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Carotenoids and Carotenoid Esters in Potatoes (*Solanum tuberosum* L.): New Insights into an Ancient Vegetable

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The carotenoid pattern of four yellow- and four white-fleshed potato cultivars (*Solanum tuberosum* L.), common on the German market, was investigated using HPLC and LC(APCI)−MS for identification and quantification of carotenoids. In each case, the carotenoid pattern was dominated by violaxanthin, antheraxanthin, lutein, and zeaxanthin, which were present in different ratios, whereas neoxanthin, β-cryptoxanthin, and β,β-carotene generally are only minor constituents. In contrast to literature data, antheraxanthin was found to be the only carotenoid epoxide present in native extracts. The total concentration of the four main carotenoids reached 175 μg/100 g, whereas the sum of carotenoid esters accounted for 41−131 μg/100 g. Therefore, carotenoid esters are regarded as quantitatively significant compounds in potatoes. For LC(APCI)−MS analyses of carotenoid esters, a two-stage cleanup procedure was developed, involving column chromatography on silica gel and enzymatic cleavage of residual triacylglycerides by lipases. This facilitated the direct identification of several potato carotenoid esters without previous isolation of the compounds. Although the unequivocal identification of all parent carotenoids was not possible, the cleanup procedure proved to be highly efficient for LC(APCI)−MS analyses of very low amounts of carotenoid esters.

KEYWORDS: Potato; *Solanum tuberosum*; carotenoid ester; carotenoid epoxide

INTRODUCTION

Potatoes (*Solanum tuberosum* L.) are grown in many countries with temperate climate all over the world. Today, potatoes are among the most commonly consumed vegetables and serve as a major source of vitamin C. Carotenoids, especially β,β-carotene, represent minor constituents. Hence, potatoes are not an important source of provitamin A (*1*). However, lutein, one of the main carotenoids, has attracted interest since high serum levels are correlated with a reduced risk for humans to be afflicted with age-related macular degeneration (AMD) (*2*, *3*). Since typical green and leafy vegetables (e.g. spinach, broccoli), the major dietary sources of lutein, are not frequently consumed, potatoes serve as additional source of lutein in the diet (*4*). Concerning the health benefits of other typical potato carotenoids (e.g. violaxanthin, neoxanthin, antheraxanthin), only little is known. With respect to the bioavailability of epoxy-carotenoids, a recently published study ascertained that epoxy-xanthophylls (e.g. violaxanthin) are not absorbed by humans (*5*). However, due to their widespread acceptability, potatoes are generally expected to be a valuable source of carotenoids in the diet, especially if intensely colored clones are selected (*4*) or modern breeding techniques are applied (*6*). The contribution of potatoes as a plant source of carotenoids was demonstrated by a study conducted in 1996 in Spain: potato belonged—together with eight other vegetables and five fruits—to the foods accounting for more than 96% of the intake of the major carotenoids in human serum, both on a yearly and on a seasonal basis (*7*).

With respect to potato carotenoids, qualitative as well as quantitative data in the literature are extremely confusing. Kasim, one of the first researchers who reported on these micronutrients in potatoes, used paper chromatography and visible spectroscopy for identification of several carotenoids in saponified potato extracts (*8*, *9*). Besides lutein and violaxanthin, he detected lutein-5,6-epoxide and β,β-carotene diepoxide, whereas zeaxanthin was not found. Pendlington et al. investigated the carotenoid pattern using alumina columns to separate carotenoids and tentatively identified eight carotenoids (e.g. β,β-carotene, lutein) as most abundant in all cultivars (*10*). Müller reported a carotenoid screening of various vegetables, applying chemical saponification before HPLC analyses (*11*). According to these results, violaxanthin is the main potato carotenoid, followed by lutein, antheraxanthin, and others. Iwanzik et al. (*1*) and Stute et al. (*12*) compared the carotenoid content of 13 potato cultivars from the same habitat. The main carotenoid was again violaxanthin, followed by lutein, lutein-5,6-epoxide, neoxanthin, and neoxanthin a; zeaxanthin was not identified. The first to mention the occurrence of carotenoid esters in amyloplast envelope membranes, the site of localization of potato carotenoids, were Fishwick and Wright (*13*). Although they did not attempt to identify the fatty acid component of the carotenoid esters, they reported 17−22% of the carotenoids to

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be present in the esterified form. As main parent carotenoids, antheraxanthin, neoxanthin, lutein/zeaxanthin, and β-cryptoxanthin were characterized. Basic work in the field of potato carotenoid ester analysis has also been carried out by Tevini and co-workers (*14*, *15*). They separated mono- and diester fractions and tentatively identified carotenoid esters (e.g. lutein-5,6-epoxide mono- and diesters) by comparing their visible spectra. Unfortunately, the individual fatty acid moiety was not investigated. However, Tevini's group analyzed the fatty acid pattern of the total carotenoid ester fraction and found palmitic, linoleic, linolenic, and stearic acid to be the main components. Recently, Lu et al. presented the carotenoid content of 11 diploid potato clones and two tetraploid cultivars (*4*). Although they used no saponification step, the existence of carotenoid esters was not mentioned, probably leading to an underestimation of the respective carotenoids. The same is true for a study dealing with orange-fleshed potatoes, establishing that the color originates from large amounts of zeaxanthin (*16*). Since saponification was used prior to analysis, no information about the occurrence of zeaxanthin esters was obtained.

