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Analytical Methods

HPLC-DAD-MS^n characterisation of carotenoids from apricots and pumpkins for the evaluation of fruit product authenticity

Christina Kurz, Reinhold Carle, Andreas Schieber *

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

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Dedicated to Prof. Dr. Rainer Wild, Heidelberg, on the occasion of his 65th birthday

Abstract

Carotenoids including carotenoid esters from six apricot (*Prunus armeniaca* L.) cultivars and from eight cultivars from three pumpkin species (*Cucurbita maxima* Duch., *Cucurbita pepo* L., and *Cucurbita moschata* Duch.) were extracted without saponification, separated on a C-30 reversed-phase column and characterised by high-performance liquid chromatography/atmospheric pressure chemical ionisation–mass spectrometry (LC–MS). The predominant free carotenoids were quantified by HPLC with diode array detection. In contrast to previously published data, α-carotene could not be detected in apricots. Although the pumpkins showed significant differences in their free carotenoid profiles, major unesterified compounds different from those found in apricots could be determined. However, due to the natural heterogeneity, authentication of the apricot products cannot be accomplished exclusively using the profile of free carotenoids. Therefore, the investigations were extended to carotenoid esters. The xanthophyll ester profiles in pumpkins significantly differed from those in apricots in that the latter also contained both saturated and unsaturated fatty acids, whereas in pumpkins exclusively saturated fatty acids were detected. Admixtures of lower cost pumpkins could be detected in quantities of $\geqslant 5\%$ by increased contents of lutein and zeaxanthin, and by the appearance of antheraxanthin and α-carotene, respectively, depending on the added pumpkin cultivar, as well as the presence of characteristic lutein and antheraxanthin esters. However, pronounced differences in the carotenoid profiles of the investigated pumpkins and the partly minor amount of characteristic compounds lead to limitations of the detection of 5% level of admixture of pumpkin to apricot and of the method in general.

Keywords: Apricot; Authenticity; Carotenoids; LC–MS; Pumpkins

1. Introduction

Fruits are the quality-determining and most costly ingredients of jams, spreads, fruit preparations and related products. As the fruit market is highly competitive with relatively narrow profit margins, unscrupulous producers may be tempted to maximise revenues by the fraudulent admixture of low cost to higher priced specified fruits. Therefore, to avoid unfair competition and to protect consumers from deception, product authentication is essential. In the past, several strategies have been described for plant species determination and for the detection of adulterations, for example profiling of phenolic compounds (Bengoechea, Sancho, Estrella Bartolomé, Gómez-Cordovés, & Hernández, 1997; Zimmermann & Galensa, 2007). However, more recent studies have shown that some polyphenols that have so far been assumed to be characteristic of certain plant species can also be found in other species (Hilt et al., 2003). While PCR methods have successfully been applied to species identification of e.g. meat and fish, degradation of DNA under heat and acidic conditions makes these methods inapplicable to jams and spreads

* Corresponding author. Tel.: +49 (0) 711 459 23125; fax: +49 (0) 711 459 24110.
E-mail address: Schieber@ualberta.ca (A. Schieber).

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Table 1
Specification of fruit samples

Fruit species	Taxonomic name	Cultivar	Geographical origin	Harvest year	Sample code
Apricot	*P. armeniaca*	Bergeron (I)	France	2006	A-B I
		Bergeron (II)	France	2006	A-B II
		Harogen	France	2006	A-H
		Moniqui	Spain	2006	A-MO
		Orangered	France	2006	A-OR
		Redsun	France	2006	A-R
Pumpkin	*Cucurbita maxima*	Bischofsmütze	Germany	2006	PK-BM
		Golden Nuggets	Germany	2005	PK-GN
		Halloween	Germany	2006	PK-HA
		Hokkaido I	Germany	2005	PK-HO I
		Hokkaido II	Germany	2006	PK-HO II
	C. pepo	Sweet Lightning	Germany	2006	PK-SL
	C. moschata	Muscade de Provence	Germany	2005	PK-MU
		Butternuts	Germany	2006	PK-BN

(Bauer, Weller, Hammes, & Hertel, 2003; Jonas et al., 2001; Moreano, Busch, & Engel, 2005). We have recently demonstrated that the neutral sugar profile of cell wall polysaccharides can also be used for authentication of fruit-derived products (Kurz, Carle, & Schieber, 2008).

Carotenoids have extensively been studied in a large number of food commodities because of their health-promoting properties. However, only little attention has been paid to their suitability as markers for the detection of food adulteration. Most work has been dedicated to the detection of fraudulently added pigments such as synthetic β-carotene or extracts from citrus peels and marigold flowers to intensify the natural colour of foods (Philip, Chen, & Nelson, 1989). Oke and Shrikhande (1977) described a method for the detection of adulteration of tomato ketchup with red pumpkin which was based on the different carotenoid profiles. While today pumpkin extracts would hardly be expected to be used in ketchup, their fraudulent addition to apricot jams and spreads would result in a more intense colour giving the impression of higher fruit contents. Dragovic-Uzelac, Delonga, Levaj, Djakovic, and Pospisil (2005) reported that admixture of pumpkin purée to apricot products can be detected by the presence of syringic acid, which could be found in pumpkins but not in apricots. However, their assessment was based on a comparatively small selection of pumpkin varieties and peak assignment was not verified by mass spectrometry. Furthermore, one analytical technique is usually insufficient to detect all kinds of adulteration commonly practised. Therefore, in most cases only combined analyses allows reliable authenticity assessment of a product. Hence, the objective of the present study was to evaluate the potential of the carotenoid profile for authenticity studies of apricot purées and jams. For this purpose, a simple extraction protocol suitable for routine analysis and a method for the simultaneous separation of carotenoids and carotenoid esters were developed.

