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# Identification and Quantitation of Carotenoids and Tocopherols in Seed Oils Recovered from Different Rosaceae Species

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**ABSTRACT:** Seed oils recovered from Rosaceae species such as dessert and cider apples (*Malus domestica* Borkh.), quince (*Cydonia oblonga* Mill.), and rose hip (*Rosa canina* L.) were analyzed for their tocopherol and carotenoid contents using HPLC-DAD-$MS^n$ following saponification. Qualitative and quantitative tocopherol and carotenoid compositions significantly differed, not only among the different genera but also among cultivars of one species. In particular, seed oils of cider apples were shown to contain higher amounts of both antioxidant classes than that of dessert apples. Total contents of tocopherols of the investigated Rosaceous seed oils ranged from 597.7 to 1099.9 mg/kg oil, while total carotenoid contents varied between 0.48 and 39.15 mg/kg oil. Thus, these seed oils were found to contain appreciable amounts of lipohilic antioxidants having health beneficial potential. The results of the present study contribute to a more economical and exhaustive exploitation of seed byproducts arising from the processing of these Rosaceous fruits.

**KEYWORDS:** *Rosaceous plants, sunflower, seed oil, saponification, antioxidants, tocopherols, carotenoids, authenticity control*

## INTRODUCTION

In the course of the production of juices, jellies, and jams, seeds of Rosaceous fruits such as apple, quince (Maloideae), and rose hip (Rosoideae) are usually removed, thus resulting in appreciable quantities of byproducts hardly exploited so far. However, there is good evidence that the recovery of lipids would be a promising alternative for an economical and exhaustive exploitation of these agro-industrial byproducts. Because of their high proportions of unsaturated fatty acids, namely, the essential linoleic and oleic acids, the recovered oils are considered nutritionally valuable edible oils. As potential ingredients, these oils may be of great interest for the pharmaceutical or cosmetic industries as well.[1−3] However, their high degree of unsaturation renders vegetable oils susceptible to lipid peroxidation and autoxidative rancidity.[4] Hence, apart from the fatty acid profile, the presence of endogenous minor constituents exerting antioxidant properties such as tocopherols and carotenoids is another crucial factor improving the oxidative stability, and consequently the shelf life, of vegetable oils and of products derived therefrom.[5,6]

Tocopherols belong to a group of lipid-soluble compounds generally referred to as vitamin E, encompassing $\alpha$-, $\beta$-, $\gamma$-, and $\delta$-isomers of the aforementioned tocopherols and the corresponding unsaturated tocotrienols, respectively. Because of their ability to scavenge free radicals involving a tocopherol−tocopheryl semiquinone redox system, tocopherols are the most important natural antioxidants. Moreover, these compounds were shown to exhibit various beneficial effects on degenerative diseases, such as atherosclerosis, cardiovascular disease, Alzheimer's disease, or certain types of cancer.[7−13] Furthermore, protective effects of vitamin E have been reported with respect to aging and UV-induced skin damage.[14,15]

Carotenoids are another class of potent antioxidants acting as free radical scavengers.[16] They form a diverse group of more than 600 structurally related polyisoprenoids (tetraterpenoids) synthesized by all higher plants, algae, and bacteria.[17] Carotenoids may be classified further as carotenes being devoid of oxygen and oxidized xanthophylls. Because of their extended system of conjugated double bonds, carotenoids are important lipophilic pigments capable of quenching singlet oxygen, thus protecting the plant from photo-oxidative damage.[18] Approximately 50 of them (e.g., $\beta$-carotene) are precursors of vitamin A, which is essential for vision and cell proliferation.[19] Furthermore, their protective role against several chronic degenerative diseases, such as cancer, cardiovascular disease, cataracts, and age-related macular degeneration, is well documented.[20−22] Because of their structural diversity, their occurrence as *cis* and *trans* isomers, and their instability toward sunlight, heat, or oxygen, the analysis of carotenoids is quite challenging.[19,23]

Despite their high degree of unsaturation, comprehensive data on the presence and amounts of antioxidative constituents in Rosaceous seed oils are scarce or even lacking in the literature. More detailed information about such health beneficial antioxidants, however, is a prerequisite for establishing economically feasible oil recovery processes, thus contributing to sustainable food production and byproduct valorization. Therefore, the aim of the present work was to investigate the profiles of tocopherols and carotenoids and to determine their contents in seed oils recovered from apple (*Malus domestica* Borkh.), quince (*Cydonia oblonga* Mill.), and rose hip (*Rosa canina* L.). For comparison, investigations of the antioxidants present in sunflower seed oil should also be included, because the latter is characterized by a fatty acid profile similar to that of apple. Therefore, a fraudulent