These inconsistent data may be traced back, on one hand, to unsuitable isolation and identification techniques and, on the other, to degradation or rearrangement reactions during the workup procedure. The lack of data concerning zeaxanthin may reflect the fact that, so far, it has been difficult to separate lutein from zeaxanthin. However, until now, little data were available about the carotenoid content of potato, especially with respect to carotenoid esters, which have not been studied using modern analytical techniques in the past 20 years. Therefore, we investigated the carotenoid content of four yellow- (Granola, Solara, Marabel, and Nicola) and four white-fleshed (Siglinde, Sante, Bintje, and Cilena) cultivars, common on the German market. For identification of free and esterified carotenoids, LC-(APCI)−MS analyses in the positive mode were applied. The carotenoid pattern of sweet potato (*Ipomoea batatas* (L.) Lam.), common in tropical areas, is dominated by β,β-carotene and β,β-carotene mono- and diepoxides and was not investigated in this study (*17*).

MATERIALS AND METHODS

Chemicals. Light petroleum (boiling fraction 40−60 °C), diethyl ether, methanol, butylated hydroxytoluene (BHT), ethyl acetate, and calcium carbonate were purchased from Merck (Darmstadt, Germany), and 3-chloroperoxybenzoic acid (~70%), β,β-carotene, sodium sulfate (anhydrous), sodium carbonate, potassium hydroxide, and methyl *tert*-butyl ether (MTBE) were purchased from Sigma-Aldrich GmbH (Taufkirchen, Germany). All solvents were distilled before use. High-purity water was prepared with a Milli-Q 185 Plus water purification system (Millipore, Eschborn, Germany). β-Cryptoxanthin was generously provided by Hoffmann-La Roche (Basel, Switzerland). Neoxanthin (c = 1.137 mg/L) and violaxanthin (c = 0.637 mg/L; certified standard solutions in ethanol) were purchased from DHI-Water&Environment (Hørsholm, Denmark). Novozym 868 L (6 kilo lipase units (KLU)/g) and Lipolase (100 KLU/g) were kindly supplied by Novo Nordisk Biotechnology GmbH (Mainz, Germany). Fresh orange peppers (for extraction of zeaxanthin) were obtained from the local market, and marigold oleoresin (for extraction of lutein) was supplied by Euram Food GmbH (Stuttgart, Germany). An extract of a genetically transformed *Escherichia coli* strain (containing the two plasmids pACCAR25delcrtX and pBBR-zep), producing antheraxanthin and zeaxanthin in high yields, was kindly supplied by G. Sandmann, Botanical Institute, J.W. Goethe University (Frankfurt, Germany).

Preparation of Samples. Potato tubers, obtained from local markets in the autumn of 2001 (grade of goods: I), were peeled and cut into small cubes. An aliquot (150 g; three to five tubers) was mixed with solid calcium carbonate (5 g) and homogenized for 2 min by an Ultra Turrax T 25 (Janke & Kunkel). Aliquots of 30−50 g were immediately extracted with light petroleum/methanol/ethyl acetate (1:1:1 v/v/v; 4 × 100 mL) until the extracts were colorless (total volume: 1.5 L). The upper layer was separated; the lower layer was shaken with water (100 mL) for phase separation and the upper layer combined with the first extract (total volume: 800 mL). The organic extract was dried with anhydrous sodium sulfate (30 g), filtered through a folded filter, and evaporated to dryness in a vacuum at 30 °C, and the residue was dissolved in light petroleum (5 mL). For determination of the native carotenoid pattern, an aliquot (1 mL) was evaporated in a gentle stream of nitrogen, and the dry residue was redissolved in MTBE/methanol/BHT (1:1:0.01 v/v/w; 1 mL) and subjected to HPLC analysis. All procedures were done under dim light.

For saponification, another aliquot of the light petroleum solution (3 mL) was transferred to a flat-bottomed flask, dried in a stream of nitrogen, dissolved in diethyl ether (100 mL), and saponified at room temperature overnight with methanolic potassium hydroxide (30% w/v; 1 mL). For complete removal of alkali, the solution was washed twice with distilled water (100 mL each). The organic layer was dried over anhydrous sodium sulfate (20 g), filtered through a folded filter, and evaporated to dryness, and the residue was redissolved in MTBE/methanol/BHT (1:1:0.01 v/v/w; 2 mL) and subjected to HPLC analysis. For quantitative determination, peak areas of carotenoids in the sample chromatograms were correlated with the concentrations on the basis of calibration curves, established as detailed earlier (*18*). UV spectra were usually recorded in the HPLC eluent by DAD. The color of the flesh (white/yellow) was evaluated by visual examination of freshly sliced tubers.