2. Experimental

2.1. Materials

All reagents and solvents used were purchased from VWR (Darmstadt, Germany) and were of analytical or HPLC grade. Antheraxanthin (5,6-epoxy-5,6-dihydro-β,β-carotene-3,3′-diol), α-carotene (β,ε-carotene), γ-carotene (β,ψ-carotene), β-cryptoxanthin (β,β-carotene-3-ol), lutein (β,ε-carotene-3,3′diol), and zeaxanthin (β,β-carotene-3,3′diol) were from CaroteNature (Lupsingen, Switzerland); β-carotene (β,β-carotene) and β-apo-8′-carotenal were from Fluka (Sigma–Aldrich, St. Louis, MO, USA). Lycopene (ψ,ψ-carotene) was purchased from AppliChem (Darmstadt, Germany). (*Z*)-Isomers of β-carotene were obtained by iodine-catalysed photoisomerisation of (*all-E*)-β-carotene (Zechmeister, 1962).

Fresh, fully ripe apricots (*Prunus armeniaca* L.) and yellow or orange fleshy pumpkins (*Cucurbita* sp.) were obtained from the local market (Stuttgart, Germany) (Table 1). Fruits were harvested in 2006, pumpkins in 2005 and 2006. Fresh apricots were manually cored at 4 °C. Pumpkins were lye peeled, manually cored at 4 °C, blanched at 85 °C for 10 min, and subsequently mashed through a sieve (mesh size: 1.5 mm). Blended products were prepared from apricot purées (cv. Bergeron I), which were produced from cored fruits treated as described above and mixed with pumpkin purée in proportions of 95:5, 90:10 and 80:20, respectively. Apricot purée for jam production was blended in proportions of 95:5, 90:10 and 85:15 with pumpkin cv. Muscade de Provence. Apricot jams were prepared by heating a defined amount of fruit purée with sucrose and highly esterified pectin (Classic AF 401, Herbstreith & Fox, Neuenbürg, Germany) under reduced pressure at 75 °C until a dry matter of 63% was obtained. Final fruit content of the jams was 45% (w/w). Samples were lyophilised in a Steris Lyovac® GT 4 Lyophiliser

(Steris, Hürth, Germany) and kept at −20 °C until analysis.

2.2. Sample preparation

Freeze-dried samples were ground with liquid nitrogen to a fine powder in a cutter (Stephan und Söhne & Co., Hameln, Germany). To avoid degradation and isomerisation of carotenoids, amber glassware was used and sample preparation was performed under dim light conditions. Aliquots of 1.5 g of powdered plant material and purées were extracted with a mixture of 50 mL of acetone and hexane (1:1, v/v) containing butylated hydroxytoluene (50 mg/100 mL) and butylated hydroxyanisole (50 mg/100 mL) as antioxidants. After flushing with nitrogen, the extraction was performed at ambient temperature for 1 h under continuous stirring. Subsequently, the extract was filtered through a Whatman No. 1 paper (Clifton, NJ, USA) and the residue was washed with 75 mL of ethyl acetate. The combined filtrates were evaporated to dryness *in vacuo* ($T < 30$ °C) and the residue was dissolved in an appropriate volume of 2-propanol (2 or 5 mL). Finally, the sample was membrane-filtered (0.2 μm) and directly used for HPLC and LC/MS analyses.

Carotenoids were extracted from 3 g of lyophilised jam with 50 mL of methanol containing 0.1% pyrogallol as an antioxidant. After flushing with nitrogen, the extraction was performed at ambient temperature for 2 h under continuous stirring. The extract was filtered and the solvent was concentrated to 15–20 mL. The residue was transferred to a separating funnel. Carotenoids were extracted from the methanol layer twice with 25 mL of hexane. The pooled organic phases were washed with 50 mL of sodium chloride solution (10 mg/100 mL) and twice with 50 mL of water to remove methanol, dried with sodium sulphate, and evaporated *in vacuo* ($T < 30$ °C). The residue was dissolved in 2-propanol and made up to 2 mL. The solution was membrane-filtered (0.2 μm) and used for HPLC and LC/MS analyses.

All samples were analysed in duplicate. Recoveries were determined by adding known amounts of internal standard solution of β-apo-8′-carotenal to the purée and jam prior to extraction.

2.3. Light microscopy

Samples of fresh raw apricots were inspected microscopically using an Axiscope 2 (Zeiss, Oberkochen, Germany) equipped with a CCD-camera (JVC, Osaka, Japan).