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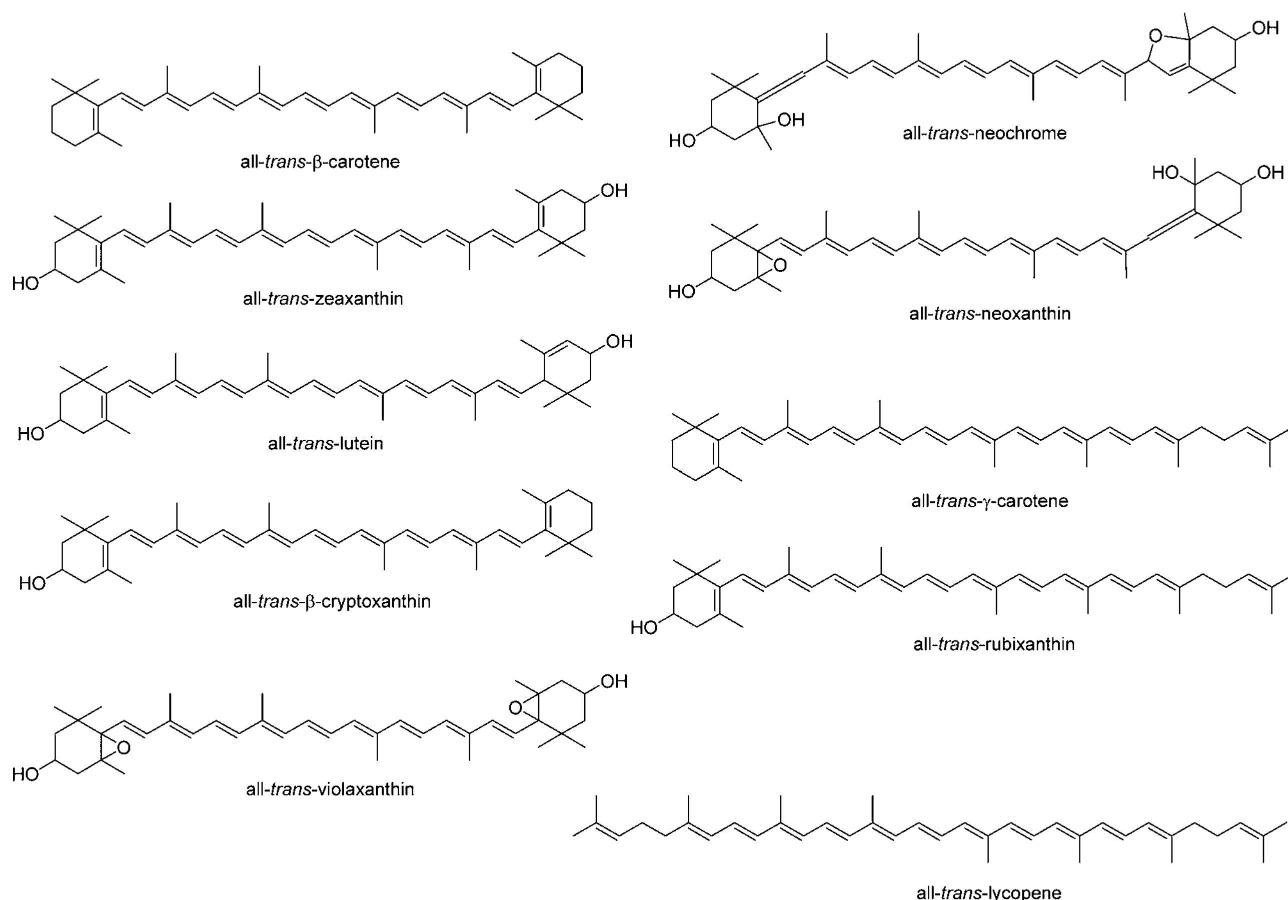


**Figure 1.** Chemical structures of the all-*trans* carotenoids detected in seed oils recovered from apple, quince, rose hip, and sunflower.

admixture of widely available sunflower oil to rare apple seed oil seems to be very likely. Hence, it should be evaluated whether their tocopherol and carotenoid compositions might be used for authenticity control.