Synthesis of Lutein, Zeaxanthin, and β,β-Carotene Epoxides. Carotenoid epoxides were synthesized on the basis of a method described by Barua (*19*, *20*) and Barua and Olson (5), comprising the chromatographic separation of the products. Lutein and zeaxanthin (1 mg each), obtained from saponified marigold oleoresin (*Tagetes erecta* L.) or saponified extracts of orange pepper (*Capsicum annuum* L.), and β,β-carotene, respectively, were dissolved in diethyl ether (10 mL). After a solution of 3-chloroperoxybenzoic acid (5 mg/10 mL) was added, each mixture was stirred at room temperature in the dark for 16 h. After the mixture was washed once with sodium carbonate solution (0.1 M, 50 mL) and twice with water (50 mL each), the ether layer was separated, dried over anhydrous sodium sulfate (2 g), filtered, and evaporated to dryness in a rotary evaporator at 30 °C. Negative starch iodine reaction in the washing water indicated complete removal of peroxides. The residue was dissolved in MTBE/methanol/BHT (1:1:0.01 v/v/w; 5 mL), membrane filtered, and subjected to HPLC and LC(APCI)−MS analyses.

Cleanup of Native Extracts for LC(APCI)−MS Analysis. Four extracts obtained as described above were pooled and evaporated to dryness in a rotary evaporator, and the residue was dissolved in light petroleum (5 mL) and subjected to column chromatography on silica gel as described earlier (*21*). Carotenoid esters were eluted using light petroleum containing different amounts of acetone (2, 5, and 10% (v/v)). Thus, polar carotenoids remained on the column and were discarded. For further purification, an enzymatic cleanup step was applied (*18*). In brief, samples cleaned by column chromatography were transferred into a sealable glass tube, the solvent was evaporated in a gentle stream of nitrogen, and 0.1 M phosphate buffer pH 7.4 (10 mL) was added. Bile salts and sodium/calcium ions were used as activators. For triacylglyceride hydrolysis, an aliquot (200 μL) of a freshly prepared lipase suspension containing Novozym 868 L (20 μL) and Lipolase (20 μL) in 5 mM calcium chloride solution (1 mL) was added, and the mixture was incubated at 37 °C for 2 h. Afterwards, the carotenoids were extracted using methanol/ethyl acetate/light petroleum (1:1:1 v/v/v; 3 × 20 mL), processed as described above, and subjected to LC(APCI)−MS analysis.

Recovery Studies. For recovery experiments, a solution of β-cryptoxanthin in MTBE/methanol (1:1 v/v) was prepared (100 μg/mL) and stored at −18 °C. Aliquots (1 mL) were added to minced potatoes (variety Granola; 150 g each), low in native β-cryptoxanthin content, and immediately subjected to the workup procedure. Thus, the resulting β-cryptoxanthin content of fortified samples (67 μg/100 g) lies in the typical range of the native concentration of a carotenoid in potato

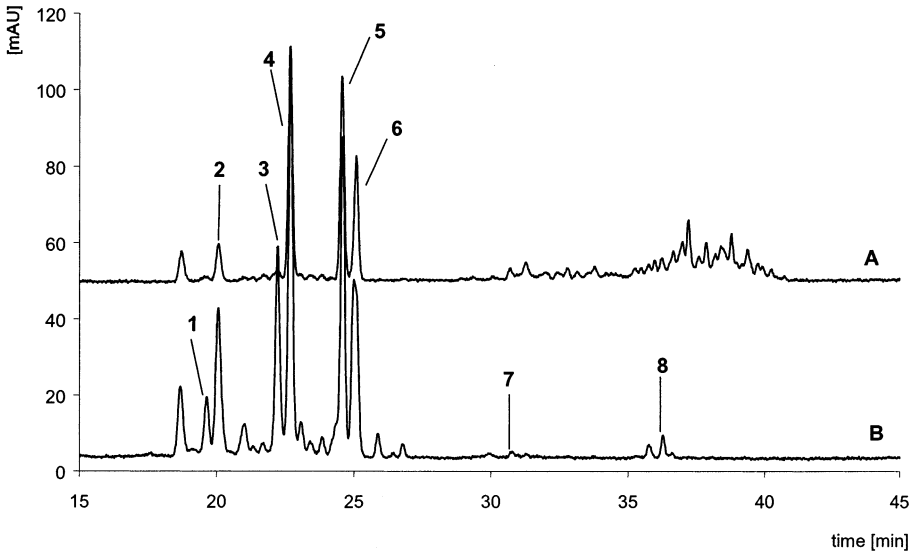

Figure 1. Typical chromatograms (DAD, 450 nm; retention time window 15–45 min) of a native (A) and a saponified (B) white-fleshed potato extract (cultivar Bintje). Peak identification: **1**, neoxanthin; **2**, violaxanthin; **3**, unidentified; **4**, antheraxanthin; **5**, lutein; **6**, zeaxanthin; **7**, β-cryptoxanthin; **8**, β,β-carotene. Due to the workup procedure, the saponified sample (B) is concentrated by a factor of 1.5.