2.4. HPLC system

HPLC analyses were performed on a model 2690 Waters separation module equipped with an autosampler injector, a model Jetstream 2 plus Waters column oven, and a model 2966 Waters UV/visible photodiode array detector controlled by a Millennium 32 (version 3.2) workstation (Waters, Milford, MA, USA). The column used was a 150 × 3.0 mm i.d., 3 μm particle size, analytical scale YMC C30 reversed-phase column (Wilmington, MA, USA) operated at 25 °C. The mobile phase consisted of methanol/methyl *tert*-butyl ether (MTBE)/water (81:15:4 v/v/v; eluent A) and methanol/MTBE/water (4:92:4, v/v/v; eluent B). The gradient used was as follows: 0% B to 80% B (60 min), 100% B isocratic (5 min), 100% B to 0% B (5 min), 0% B isocratic (5 min). Total run time was 75 min. The injection volume was 10–50 μL. Carotenoids were monitored at 450 nm (all-*trans*-β-carotene, zeaxanthin) and 445 nm (antheraxanthin, α-carotene, and lutein) at a flow rate of 0.42 mL/min. UV/vis spectra were recorded in the range of 200–700 nm. Carotenoids were quantified using calibration curves of the corresponding standard compounds at the specific absorption maximum. Concentrations of stock solutions were determined spectrophotometrically using their specific absorption coefficients ($A^{1\%}_{1\ cm}$) as published previously (Britton, 1995).

2.5. LC/mass spectrometry

LC/MS analyses were performed using an Agilent HPLC series (Agilent, Waldbronn, Germany) equipped with ChemStation software, a model G1322A degasser, a model G1312A binary pump, a model G1313A autosampler, a model G1316 column oven, and a model G1315A DAD system. The analyses were performed with the column and mobile phase described above. The injection volume was 10–50 μL. All carotenoids and carotenoid esters were monitored at 450 nm at a flow rate of 0.42 mL/min. UV/vis spectra were recorded in the range of 200–700 nm at a spectral acquisition rate of 1.25 scans/s (peak width 0.2 min).

The HPLC system was coupled on-line to a Bruker (Bremen, Germany) model Esquire 3000+ ion trap mass spectrometer fitted with an APcI source. Data acquisition and processing were performed using Esquire Control software. Positive ion mass spectra of the column eluate were recorded in the range of *m*/*z* 100–1100 at a scan speed of 13 000 Th/s (peak width 0.6 Th, FWHM). Nitrogen was used both as the drying gas at a flow rate of 4.0 L/min and as the nebulising gas at a pressure of 50 psi. The nebuliser temperature was set at 350 °C and a potential of 2779 kV was used on the capillary. Corona was set at 4000 nA in the positive ion mode, and the vaporiser temperature was set at 400 °C. Helium was used as the collision gas for CID at a pressure of 4.9×10^{-6} mbar. CID spectra were obtained with an isolation width of 2.0 Th for precursor ions and a fragmentation amplitude of 1.0 V.

3. Results and discussion

3.1. Methodology

The objective of the present study was to characterise fruit specific carotenoid fingerprints in several apricot and pumpkin cultivars for the evaluation of fruit authenticity.

Sample preparation was performed by extraction of the carotenoids with acetone/hexane or methanol without saponification before HPLC and LC/MS analysis. The analysis of unsaponified extracts presents several advantages, such as a high throughput and minimal artefact formation (Schiedt & Liaaen-Jensen, 1995). The chromatograms obtained are more complex but provide more information, in particular with respect to the presence of esterified xanthophylls which are included in the fingerprint.

Because of the high pectin and sugar content, the extraction of carotenoids from jams needed to be performed successively. For removal of sugars and pectins, jams were first extracted with methanol. Subsequently, the carotenoids were extracted with hexane, while the sugars remained in the methanol layer. This procedure allowed carotenoid extraction even from products with high sugar contents. The recoveries of the internal standard obtained using the extraction methods described above were satisfactory both for purées (86.8–93.2%) and jams (77.1–88.6%). For authentication purposes only the predominant carotenoid peaks were considered, so that even small ratios of pumpkin admixture could be determined. For carotenoid characterisation several LC/MS methods have been reported. Positive atmospheric pressure chemical ionisation ($APcI^+$) proved to be the most suitable technique (van Breemen, 1997) and was therefore used in this study.

The identification of the carotenes and xanthophylls investigated in the present study (antheraxanthin, lutein, lycopene, zeaxanthin, α-carotene, β-carotene, γ-carotene and β-cryptoxanthin) was based on the comparison of retention times, UV/vis and mass spectrometric data of reference standards. β-Carotene *Z*-isomers were identified according to a former investigation (Lacker, Strohschein, & Albert, 1999) by their retention time and their UV/vis spectral characteristics in comparison with the isomers obtained by iodine-catalysed conversion of *all-E*-β-carotene. Xanthophyll esters were characterised based on their retention times, UV/vis spectra and fragmentation patterns in LC–MS experiments.

3.2. Predominant carotenoids of apricots

Although the carotenoid contents varied, the qualitative profile of the major carotenoids was comparable in all apricot cultivars investigated. The chromatograms of carotenoid extracts from apricots are shown in Fig. 1A. In agreement with previous reports (Dragovic-Uzelac, Levaj, Mrkic, Bursac, & Boras, 2007; Fraser & Bramley, 2004; Ruiz, Egea, Tomás-Barberán, & Gil, 2005), the most abundant carotenoid in apricot fruits is β-carotene (peak 9), ranging from 1.44 ± 0.01 μg/g in the white fleshy cv. Moniqui to 39.07 ± 2.93 μg/g in cv. Harogen (Table 2). Mass spectrometric analysis revealed a base peak at *m/z* 537 $[M+H]^+$ and a fragment at *m/z* 444 $[M^{+\cdot}-92]$ resulting from elimination of toluene.