## ■ MATERIALS AND METHODS

**Chemicals.** All reagents and solvents were purchased from Merck (Darmstadt, Germany) and were of analytical and HPLC grade, respectively. $\alpha$-, $\beta$-, $\gamma$-, and $\delta$-Tocopherol and all-*trans*-$\beta$-carotene used for quantitation were purchased from Sigma-Aldrich (Steinheim, Germany). Solutions of additional carotenoids (CaroteNature, Lupsingen, Switzerland) were available from a previous work.[24] However, because of their limited quantities and purities, they were used for qualitative purposes only.

**Plant Material.** Authenticated apple cultivars harvested in 2009 were provided by local growers and the Hohenheim University research station for horticulture (Stuttgart, Germany). Different batches of quince fruits (II, IV, both harvested in 2009; III, harvested in 2010) and rose hip fruits (harvested in 2010) were obtained from local growers of the same region. Additionally, sun-dried quince seeds (quince I, harvested in 2010) originated from Northern India (Himalaya). Seeds of the Rosaceous plants under investigation were separated manually, lyophilized, and finely ground as recently reported in detail.[3] For comparative analyses, sunflower seeds obtained from a local oil mill were also included.

**Lipid Extraction.** Approximately 10 g of seed flour was extracted with 100 mL of *n*-hexane by continuous stirring for 10 min at ambient temperature. This extraction procedure was repeated three times. To avoid degradation and isomerization of tocopherols and carotenoids, respectively, extraction was performed under nitrogen atmosphere using amber glassware. The filtered and combined organic filtrates were evaporated at 30 °C in vacuo to constant weight. The recovered oils were stored in the dark at −80 °C under nitrogen until further analyses.

**Saponification of the Oils.** For removal of triacylglycerides and chlorophylls, oil samples were saponified prior to analyses of tocopherols and carotenoids. Briefly, approximately 0.5 g of oil was saponified by adding 50 mL of 10% (w/w) potassium hydroxide in methanol and 0.25 g of ascorbic acid to prevent the loss of lipid-soluble vitamins and their precursors. For complete saponification, samples were gently stirred at ambient temperature under nitrogen for 1 (apple, quince) and 24 h (rose hip), respectively. Subsequently, 50 mL of deionized water was added. The resulting suspension was transferred to a separatory funnel and extracted four times with proportions of 50 mL of *n*-hexane−ethyl acetate (85:15; v/v). The combined organic fractions were washed with deionized water to neutral pH. After they were dried with anhydrous sodium sulfate, the organic solvents were evaporated to dryness at 30 °C in vacuo, and the residue was redissolved in 2-propanol. Finally, samples were membrane-filtered (0.45 $\mu$m) and stored at −80 °C until high-performance liquid chromatography (HPLC) analyses. Amber glassware was used throughout under dim light conditions to minimize decomposition and isomerization of carotenoids and tocopherols.

**Determination of Tocopherols and Carotenoids Using Reversed-Phase HPLC−Diode Array Detection (DAD).** All HPLC analyses were performed using 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/vis photodiode array detector controlled by a Millennium 32 workstation (version 3.2) (Waters, Milford, MA). Separation was carried out on a 150 mm × 3.0 mm i.d., 3 $\mu$m particle size, analytical scale YMC C-30 reversed-phase column (Wilmington, MA) operated at 25 °C. Spectra were recorded in the range of 200−700 nm at a flow rate of 0.42 mL/min.

Table 1. Contents of Individual Tocopherols in Oils Recovered from Seeds of Different Apple Cultivars, Quince, Rose Hip, and Sunflower (mg/kg Oil)[a]