samples. Recoveries were calculated according to AOAC methods (*22*) as follows: % Recovery = $[(c_A - c_U)/c_B] \times 100$, where c_A is the concentration of β-cryptoxanthin measured in the fortified sample, c_U is the concentration of β-cryptoxanthin measured in the unfortified sample, and c_B is the concentration of β-cryptoxanthin added in fortified samples. The following recoveries were obtained ($n = 4$): without saponification, 83.4 ± 2.7%; after saponification, 81.5 ± 4.8%. These values are regarded as acceptable since they lie between a range of 80−110%, which is accepted for concentrations of approximately 1 mg/kg by regulatory agencies in the United States (*23*).

HPLC and LC(APCI)−MS Analyses. Analyses were performed with a modular HPLC system HP1050 (Hewlett-Packard, Waldbronn, Germany) and a column thermoregulator (Mistral, Spark, The Netherlands). A 250 mm × 4.6 mm i.d., 5 μm YMC C30 reverse-phase column from YMC Europe (Schermbeck, Germany), equipped with a 10 mm × 4.6 mm i.d., 5 μm Nucleosil C18 precolumn (Bischoff, Leonberg, Germany), was used and kept at 35 °C. Two mobile-phase systems were used. For HPLC analyses, system I was used: mobile phases methanol/water/triethylamine (90:10:0.1 v/v/v) (A) and methanol/MTBE/water/triethylamine (6:90:4:0.1 v/v/v/v) (B); gradient (min/%A) 0/99, 8/99, 45/0, 50/99, and 55/99. For analysis of carotenoid esters by LC(APCI)−MS, system II was applied: mobile phases methanol/MTBE/water/triethylamine [81:15:4:0.1 v/v/v/v (A) and 6:90:4:0.1 v/v/v/v (B)]; gradient (min/%A) 0/99, 39/44, 45/0, 50/99, and 55/99. The flow rate was 1 mL/min, the injection volume 20 μL, and the detection wavelength (DAD) 450 nm in both systems. LC(APCI)−MS was run on an HP1100 HPLC system, coupled to a Micromass (Manchester, UK) VG platform II quadrupole mass spectrometer equipped with an $APCI^+$ interface; the MS parameters have been previously reported (*24*).

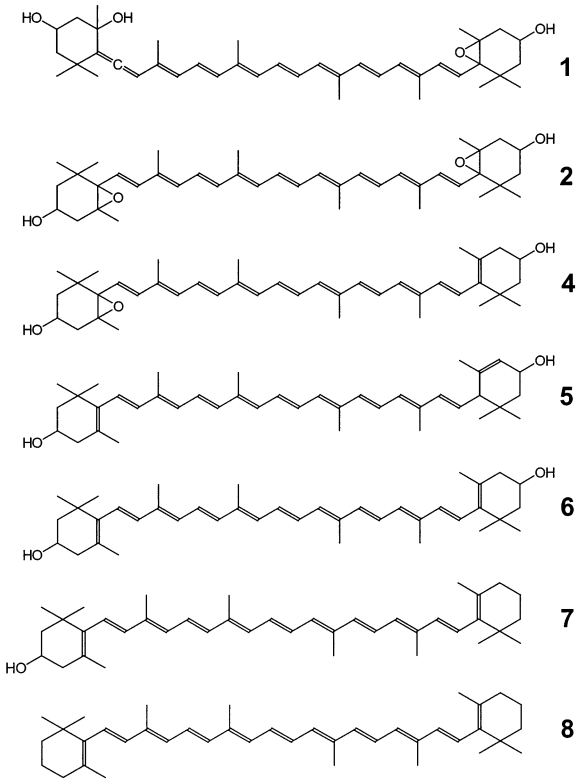

Figure 2. Structures of the most important carotenoids found in potatoes. The numbering corresponds to the carotenoids indicated in **Figure 1**.

RESULTS AND DISCUSSION

Qualitative and Quantitative Results. Since violaxanthin, a carotenoid which is rather susceptible to acidic conditions, may occur in potatoes, we mixed the potato paste immediately after homogenization with solid calcium carbonate to raise the pH of the mixture. As preliminary tests showed, this procedure, suggested previously by Lu et al. (*4*), enabled us to determine native violaxanthin without the risk of destruction in potato extracts. We investigated the carotenoid content of four yellow- (Granola, Solara, Marabel, and Nicola) and four white-fleshed (Siglinde, Sante, Bintje, and Cilena) cultivars, common in Germany. The carotenoid pattern of native extracts, shown in **Figure 1A**, was dominated by violaxanthin (**2**), antheraxanthin (**4**), lutein (**5**), and zeaxanthin (**6**) in different ratios, followed by β-cryptoxanthin (**7**) and β,β-carotene (**8**), being only byproducts; neoxanthin (**1**), a carotenoid eluting just in front of violaxanthin, represented in most cases another minor constituent (for structures of the carotenoids, see **Figure 2**). The identification of **1**, **2**, **5**, **6**, **7**, and **8** was confirmed by comparison of their retention times and UV spectra with those of commercial authentic reference material; UV data were consistent with those reported (*25*), as well as the fragmentation pattern in LC(APCI)−MS analyses. In principle, these results are in accordance with those of Müller (*11*), who found violaxanthin to be the main carotenoid (180 μg/100 g), followed by mutato-/