In contrast to the previously published results (Dragovic-Uzelac et al., 2007; Fraser & Bramley, 2004; Yano et al., 2005), we could not confirm the presence of α-carotene in apricots. *Z*-Isomers of β-carotene, which have been detected in considerable amounts in the unprocessed fruits in different forms (Fig. 1A, peaks 5, 6, 7, 10), could readily

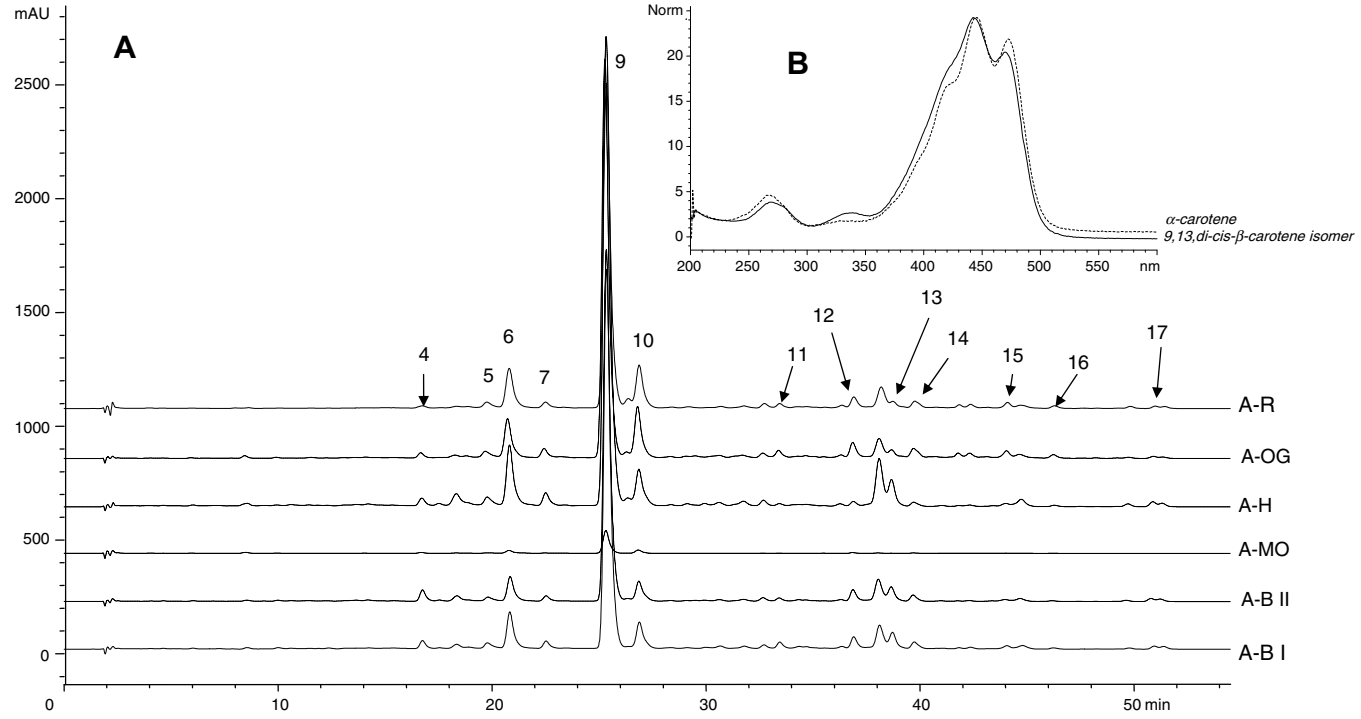

Fig. 1. (A) HPLC chromatograms (450 nm) of carotenoid extracts from the apricot cultivars investigated; for sample codes see Table 1, for peak assignment see Table 4; (B) UV–Vis spectrum of a 9,13-di-*cis*-isomer of β-carotene (peak 7) compared with the spectrum of α-carotene.

Table 2
Genuine carotenoid contents [μg/g ± SD] and absorption maxima [nm] of the non-esterified carotenoids in the raw materials used for authenticity studies and β-carotene

Sample[a]	Antheraxanthin λ_{max}, 422sh, 445, 473	Lutein λ_{max}, 422sh, 445, 473	Zeaxanthin λ_{max}, 426sh, 450, 477	α-Carotene λ_{max}, 422sh, 445, 473	β-Carotene λ_{max}, 426sh, 450, 477
A-B I	nd[b]	0.13 ± 0.01	tr[c]	nd	24.47 ± 0.86
A-B II	nd	0.16 ± 0.01	0.13 ± 0.01	nd	25.36 ± 0.17
A-H	nd	0.36 ± 0.02	0.46 ± 0.03	nd	39.07 ± 2.93
A-MO	nd	0.06 ± 0.002	nd	nd	1.44 ± 0.01
A-OR	nd	0.14 ± 0.01	0.11 ± 0.01	nd	28.48 ± 0.66
A-R	nd	0.10 ± 0.01	0.13 ± 0.02	nd	21.39 ± 0.28
PK-BM		0.32 ± 0.01			4.73 ± 0.11
PK-GN	4.87 ± 0.03	22.75 ± 0.23	3.37 ± 0.47		18.64 ± 0.01
PK-HA		8.13 ± 0.60	0.57 ± 0.05		8.13 ± 0.84
PK-HO I	10.19 ± 0.14	29.74 ± 1.56	5.78 ± 0.09		27.14 ± 0.92
PK-HO II	21.99 ± 1.90	38.67 ± 3.28	22.45 ± 1.94		70.92 ± 4.24
PK-SL		1.31 ± 0.06			7.00 ± 0.17
PK-MU				10.60 ± 0.42	8.97 ± 0.69
PK-BN		1.40 ± 0.08		0.58 ± 0.003	11.40 ± 0.06