| tocopherols | α- | β- | γ- | δ- | total |
|---|---|---|---|---|---|
| | | | apple cultivars | | |
| Bittenfelder | 462.3 ± 16.4 cd | 564.1 ± 18.3 a | 10.9 ± 0.7 d | 15.0 ± 0.2 e | 1052.4 ± 24.6 a |
| Gewürzluike | 274.7 ± 13.5 ef | 410.2 ± 21.6 b | 82.4 ± 5.6 b | 110.5 ± 7.0 a | 877.8 ± 27.0 bc |
| Brettacher | 536.1 ± 33.1 c | 391.0 ± 20.8 b | 15.1 ± 2.0 cd | 12.1 ± 0.3 ef | 954.3 ± 39.1 ab |
| Bohnapfel | 437.4 ± 13.2 cd | 569.1 ± 15.0 a | 25.1 ± 1.2 bcd | 34.3 ± 0.6 d | 1065.9 ± 20.0 a |
| Royal Gala | 290.7 ± 20.2 e | 380.2 ± 23.3 b | 79.3 ± 6.0 bc | 83.0 ± 4.9 c | 833.2 ± 31.8 bc |
| Pinova | 273.5 ± 12.0 ef | 367.8 ± 10.2 b | 87.6 ± 4.1 b | 96.8 ± 5.8 b | 825.7 ± 17.3 bc |
| quince I | 525.4 ± 15.0 c | 31.7 ± 2.4 c | 28.2 ± 0.4 bcd | 12.4 ± 0.9 ef | 597.7 ± 15.2 d |
| quince II | 710.6 ± 54.8 ab | 26.1 ± 0.7 c | 16.9 ± 0.3 cd | 2.5 ± 0.0 ef | 756.1 ± 54.8 dc |
| quince III | 780.7 ± 18.1 a | 33.5 ± 0.6 c | 26.4 ± 1.4 bcd | 2.5 ± 0.3 ef | 843.1 ± 18.2 bc |
| quince IV | 667.0 ± 35.4 b | 22.7 ± 0.5 c | 27.9 ± 2.9 bcd | 2.3 ± 0.4 ef | 719.9 ± 35.5 dc |
| rose hip | 173.4 ± 5.1 f | ND | 895.4 ± 55.6 a | 31.2 ± 3.7 d | 1099.9 ± 55.9 a |
| sunflower | 373.3 ± 17.4 de | 39.0 ± 3.1 c | 7.4 ± 0.1 d | 4.1 ± 0.2 ef | 423.8 ± 17.6 e |

[a]Results are expressed as means ± standard deviations ($n$ = 2). Identical letters within the same row indicate that samples did not significantly differ.

**HPLC System I (Tocopherols).** A mobile phase consisting of methanol/water (96:4; v/v, eluent A) and methanol/methyl *tert*-butyl ether (MTBE)/water (4:92:4; v/v, eluent B) was employed for the separation of tocopherols. The gradient used was as follows: 0−58% B (40 min), 58−100% B (1 min), 100% B isocratic (5 min), and 0% B isocratic (9 min). The total run time was 55 min. The injection volume of all samples was 10 μL. Tocopherols were monitored at 292, 296, and 298 nm for the α-, β-, γ-, and δ-isomers, respectively. Individual tocopherols were quantitated using calibration curves of the corresponding compounds at their specific absorption maximum. Concentrations of stock solutions were determined spectrophotometrically using their specific absorption coefficients ($A^{1\%}_{1cm}$) as previously published.[25]

**HPLC System II (Carotenoids).** The carotenoid determination was performed according to the methods previously reported[24,26] with some modifications. To improve the separation of the more polar xanthophylls, the proportion of MTBE in eluent A had to be reduced. Thus, the mobile phase employed consisted of methanol/MTBE/water (91:5:4; v/v, eluent A) and methanol/MTBE/water (4:92:4; v/v, eluent B). For the analysis of rose hip samples, the same gradient was used as described previously.[24] The stop time was set at 75 min. For the remaining samples, however, the following gradient was used: 0−60% B (45 min), 100% B isocratic (5 min), and 0% B isocratic (5 min). The total run time was 55 min.

Carotenoids were quantitated using a calibration curve of all-*trans*-β-carotene at 450 nm. The concentration of the stock solution was verified spectrophotometrically using the specific absorption coefficient ($A^{1\%}_{1cm}$) as previously published.[27]