Table 1. Concentration of Carotenoids [μg/100 g Fresh Weight] of Different Potato Cultivars (Edible Part), Concentration of Carotenoid Esters, Calculated as Lutein Dimyristate, and Color of the Flesh

		carotenoids [μg/100 g][a]						
cultivar	color	violaxanthin	antheraxanthin[b]	lutein	zeaxanthin	β-cryptoxanthin	*all-trans*-β-carotin	diesters[c]
Granola	yellow	66.3 ± 13.6	28.8 ± 5.4	26.7 ± 4.5	8.5 ± 1.3	4.9 ± 1.0	1.8 ± 0.4	56.0 ± 5.1
		70.6 ± 11.2	33.5 ± 2.1	27.3 ± 4.4	8.6 ± 2.1	4.8 ± 1.5	2.2 ± 0.8	
Solara	yellow	15.0 ± 3.3	27.9 ± 2.8	27.0 ± 2.6	20.7 ± 4.0	3.9 ± 0.6	3.4 ± 0.6	96.2 ± 4.6
		23.8 ± 2.5	30.6 ± 2.3	32.0 ± 3.8	23.3 ± 1.7	5.8 ± 0.4	3.6 ± 0.8	
Marabel	yellow	7.8 ± 1.6	48.0 ± 7.0	40.5 ± 5.0	78.3 ± 8.9	4.8 ± 0.7	2.2 ± 0.5	131.2 ± 2.8
		40.6 ± 7.0	66.1 ± 4.1	48.9 ± 4.9	107.4 ± 5.5	2.2 ± 0.6	2.3 ± 0.5	
Nicola	yellow	9.1 ± 1.8	21.6 ± 3.5	16.8 ± 1.3	10.0 ± 1.6	2.5 ± 0.5	2.0 ± 0.6	116.8 ± 7.2
		27.4 ± 2.3	41.8 ± 4.6	24.4 ± 1.8	33.6 ± 2.8	1.1 ± 0.1	1.6 ± 0.6	
Siglinde[d]	white	12.8 ± 2.0	11.8 ± 1.3	20.7 ± 2.0	6.1 ± 0.8	2.4 ± 0.7	3.4 ± 0.3	100.0 ± 7.1
		24.5 ± 2.1	17.8 ± 0.9	23.8 ± 2.6	10.8 ± 0.9	1.8 ± 0.4	2.6 ± 0.9	
Sante	white	7.7 ± 1.7	7.7 ± 2.3	20.8 ± 1.7	5.6 ± 0.6	2.4 ± 0.5	1.6 ± 0.6	62.6 ± 9.8
		19.7 ± 2.5	12.2 ± 2.0	22.1 ± 0.6	6.4 ± 1.1	2.5 ± 0.8	1.4 ± 0.6	
Bintje	white	3.3 ± 0.7	20.9 ± 3.8	21.0 ± 2.9	16.9 ± 5.7	0.8 ± 0.2	1.0 ± 0.2	40.7 ± 4.8
		6.0 ± 0.2	26.2 ± 3.1	24.2 ± 2.3	19.6 ± 1.7	0.3 ± 0.1	1.0 ± 0.1	
Cilena	white	4.9 ± 1.2	9.8 ± 2.0	20.6 ± 4.8	2.7 ± 0.8	2.3 ± 0.3	2.4 ± 0.2	94.3 ± 8.2
		20.3 ± 2.4	23.7 ± 2.1	29.5 ± 1.2	16.7 ± 3.6	0.5 ± 0.2	2.2 ± 0.2	

[a] The given values represent means ± standard deviations of five independent determinations (based on the workup of different tubers). The first line lists the concentration of free carotenoids. The second line gives the carotenoid content after saponification. [b] Antheraxanthin was calculated as zeaxanthin. [c] Calculated as lutein dimyristate. [d] Exact cultivar: Siglinde Hochmoor.

