0.92 ± 0.12	
C 18/C18 – Z (**8**)				0.15 ± 0.01	
C12/C14 – Z (**12**)				0.81 ± 0.06	
C14/C16 – Z (**13**)	1.02 ± 0.02	3.91 ± 0.59		0.84 ± 0.09	0.007 ± 0.004
C16/C18 – Z				1.02 ± 0.13	
free Z (**11**)	0.12 ± 0.01	0.48 ± 0.14	1.22 ± 0.19	0.46 ± 0.07	0.001 ± 0.001
total Z	6.39 ± 0.21	54.89 ± 7.23	82.4 ± 2.19	3.27 ± 0.42	0.044 ± 0.003
Z/C[b] (%)	69	58	89	19	5

[a] Quantitative amounts ($n = 3$) were calculated on the basis of the respective molecular mass. Numbers of the compounds correspond to **Figures 2** and **4**. [b] Z/C [%] = area zeaxanthin × 100/area total carotenoids (detected at 450 nm, DAD). [c] Dried berries.

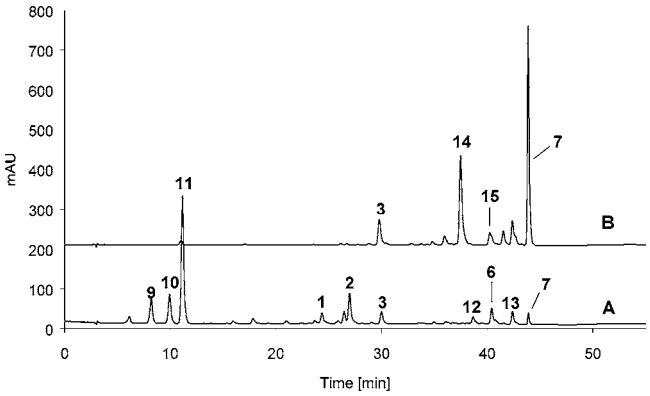

Figure 4. HPLC chromatograms (DAD, 450 nm) of an extract of orange pepper (*Ca. annuum* L.) (**A**) and Chinese lantern husks (*P. alkekengi*) (**B**). Peak assignment (Z = zeaxanthin): Z laurate (**1**), Z myristate (**2**), Z palmitate (**3**), Z dimyristate (**6**), Z dipalmitate (**7**), antheraxanthin (**9**), lutein (**10**), Z (**11**), Z laurate/myristate (**12**), Z myristate/palmitate (**13**), β-cryptoxanthin palmitate (**14**), and antheraxanthin dipalmitate (**15**).

technological importance (**Table 2**). In particular, dried wolfberries (82.4 mg/100 g of dried berries) are an interesting source for zeaxanthin dipalmitate, especially in terms of the transport of raw material and the simplified extraction process, as only a milling step is needed before solvent extraction can be performed. A rather uncommon, but nevertheless valuable, source for zeaxanthin dipalmitate are the husks of Chinese lanterns as they are normally discarded. With contents of 54.9 mg/100 g of sepals, these could be of interest as raw material for the extraction of zeaxanthin dipalmitate. However, outstanding xanthophyll concentrations of *T. erecta* oleoresins cannot be achieved using any of the plants investigated.

Zeaxanthin esters can be isolated from a variety of different fruits of which some could be of technological interest for the extraction of oleoresins. Certainly, the solvent system used in this study has to be replaced by an appropriate solvent (e.g., *n*-hexane) according to law. Further on, extraction yields have to be optimized. As consumers are sensitive to food additives in general, products with a "natural dyed" label may attract interest. Thus, it is anticipated that the commercial use of zeaxanthin ester oleoresins obtained from several plants mentioned in this study will soon be realized.

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