**HPLC-DAD-MS$^n$.** 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 gradient pump, a model G1329/G1330A thermo-autosampler, a model G1316A column oven, and a model G1315A diode array detector. The analyses were performed under the conditions described above. Injection volumes were from 10 to 50 μL. 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 online to a Bruker (Bremen, Germany) model Esquire 3000+ ion trap mass spectrometer fitted with an atmospheric pressure chemical ionization (APCI) source. Positive ion mass spectra of the column eluate were recorded in the range of $m/z$ 50−1100. The flow rate of nitrogen as drying gas was 4.0 L/min, and the nebulizer pressure was 50 psi. The nebulizer temperature was set at 350 °C, and a potential of 2779 kV was used on the capillary. The corona was set at 4000 nA in the positive ion mode, and the vaporizer temperature was set at 400 °C. Helium was used as the collision gas for collision-induced dissociation (CID) at a pressure of $4.9 \times 10^{-6}$ mbar. CID spectra were obtained with an isolation width of 2.0 Th for precursor ions and a fragmentation amplitude of 1.0 V. The identification of the carotenes and xanthophylls was based on the comparison of chromatographic properties on a $C_{30}$ column, UV/vis data (maximum absorption wavelengths ($\lambda_{max}$), spectroscopic fine structures (% III/II), *cis*-peak intensities (% $A_B/A_{II}$)), mass spectrometric characteristics, and literature data available.

**Statistical Analysis.** All experiments were performed in duplicate. Significant differences ($\alpha$ = 0.05) were determined using the Tukey test for different independent samples. Data evaluation was performed with SAS software package (SAS Institute, Cary, NC, software version 9.1).

## RESULTS AND DISCUSSION

**Qualitative and Quantitative Composition of Tocopherols.** The tocopherol compositions of the apple seed oils, quince, rose hip, and sunflower seed oils analyzed using HPLC are shown in Table 1. α-, β-, γ- and δ-Tocopherols were identified by comparison of their retention times with those of authentic reference compounds. Not only the tocopherol profile but also their individual amounts in the different seed oils significantly differed.

With the exception of the cv. 'Brettacher', β-tocopherol was the prevailing tocopherol isomer present in apple seed oils, showing contents between 367.8 and 569.1 mg/kg. α-Tocopherol amounts were only somewhat lower ranging from 273.5 (cv. 'Pinova') to 536.1 mg/kg (cv. 'Brettacher'). Hence, the ratio of α- and β-tocopherols was approximately 1:1. Interestingly, the total contents of tocopherols were lowest for the seed oils recovered from the dessert apple cultivars 'Royal Gala' (833.2 mg/kg) and 'Pinova' (825.7 mg/kg), whereas cider apple cultivars such as 'Rheinischer Bohnapfel' and 'Bittenfelder' exhibited significantly higher contents (1065.9 and 1052.4 mg/kg, respectively). Similar findings were reported when comparing the phenolic contents of cider and dessert apple also demonstrating the first to exhibit significantly higher amounts.[28] γ- and δ-Isomers were present as minor constituents with amounts ranging from 10.9 to 87.6 mg/kg and 12.1 to 110.5 mg/kg, respectively. These results are in accordance with those of a previous study investigating different types of vegetable oils for their tocopherol contents.[29] The authors

reported a comparable tocopherol composition for apple seed oil. Moreover, the results of the aforementioned study demonstrated this type of oil to be richer in health-promoting tocopherols than almond oil, which is generally considered a most valuable cosmetic oil. Consequently, the application of apple seed oils in cosmetics seems to be a promising alternative to widely used almond oil.

The tocopherol profile of quince seed oils significantly differed from those of apple and rose hip seed oils with $\alpha$-tocopherol being clearly the preponderant isomer making up 88−94% of the total tocopherol content. The total contents ranged from 597.7 (quince I) to 843.1 mg/kg (quince III), thus being comparable to those of the dessert apple cultivars.

In contrast to the tocopherol profile of apple and quince seed oils, $\gamma$-tocopherol was most abundant in the seed oil recovered from rose hip, showing a content of 895.4 mg/kg. Thus, $\gamma$-tocopherol contributed to 88% of total tocopherol content, which was found to be 1099.9 mg/kg.

Additional analysis of sunflower seed oil revealed this type of oil to contain significantly lower amounts of vitamin E isomers, resulting in the lowest total tocopherol content (423.8 mg/kg) being approximately half that of the apple seed oils under investigation. $\alpha$-Tocopherol was clearly the preponderant constituent amounting to 373.3 mg/kg (88%) in sunflower seed oil, which was consistent with previous findings on different types of sunflower oils.[29] Hence, tocopherol profiles are likely to be a suitable tool to distinguish between seed oils originating from apple and sunflower, respectively. This might be useful in the detection of adulterations of higher priced apple seed oils with widely available sunflower seed oil, thus allowing their authentication. However, further corroboration of these findings based on a broader range of samples is required.