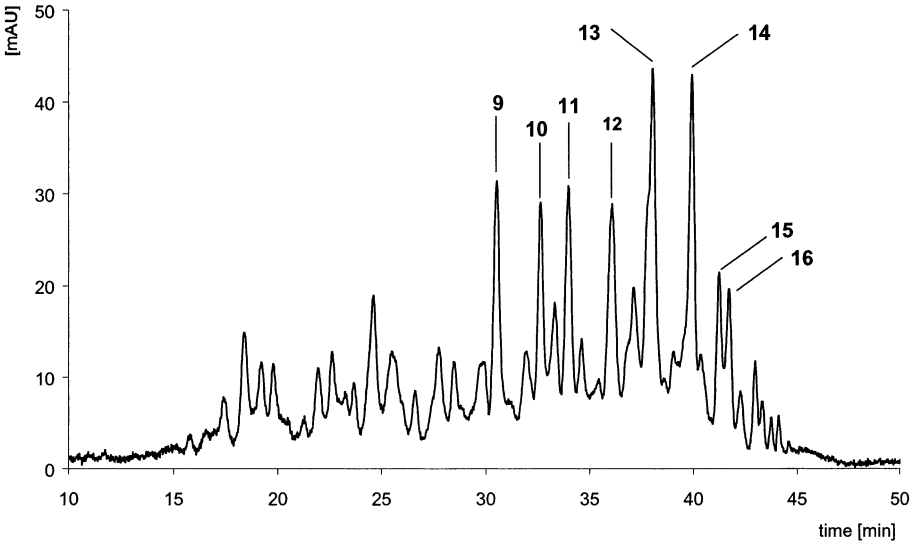

Figure 3. HPLC chromatogram (DAD, 450 nm; retention time window 10–50 min) of a native potato extract (cultivar Nicola) after application of a two-stage cleanup procedure (for conditions, see Materials and Methods; for precise peak assignment, see **Table 2**).

antheraxanthin, lutein, zeaxanthin, and neoxanthin; β,β-carotene and β-cryptoxanthin are minor components. Additionally, carotenoid esters were found in varying amounts in each cultivar. Analytical proof is provided by the disappearance of the respective peaks after applying alkaline saponification. As expected, the carotenoid pattern of the resulting saponified samples was more complex (**Figure 1B**) than that of native samples: on one hand, amounts of xanthophylls already present in native samples increased, while on the other hand, several new minor carotenoids appeared in the more polar region. In all cases, a more or less intense compound (**3**; *m*/*z* 601 $[M + H]^+$; λ_{max} 414, 436, 466 nm) appeared in front of antheraxanthin (**4**). Moreover, the mass spectra of the peak at the retention time of zeaxanthin (**6**) showed in some cases (e.g. Nicola, Marabel) an additional but less intense ion signal (*m*/*z* 585). This signal does definitely not belong to the spectrum of the pure compound (**6**), pointing to a coeluting carotenoid. Attempts to separate this substance by variation of the gradient failed. However, lutein-5,6-epoxide (see below) can be ruled out as responsible for the two phenomena mentioned above. Unfortunately, currently an unequivocal identification of both naturally esterified carotenoids cannot be provided.

On the basis of the carotenoid pattern observed, it was difficult to differentiate between white- and yellow-fleshed cultivars. In our investigations, neither white- nor yellow-fleshed potatoes showed typical carotenoids as being responsible for their inherent color. For example, the main carotenoid in yellow-fleshed cultivars can be antheraxanthin (Solara, Nicola) or zeaxanthin (Marabel), whereas in Granola violaxanthin dominated the spectrum. The total concentration of carotenoids seems to be a good tool to differentiate between the two groups: the sum of the four main carotenoids determined in native samples of yellow-fleshed cultivars was in the range of 58−175 μg/100 g, whereas that of white-fleshed cultivars was between 38 and 62 μg/100 g, respectively. Iwanzik et al. (*1*) reported concentrations in the range of 171−343 μg/100 g (intensively yellow) and 27−74 μg/100 g (white), verifying the overall tendency observed in our study. Carotenoid esters, calculated in native samples as lutein dimyristate, accounted for between 41 μg/

Table 2. LC(APCI)–MS Data and Identification of Carotenoid Diesters Originating from the Cultivar Nicola

	m/z (relative intensity)[a]							
	9	**10**	**11**	**12**	**13**	**14**	**15**	**16**
$[M + H]^+$	1021	1049	1021	1049	1005		989	
	(15)	(28)	(100)	(100)	(100)		(100)	
$[M + H - H_2O]^+$	1003	1031	1003	1031	987			
	(67)	(91)	(46)	(54)	(17)			
$[M + H - H_2O - FA_1]^+$	775	803/775	775	803	759			
	(100)	(100/95)	(10)	(7)	(26)			
$[M + H - H_2O - FA_1 - FA_2]^+$	547	547	547	547	531			
	(30)	(32)	(5)	(5)	(13)			
$[M + H - FA_1]^+$	793	821/793	793	821/793	777	761	761	789/761
	(12)	(16/22)	(10)	(6/5)	(28)	(100)	(47)	(100/67)
$[M + H - FA_1 - FA_2]^+$	565	565	565	565	549	533	533	533
	(5)	(15)	(10)	(12)	(10)	(38)	(5)	(57)
λ_{max} (DAD)	418/	418/	402/	402/	428/	417/	420/	417/
	438/	438/	422/	422/	454	444/	452/	444/
	466	466	446	446		471	480	471
type of diester	C14:0	C14:0	C14:0	C14:0	C14:0	C14:0	C14:0	C14:0
	C14:0	C16:0	C14:0	C16:0	C14:0	C14:0	C14:0	C16:0
parent carotenoid	violaxanthin	violaxanthin	*b*	*b*	*b*	lutein	zeaxanthin	lutein

[a] The numbers of the compounds **9**–**16** correspond to the peak numbering in **Figure 3**. [b] The parent carotenoid was not unequivocally identified.