[a] For sample codes see Table 1.
[b] nd: not detectable.
[c] tr: traces (<0.1).

be mistaken for α-carotene if identification relies solely on the retention time, and it is even difficult to distinguish the isomers by their UV/vis spectral data. Since isomerisation of β-carotene leads to a hypsochromic shift of the absorbance the maxima are similar to those of α-carotene (Fig. 1B). When a peripheral double bond is converted into *cis*-conformation, the intensity of the main band decreases and a "*cis* peak" appears at 340 nm, as could be observed for peaks 7 and 10 (the 9,13-di-*cis* and 9-*cis* isomers) in Fig. 1A. Maximal intensity of the "*cis* peak" is observed when the *Z*-double bond is located at or near the centre of the chromophore (the 15- and 13-*cis* isomer, Fig. 1A, peaks 5 and 6). c*is*-Isomerisation may occur as a consequence of light exposure and thermal treatment. However, both effects could be excluded during drying, milling, and sample preparation. Therefore, it is concluded that the *cis*-forms of β-carotene are genuinely present in apricot fruits. This assumption was corroborated by microscopic investigations which revealed globulous apricot chromoplasts containing yellow carotenoid-carrying lipid droplets similar to the findings of Vasquez-Caicedo, Heller, Neidhart, and Carle (2006) (data not shown). Partial solubilisation of β-carotene in lipid globules is considered the main reason for the presence of *cis*-isomers (Pott, Marx, Neidhart, Mühlbauer, & Carle, 2003; Vasquez-Caicedo et al., 2006), whereas carotenes sequestered in crystalline chromoplasts, e.g. in carrot roots, were shown to be fairly stable toward isomerisation (Marx, Stuparic, Schieber, & Carle, 2003). The literature data on the contents of carotenoids, particularly zeaxanthin, lutein and α-carotene, in apricots have mainly been based on HPLC-DAD with UV/vis analysis and comparison of retention times, and are even contradictory (Bureau & Bushway, 1989; Dragovic-Uzelac et al., 2007; Sass-Kiss, Kiss, Milotay, Kerek, & Toth-Markus, 2005). Other investigations performed on saponified extracts cannot be compared to our results since alkaline hydrolysis causes degradation of xanthophyll esters.

Among the carotenoids mentioned above, γ-carotene (λ_{max} 435, 460, 491) and lycopene (λ_{max} 445, 472, 503) with molecular mass ions at *m/z* 537 were detected as minor components. Lycopene showed a unique fragment ion at *m/z* 467 (Pajkovic & van Breemen, 2005) due to the presence of a ψ end group. The occurrence of lycopene and γ-carotene has also been reported in previous investigations (Dragovic-Uzelac et al., 2007; Khachik, Beecher, Goli, & Lusby, 1992; Müller, 1997; Ruiz et al., 2005; Sass-Kiss et al., 2005).

3.3. Predominant carotenoids of pumpkins

As can be seen from Fig. 2, the carotenoid profiles of the different pumpkin cultivars showed marked differences both in their qualitative and quantitative distribution, even in pumpkins of the same species, which is consistent with the results reported by others (Arima & Rodriguez-Amaya, 1988; Azevedo-Meleiro & Rodriguez-Amaya, 2007; Kreck, Kürbel, Ludwig, Paschold, & Dietrich, 2006). The carotenoid composition also influenced the visual appearance of the pumpkins. In line with a former study (Murkovic, Mülleder, & Neunteufl, 2002) we observed that varieties such as *Cucurbita moschata* with a high content of carotenes had an orange appearance, while those with a high lutein and a low carotene content like Halloween showed a bright yellow colour. This property renders pumpkins likely candidates to be used for fraudulent purposes in apricot based products. The differences in the carotenoid profiles of the *Cucurbita* cultivars investigated may be due to the long period of pumpkin harvest. In contrast to other vegetables or fruits, pumpkins can be stored for longer time periods, hence ripening processes can continue (Niewczas, Szweda, & Mitek, 2005). A commonality observed for all