**Carotenoids.** Identification of the carotenoids was based on the combined information obtained from their chromatographic behavior on a $C_{30}$ column, UV/vis and mass spectrometric characteristics compared to literature data, and available authentic reference compounds. The chemical structures of the all-*trans* isomers of the carotenoids detected in the Rosaceous seed oils under study are presented in Figure 1. Because of the variation of the carotenoids among the different samples and due to the lack of authentic reference compounds suitable for quantitation purposes, their contents were quantitated solely using all-*trans*-$\beta$-carotene, providing an overview of their contents.

**Carotenoid Profiles (Maloideae, Sunflower).** Analysis of carotenoids was conducted because the oils recovered from the seed byproducts of apple and quince exhibited marked yellow color hues, indicating the presence of appreciable amounts of these lipophilic pigments. Moreover, to the best of our knowledge, there are no data available dealing with carotenoids of seed oils from these Rosaceae. Economically important sunflower seed oil was also included in our investigations because its fatty acid distribution is very similar to that of apple seed oils. Hence, differences in the carotenoid profile might be instrumental in authenticity assessment. Saponification of the seed oils proved to be indispensible, since the removal of abundant neutral lipids and the hydrolysis of carotenoid esters allow considerable enrichment of the parent carotenoids, thus facilitating their isolation and determination. Figure 2 shows typical carotenoid profiles of apple (A), quince (B), and sunflower (C) seed oils after alkaline hydrolysis recorded at 450 nm, illustrating the presence of a restricted number of parent carotenoids (Table 2).

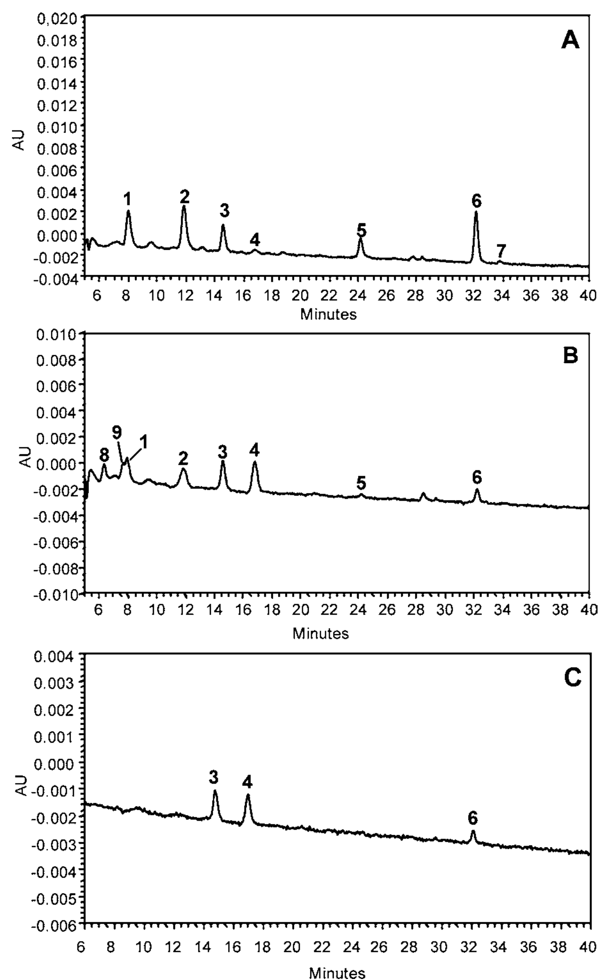


Figure 2. HPLC chromatograms of carotenoids in seed oils recovered from apple (A), quince (B), and sunflower (C) at a detection wavelength of 450 nm, respectively. For peak assignment, see Table 2.

Compound **1** eluting at 7.9−8.0 min revealed a protonated molecular ion $[M + H]^+$ at $m/z$ 601 producing a fragment ion at $m/z$ 583 in subsequent $MS^2$ experiments, thus indicating the loss of a water molecule as already reported for xanthophylls.[30,31] Taking also into account its UV/vis characteristics ($\lambda_{max}$, % III/II, and % $A_B/A_{II}$) and its eluting behavior, compound **1** was identified as all-*trans*-violaxanthin. This assignment was confirmed by coelution with the respective reference compound. Because of the similar fragmentation pathway in MS/MS experiments in combination with the reduced spectroscopic fine structure as compared to all-*trans*-violaxanthin, peak **2** was assigned to a *cis*-isomer of violaxanthin. This assumption was corroborated by the observation of a hypsochromic shift of $\lambda_{max}$ (∼3 nm) and by the appearance of an additional "*cis*-peak" with low intensity at 326 nm, indicating the presence of a peripheral double bond. In agreement with previous findings on violaxanthin isomers,[32,33] compound **2** eluting after all-*trans*-violaxanthin was identified as 9-*cis*-violaxanthin.