100 g (Bintje) and 131 μg/100 g (Marabel) and, therefore, are quantitatively significant compounds in the potato carotenoid spectrum.

The quantitative results of all cultivars investigated are presented in **Table 1**. The values given represent means of five independent determinations. Thus, the standard deviations reflect the native variation of the carotenoid content of the samples, obtained from three to five tubers each. The wide variation observed in some cases (especially if considering very low concentration carotenoids, such as β-cryptoxanthin and β,β-carotene) may be the result of this small number of tubers used for a single determination, the stage of ripeness, and the weather conditions during growth. The results of recovery studies (native samples, 83.4 $\pm$ 2.7%; after saponification, 81.5 $\pm$ 4.8%) demonstrate that there is only minor loss of major carotenoids during the extraction process or the saponification procedure, pointing out that the standard deviations are due to differences in the carotenoid content of the respective samples. However, for cases of extremely low concentrated carotenoids (β-cryptoxanthin and β-carotene; see **Table 1**), the lower concentrations obtained in some cases after saponification may actually be a result of losses or degradation reactions during this workup step.

Epoxides in Potato. Special attention was paid to the question of whether lutein, zeaxanthin, or β,β-carotene epoxides are present in native extracts. The epoxidation/de-epoxidation reaction sequence of violaxanthin to zeaxanthin via antheraxanthin is known as the violaxanthin cycle. Thus, antheraxanthin represents an intermediate in both directions and can be expected in plants showing the respective enzymatic activity, e.g. potatoes and tobacco (*26*). To confirm this theory, we independently synthesized the epoxides of lutein, zeaxanthin, and β,β-carotene and investigated the resulting solutions using LC(APCI)−MS. The respective chromatograms of lutein and zeaxanthin epoxides showed four peaks each. MS analyses proved two peaks to correspond to diepoxy xanthophylls, the others representing monoepoxides. In the case of lutein monoepoxides, the quasi-molecular ions (*m/z* 585 $[M + H]^+$) were of minor intensity (36%), whereas the fragment ions generated by loss of water (*m/z* 567) formed the base peak. This water elimination is typical for lutein (*m/z* 569 $[M + H]^+$ (8%), 551 (100%); λ_{max} 417, 444, 471 nm), since the allylic cation generated at the C-3′ position is stabilized by resonance (*21*). The UV spectra of lutein monoepoxides are characterized by three maxima (λ_{max} 416, 440, 470 nm), which are consistent with previous data (*25*). The twin peaks are probably due to diastereoisomers formed during epoxidation; the diastereoisomeric profile was not studied in detail. In the case of zeaxanthin monoepoxides, the quasi-molecular ion (*m/z* 585 $[M + H]^+$) formed the base peak, whereas the fragment ion produced by loss of water was of low intensity (13%); this is in line with the behavior of zeaxanthin (*m/z* 569 $[M + H]^+$ (100%), 551 (7%); λ_{max} 420, 452, 480 nm). As expected, the spectroscopic characteristics (λ_{max} 419, 446, 474 nm) show a hypsochromic shift compared to zeaxanthin. Since **4** (**Figure 1**) showed the same characteristics as zeaxanthin monoepoxide, also designated antheraxanthin (3,3′-dihydroxy-5,6-epoxy-β,β-carotene), it can be concluded that this peak corresponds to this compound. For verification, we used an extract of a genetically modified *E. coli* strain, producing antheraxanthin in addition to zeaxanthin. LC(APCI)−MS analysis of both compounds clearly proved **4** to be antheraxanthin. For further evidence, one potato extract was evaporated, and the residue was dissolved in ethanol and treated with a few drops of diluted hydrochloric acid to convert the presumed 5,6-epoxide (antheraxanthin) to its 5,8-isomer (mutatoxanthin) (*27*). An LC(APCI)−MS analysis proved that the new main peak which appeared in the chromatogram represents the expected carotenoid (*m/z* 585 $[M + H]^+$ (100%), 567 (11%); λ_{max} 405 (sh), 426, 453 nm). The occurrence of antheraxanthin is in contrast to reports of Iwanzik et al. (*1*) and Kasim (*8*, *9*), who found lutein-5,6-epoxide in potato extracts.

Epoxides of β,β-carotene are easily accessible via epoxidation with 3-chloroperoxybenzoic acid, yielding both mono- and diepoxides in high amounts. Neither β,β-carotene monoepoxide (*m/z* 553 $[M + H]^+$ (100%), 535 (24%); λ_{max} 420, 445, 472 nm) nor β,β-carotene diepoxide (*m/z* 569 $[M + H]^+$ (100%), 551 (46%); λ_{max} 415, 439, 468 nm) was present in the potato extracts examined in this study.