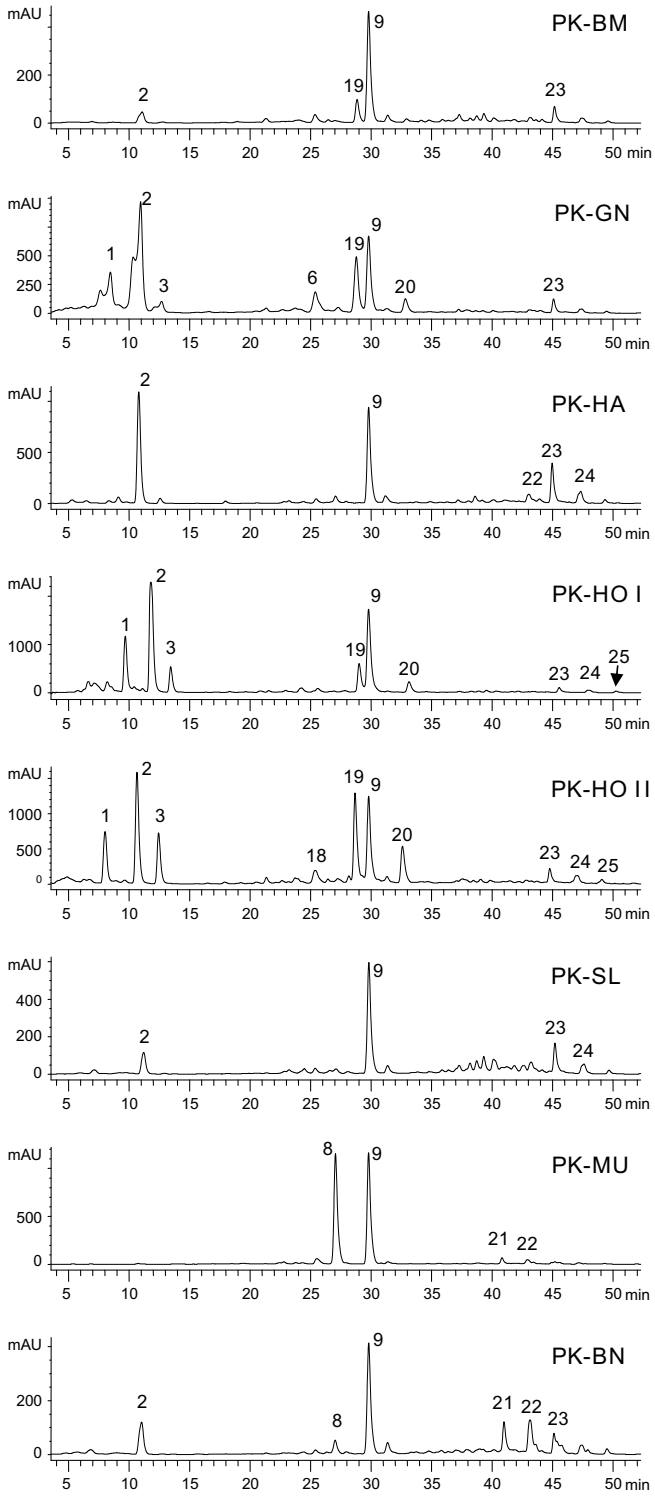

Fig. 2. Chromatographic patterns at 450 nm of pumpkin carotenoids; for sample codes see Table 1; for peak assignment see Table 4.

cultivars was the presence of relatively high contents of β-carotene, ranging from 4.73 ± 0.11 μg/g in the bright yellow fleshy cv. Bischofsmütze to 70.92 ± 4.24 μg/g in the intensely orange coloured cv. Hokkaido (II). Lutein and β-carotene were the main free carotenoids of the cultivars Bischofsmütze, Halloween, Sweet Lightning, and Butternuts. In the cultivars Hokkaido (I and II) and Golden Nuggets also antheraxanthin was detected. Although lutein and antheraxanthin showed identical UV/vis spectra, they could easily be differentiated by mass spectrometry. Fragmentation of *m/z* 585 for antheraxanthin and *m/z* 569 for zeaxanthin yielded dehydrated product ions at *m/z* 567 and 551, respectively. In the MS^3 experiment the loss of a second H_2O molecule was observed, resulting in product ions at *m/z* 549 and 533. Dehydrated fragment ions from protonated molecules $[M+H-nH_2O]^+$ have been described for all hydroxylated carotenoids (Maoka, Fujiwara, Hashimoto, & Akimoto, 2002). Relative intensities of the dehydrated fragment ions may differ, thus reflecting the structural characteristics of the hydroxylated end groups of isomeric carotenoids. This different mass spectrometric behaviour was used for the distinction of lutein and zeaxanthin. Lutein, which possesses a 3-hydroxy-ε end group, showed an $[M+H-H_2O]^+$ ion at *m/z* 551 as the most abundant signal and another product ion at *m/z* 533 ($[M+H-2H_2O]^+$) in the MS^1 experiment, which was hardly observed for zeaxanthin, thus confirming the data of other studies (Pajkovic & van Breemen, 2005). Also in accordance with former studies on *C. moschata* cv. Muscade de Provence (Kreck et al., 2004) comparable quantities of α-carotene and β-carotene were found (Fig. 2, PK-MU). Having identical chromophores, α-carotene and β-carotene showed absorption spectra similar to those of lutein and zeaxanthin, respectively. Both had mass spectra with molecular mass ions at *m/z* 537 and a fragment ion at *m/z* 444. However, β-carotene displayed the expected $[M-137]^+$ ion, whereas α-carotene was characterised by the losses of 56, 123 and 148 mass units from $M^{+\cdot}$ characteristic of the ε end group (Britton, 1995). α-Carotene was also found in low amounts in cv. Butternuts also belonging to *C. moschata.* Zeaxanthin, which was found in varieties of *C. maxima*, was absent in those of *C. moschata* (Hidaka, Anno, & Nakatsu, 1987). However, in contrast to an earlier study (Azevedo-Meleiro & Rodriguez-Amaya, 2007) we could not confirm violaxanthin as major carotenoid in the pumpkins investigated.