On the basis of their elution order and differing UV/vis spectroscopic data, compounds **3** and **4** were assumed to be all-*trans*-lutein and all-*trans*-zeaxanthin. However, under the conditions applied, both compounds provided identical

Table 2. Chromatographic, UV/vis, and Mass Spectrometric Characteristics of Carotenoids in Seed Oils of Apple, Quince, and Sunflower

| no.[a] | carotenoid | $t_R$ (min)[b] | $\lambda_{max}$ (nm) | % III/II | % $A_B/A_{II}$ | $[M + H]^+$ ($m/z$) | MS/MS fragment ions ($m/z$) |
|---|---|---|---|---|---|---|---|
| **8** | all-*trans*-neoxanthin | 7.4−7.5 | 416/440/470 | 88 | | 601 | 583 [M + H − 18] |
| **9** | 9-*cis*-neoxanthin | 7.7−7.8 | 326/416/439/468 | 55 | 24 | 601 | 583 [M + H − 18] |
| **1** | all-*trans*-violaxanthin[c] | 7.9−8.0 | 416/439/468 | 100 | | 601 | 583 [M + H − 18] |
| **2** | 9-*cis*-violaxanthin | 11.8−11.9 | 326/412/435/464 | 90 | 6 | 601 | 583 [M + H − 18] |
| **3** | all-*trans*-lutein[c] | 14.5−14.6 | 423/445/472 | 63 | | 569 | 551 [M + H − 18], 495 [M + H − 18−56],[d] 430 [M + H − 139][d] |
| **4** | all-*trans*-zeaxanthin[c] | 16.7−16.8 | 425/450/477 | 33 | | 569 | 551 [M + H − 18] |
| **5** | all-*trans*-*β*-cryptoxanthin | 24.1−24.2 | 425/451/477 | 28 | | 553 | 535 [M + H − 18] |
| **6** | all-*trans*-*β*-carotene | 32.1−32.2 | 426/451/477 | 19 | | 537 | 444 [M − 92] |
| **7** | 9-*cis*- *β*-carotene | 33.7−33.8 | 342/423/446/471 | 25 | 8 | 537 | 444 [M − 92] |

[a]Peak number in Figure 2. [b]Range of retention times for different sample types. [c]Cochromatography with corresponding reference compound. [d]According to ref 19.

Table 3. Contents of Individual Carotenoids (mg/kg Oil) in Seed Oils Recovered from Different Apple Cultivars[a]

| | Bittenfelder | Gewürzluike | Brettacher | Bohnapfel | Royal Gala | Pinova |
|---|---|---|---|---|---|---|
| all-*trans*-neoxanthin | ND | ND | 0.96 ± 0.02 a | ND | ND | ND |
| 9-*cis*-neoxanthin | ND | ND | ND | ND | ND | ND |
| all-*trans*-violaxanthin | 1.66 ± 0.23 b | 0.14 ± 0.03 d | 4.26 ± 0.03 a | 0.86 ± 0.09 c | 1.75 ± 0.06 b | 0.71 ± 0.03 c |
| 9-*cis*-violaxanthin | 2.07 ± 0.15 c | 0.23 ± 0.05 gh | 5.76 ± 0.09 a | 1.33 ± 0.02 e | 2.78 ± 0.03 b | 1.64 ± 0.04 d |
| all-*trans*-lutein | 0.94 ± 0.09 b | 0.33 ± 0.02 de | 1.21 ± 0.06 a | 0.41 ± 0.01 cd | 0.46 ± 0.02 cd | 0.36 ± 0.00 cde |
| all-*trans*-zeaxanthin | 0.15 ± 0.02 c | tr | 0.10 ± 0.00 c | 0.08 ± 0.01 c | tr | 0.06 ± 0.00 c |
| all-*trans*-*β*-cryptoxanthin | 0.79 ± 0.06 a | 0.14 ± 0.01 cd | 0.27 ± 0.02 b | 0.07 ± 0.01 de | 0.12 ± 0.01 cd | 0.23 ± 0.03 b |
| all-*trans*-*β*-carotene | 2.03 ± 0.14 b | 0.12 ± 0.03 efg | 3.10 ± 0.12 a | 0.40 ± 0.04 d | 0.08 ± 0.01 fg | 0.07 ± 0.00 fg |
| 9-*cis*-*β*-carotene | tr | ND | 0.13 ± 0.02 | tr | ND | tr |
| total | 7.63 ± 0.33 b | 0.95 ± 0.07 e | 15.80 ± 0.17 a | 3.15 ± 0.10 d | 5.19 ± 0.07 c | 3.01 ± 0.05 d |