LC(APCI)−MS Analyses of Carotenoid Esters. As an example, four native extracts of the cultivar Nicola, corresponding to 600 g of fresh material, were pooled, purified using column chromatography on silica gel as described earlier (*21*), and analyzed by LC(APCI)−MS. Unfortunately, the presence of residual triacylglycerides resulted in a high background noise, which forestalled monitoring of target compound spectra. To avoid this interference, an additional enzymatic cleanup pro-

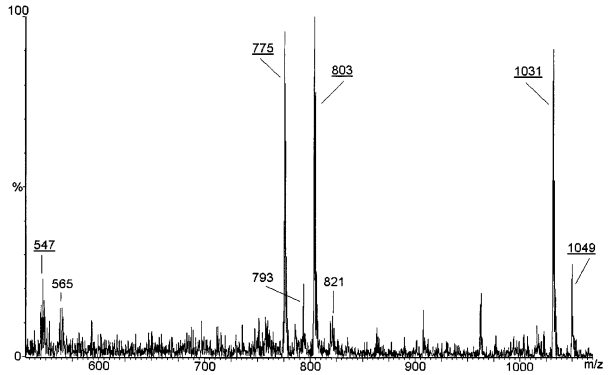

Figure 4. Mass spectrum (APCI$^+$ mode) of violaxanthin myristate–palmitate (corresponding to **10**, **Figure 3**) as an example of the fragmentation pattern of a mixed carotenoid diester possessing epoxy groups. Fragments belonging to the main fragmentation pathway are underlined.

cedure, using lipases for hydrolysis of triacylglycerides, was applied. To ensure nonspecific hydrolysis of triacylglycerides, a mixture of two commercial microbial lipases was used. Both lipases were proven not to cleave carotenoid esters in earlier experiments (data not shown), ensuring carotenoid ester stability during the cleanup procedure. The extract cleaned in this way was subjected to LC(APCI)−MS analysis again and showed the chromatogram depicted in **Figure 3**, pointing to an extremely complex carotenoid ester spectrum. The MS and the UV data of several peaks (**9**−**16**) are summarized in **Table 2**. An exemplary mass spectrum, obtained from a mixed xanthophyll diester, is given in **Figure 4**; it represents violaxanthin myristate−palmitate (peak **10**, **Figure 3**). The fragmentation pattern is charaterized by two pathways, showing daughter ions with diverse intensity; signals with high intensity belong to the main pathway (the respective fragment masses are underlined in **Figure 4**), which is characterized by the following cascade: *m*/*z* 1031 is generated by the loss of water from the quasimolecular ion (*m*/*z* 1049 $[M + H]^+$); two daughter ions (*m*/*z* 803 and 775) from *m*/*z* 1031 are formed by the loss of myristic (228 Da) or palmitic (256 Da) acids, respectively; *m*/*z* 547 is generated by release of the remaining fatty acid. A minor fragmentation pathway starts likewise from the quasimolecular ion at *m*/*z* 1049 and leads to the corresponding fragment ions *m*/*z* 821 (loss of C14:0), 793 (loss of C16:0), and 565, which represents the backbone of violaxanthin. A comparable fragmentation scheme is observed for **9**−**13**, allowing for identification of the nature of the fatty acids bound to the parent carotenoid. Of course, in the case of lutein and zeaxanthin diesters (**14**−**16**), the fragmentation pathway initiated by the loss of water from $[M + H]^+$ does not appear. The assignment of the parent carotenoid is sometimes difficult, but making use of the absorption spectra obtained by diode array detection led to the unequivocal identification of **9**, **10**, **14**, **15**, and **16** (see **Table 2**). Concerning **11**−**13**, the UV/vis spectra did not exactly match the spectroscopic data of the major carotenoids mentioned above, particularly not the spectrum of antheraxanthin, one of the major carotenoids. Although the parent carotenoids of **11**−**13** were not unequivocally identified, the cleanup procedure proved to be highly efficient, allowing for the LC(APCI)−MS-based identification of fatty acids acylating carotenoids, which occur in minute amounts in plant extracts.

In contrast to previous results (*14*, *15*), we detected in a native extract from the cultivar Nicola myristic and palmitic acids as the main fatty acids bound to carotenoids. It is apparent that only several major carotenoid esters of one single cultivar were analyzed. Various other fatty acids may acylate minor carotenoids. Thus, the complete fatty acid pattern of carotenoid esters is not known. Analyses of the triacylglyceride fatty acid distribution of the same cultivar by gas chromatography (*24*) showed linolic (44 area%), palmitic (25 area%), and linolenic acids (20 area%) to be the main components, matching the data reported by Tevini et al. concerning the total lipid fraction (*15*). Interestingly, this distribution seems not to be reflected by the native fatty acid pattern of carotenoid diesters but underlines an overall tendency, previously found in other plants, such as *T. erecta* (*21*). Further studies will have to clarify if there exists an enzyme system that is responsible for the highly specific esterification step in the plant carotenoid ester biosynthesis.

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