3.4. Determination of carotenoids in apricot products blended with pumpkin purée

The suitability of the extraction methods developed for the detection of an unspecified admixture of pumpkin purée to apricot purées was determined using mixtures of apricot purée of the cultivar Bergeron (I) with increasing proportions of the *C. maxima* cultivars Bischofsmütze, Hokkaido, Halloween, Golden Nuggets, *C. moschata* cv. Muscade de Provence, Butternuts and *C. pepo* cv. Sweet Lightning. Additionally, for the evaluation of the extraction method for products containing high amounts of sugars, apricot jams prepared with Muscade de Provence pumpkin admixtures were examined.

The initial contents of the predominant carotenoids in the raw materials used for authenticity studies are shown in Table 2. The carotenoid amounts resulting from

Table 3
Carotenoid contents of apricot purées and jams blended with increasing proportions of pumpkins

Added pumpkin[a]	Carotenoid	Crotenoid content [μg/g FW[b] ± SD]		
		Pumpkin proportion		
		5%	10%	(15%) 20%
PK-BM	Lutein	0.11 ± 0.01	0.12 ± 0.01	0.15 ± 0.01
PK-GN	Lutein	0.53 ± 0.01	0.68 ± 0.01	1.43 ± 0.05
	Zeaxanthin	0.14 ± 0.01	0.14 ± 0.004	0.25 ± 0.03
	Atheraxanthin	0.14 ± 0.01	0.18 ± 0.01	0.45 ± 0.10
PK-HA	Lutein	0.54 ± 0.05	1.15 ± 0.10	2.00 ± 0.12
	Zeaxanthin	0.10 ± 0.02	0.15 ± 0.03	0.17 ± 0.01
PK-HO I	Lutein	1.71 ± 0.16	4.83 ± 0.38	9.93 ± 1.06
	Zeaxanthin	0.51 ± 0.05	1.26 ± 0.08	2.66 ± 0.24
	Antheraxanthin	0.79 ± 0.06	2.22 ± 0.19	4.96 ± 0.39
PK-HO II	Lutein	2.35 ± 0.36	4.81 ± 0.07	9.55 ± 0.20
	Zeaxanthin	0.99 ± 0.15	1.99 ± 0.01	3.94 ± 0.11
	Antheraxanthin	0.84 ± 0.17	1.68 ± 0.21	3.38 ± 0.08
PK-SL	Lutein	0.10 ± 0.01	0.13 ± 0.01	0.23 ± 0.05
PK-MU	α-Carotene	2.46 ± 0.03	4.52 ± 0.32	5.72 ± 0.26
PK-MU jam	α-Carotene	1.39 ± 0.21	2.28 ± 0.03	(3.68 ± 0.05)
PK-BN	Lutein	0.12 ± 0.03	0.20 ± 0.01	0.30 ± 0.001

[a] For sample codes see Table 1.
[b] FW: fresh weight.

increasing proportion of pumpkins in apricot purée are summarised in Table 3. Most of the pumpkin cultivars contained significant amounts of xanthophyll esters. However, this study focused on the quantification of free carotenoids due to the complex profile and the lack of standard compounds of xanthophyll esters.

Owing to their high carotenoid contents, admixtures of the cv. Golden Nuggets, Halloween, Hokkaido (I and II), and Muscade de Provence to apricot purée could be detected from ⩾5%. Apart from purées, also apricot jam was blended with pumpkin purée of the cv. Muscade de Provence showing a significant increase of the α-carotene content in the blend, allowing the detection of pumpkin admixture in proportions exceeding 5%. Taking into account that fraudulent admixtures of pumpkins could also be practised to improve the colour of apricot purées and apricot based fruit products, the cultivars most 'suitable' for this purpose such as Hokkaido and Muscade de Provence would easily be detected by analysing the predominant carotenoids.

Because of their lower genuine lutein contents cv. Sweet Lightning, Butternuts and particularly Bischofsmütze, the increase in the lutein content of the purée blend was less pronounced. The lutein contents of apricot purées blended with Butternuts and Sweet Lightning rose by admixtures exceeding 10% and 20%, respectively. Although α-carotene was found in cv. Butternuts, its quantification in purée blends failed because of the minor contents.

3.5. Characterisation of the xanthophyll ester profiles of apricots and pumpkins

From the results obtained from the determination of carotenes and xanthophylls it becomes evident that authentication of apricot products exclusively via non-esterified carotenoids can hardly be accomplished, owing to the marked heterogeneity of the carotenoid profile found in the pumpkins. Therefore, we extended our studies to the characterisation of the xanthophyll esters using HPLC and LC–MS. Previous reports on the presence of carotenoid esters in apricots are contradictory. While in earlier studies dating back to the late 1980s no carotenoid esters were found in fresh, dried, and canned fruits (Khachik, Beecher, & Lubsy, 1989; Philip & Chen, 1988), a comprehensive screening of fruits and vegetables carried out more recently indicated the presence of β-cryptoxanthin and lutein esters in apricots and of lutein, zeaxanthin and violaxanthin esters in pumpkin (*C. pepo*) (Breithaupt & Bamedi, 2001). However, in the latter study only the total

Table 4
Carotenoids and carotenoid esters characterised in apricots and pumpkins

Peak	Carotenoid ester	$[M+H+2FA^a]^+$ *m/z*	$[M+H+1FA]^+$ *m/z*	$[M+H]^+$ *m/z*
1	Antheraxanthin	–	–	585
2	Lutein	–	–	569
3	Zeaxanthin	–	–	569
4	β-Cryptoxanthin	–	–	553
5	15-*cis*-β-Carotene	–	–	537
6	13-*cis*-β-Carotene	–	–	537
7	9,13-di-*cis*-β-Carotene	–	–	537
8	α-Carotene	–	–	537
9	β-Carotene	–	–	537
10	9-*cis*-β-Carotene	–	–	537
11	β-Cryptoxanthin–laurate (C12:0)	–	735	535
12	β-Cryptoxanthin–oleate (C18:1)	–	817	535
13	γ-Carotene	–	–	537
14	β-Cryptoxanthin–palmitate (C16:0)	–	791	535
15	Lutein-di-oleate (2 × C18:1)	1097	815	533
16	Lutein–palmitate–oleate (C16:0; C18:1)	1071	789/815	533
17	Lycopene	–	–	537
18	Antheraxanthin–laurate (C12:0)	–	767	567
19	Antheraxanthin–myristate (C 14:0)	–	795	567
20	Antheraxanthin–palmitate (C16:0)	–	823	567
21	Lutein-di-laurate (2 × C12:0)	933	733	533
22	Lutein–laurate–myristate (C12:0; C 14:0)	961	733/761	533
23	Lutein-di-myristate (2 × C 14:0)	989	761	533
24	Lutein–myristate–palmitate (C 14:0; C 16:0)	1017	761/789	533
25	Lutein-di-palmitate (2 × C16:0)	1045	789	533

[a] Fatty acids.

contents were given without information on the nature of the acylating fatty acid being obtained. As can be seen from Fig. 1A and Table 4, apricots contained β-cryptoxanthin in its free form and esterified with saturated and unsaturated fatty acids, respectively. The lutein esters identified in *P. armeniaca* were esterified with at least one monounsaturated fatty acid (oleic acid). Due to their more hydrophobic character, esters eluted later than non-esterified xanthophylls. Their identification was based on the molecular ion $[M+H]^+$ and the fragments obtained after loss of one and two fatty acid moieties, respectively. Consistent with former investigations (Tian, Duncan, & Schwartz, 2003) fragment ions resulting from the loss of the longer chain fatty acids were more abundant than those produced by the loss of the shorter chain fatty acid (data not shown).

In contrast to apricots, pumpkins contained antheraxanthin monoesters beside lutein diesters as the predominant esterified carotenoids (Fig. 2, peak identification is given in Table 4). Antheraxanthin was found to be monoacylated with lauric, myristic and palmitic acids. While antheraxanthin esters were detected only in the cultivars Bischofsmütze, Golden Nuggets and Hokkaido I & II, lutein esters were present in all pumpkin cultivars investigated, with lutein-di-myristate most frequently being found. The nature of the fatty acid attached to the xanthophylls proved to be distinctive clearly characteristic of pumpkin. As can be seen from Fig. 2 and Table 4, pumpkins exclusively contained xanthophyll esters acylated with saturated fatty acids, whereas in apricots also oleic acid was detected. The presence of dimyristoyl–lutein, myristoyl–palmitoyl–lutein and dipalmitoyl–lutein in *C. pepo* has also been reported by Khachik and Beecher (1988), Khachik, Beecher, and Lusby (1988) and Breithaupt, Wirt, and Bamedi (2002).

To assess the suitability of carotenoid esters for authentication, apricot purée blended with pumpkin mash was investigated. Using LC-MS, the admixture of pumpkin could unambiguously be detected in purées containing 5% pumpkin mash of the cultivars Bischofsmütze, Golden Nuggets and Hokkaido I and II by the presence of the characteristic ions of antheraxanthin myristate [*m/z* 795, $M+H^+$]. Accordingly, lutein-di-myristate (peak 23) could be detected in all samples containing 5% pumpkin purée except for the pumpkin cultivar Muscade de Provence which did not contain lutein-di-myristate as a major compound. However, admixtures of 5% Muscade de Provence could be identified by the presence of lutein-di-laurate and α-carotene, as mentioned previously. Further carotenoid esters (lutein–laurate–myristate, lutein–myristate–palmitate) originating from pumpkin were found in instances where $\geqslant 10\%$ pumpkin mash was added to the apricot purée.

4. Concluding remarks

From the present study it becomes evident that the carotenoid fingerprints of apricots differed from that of most pumpkin cultivars investigated. However, within the pumpkin samples a pronounced variability in the quantitative composition of the predominant carotenoids was observed. As a consequence, it is difficult to provide a general strategy for the detection of fraudulent admixtures of pumpkin purée to apricot products solely based on carotenoids. Our findings that apricot and pumpkin xanthophyll esters show a differing profile of fatty acids, with oleic acid esters being present exclusively in apricot, suggests that esterified carotenoids should also be included in authenticity studies. However, given that characteristic esters involve minor compounds, detection of 5% of pumpkin addition is limited by the minor nature of detectable differences in the acylated fatty acids. Again, it should also be stressed that such studies should not be limited to one particular class of compounds. We have recently demonstrated that the neutral sugar profile of the hemicellulose fraction represents a diagnostically important tool for plant species determination (Fügel, Carle, & Schieber, 2004; Kurz et al., 2008). The carotenoid fingerprint could be another valuable contribution to the detection of adulterations of apricot based products by addition of low cost pumpkins. Due to the complex carotenoid composition and their structural similarity, the use of hyphenated techniques such as HPLC with diode array and mass spectrometric detection is strongly recommended.

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