[a]Results are expressed as means ± standard deviations ($n$ = 2). Identical letters within the corresponding lines in Tables 3 and 4 indicate that samples did not significantly differ.

protonated molecular ions $[M + H]^+$ at $m/z$ 569 and characteristic dehydrated fragment ions at $m/z$ 551 in subsequent MS/MS experiments. Nevertheless, lutein and zeaxanthin could be distinguished according to recently reported data,[19] demonstrating the $MS^2$ fragments at $m/z$ 430 and at $m/z$ 495 to be indicative of lutein. Furthermore, additional cochromatography experiments with the corresponding standards verified the assignment of peaks **3** and **4** to the all-*trans* isomers of lutein and zeaxanthin, respectively.

By comparison of its $\lambda_{max}$ values (425/451/477) and spectroscopic fine structure with literature data,[34,35] compound **5** was identified as all-*trans*-*β*-cryptoxanthin, showing an identical retention time as the corresponding reference compound. This assignment was corroborated by the fragmentation pattern of compound **5**, revealing an $[M + H]^+$ at $m/z$ 553 and a typical fragment ion at $m/z$ 535, resulting from the loss of water, thus being in accordance with published data.[35]

Compound **6** eluting relatively late ($t_R$ ~ 24 min) exhibited UV/vis and mass spectrometric characteristics being similar to those reported in literature for all-*trans*-*β*-carotene.[26,27,36] Furthermore, compound **6** exhibited identical retention behavior as the corresponding standard. Therefore, peak **6** was assigned to the all-*trans* isomer of *β*-carotene. Mass spectrometric analysis of compound **7** shortly eluting thereafter revealed a protonated molecular ion $[M + H]^+$ at $m/z$ 537 and a $MS^2$ fragment at $m/z$ 444, resulting from the elimination of toluene. Hence, compound **7** was concluded to be another *β*-carotene isomer. This assumption was confirmed by its UV/vis spectrum showing a hypsochromic shift (~6 nm) and an additional "*cis*-peak" at 342 nm. Considering the elution order and the low intensity of the "*cis*-peak", compound **7** was identified as 9-*cis*-*β*-carotene.

Two additional carotenoid structures were detected only in samples deriving from quince. In our MS/MS experiments, compounds **8** and **9** exhibited comparable $\lambda_{max}$ values and identical fragmentation patterns, most likely indicating a neoxanthin structure. Taking into account their chromatographic properties under the conditions applied for separation, **8** was tentatively identified as the all-*trans* isomer of neoxanthin, whereas compound **9** coeluting with all-*trans*-violaxanthin was assumed to be 9-*cis*-neoxanthin. This assumption was consistent with the appearance of an additional "*cis*-peak" at 326 nm of relatively low intensity as already reported for the fruit pulp of *Artocarpus heterophyllus*.[36]

In summary, all-*trans*-violaxanthin, 9-*cis*-violaxanthin, all-*trans*-lutein, all-*trans*-zeaxanthin, all-*trans*-*β*-cryptoxanthin, all-*trans*-*β*-carotene, and 9-*cis*-*β*-carotene were identified in apple and quince seed oils. Seed oil recovered from quince additionally contained all-*trans*-neoxanthin and 9-*cis*-neoxanthin, while sunflower seed oil only contained the all-*trans* isomers of lutein, zeaxanthin, and *β*-carotene. The pigments detected are the predominant carotenoids in chloroplasts of higher plants, probably originating from photosynthetic tissues of the embryo in the seeds.[37]

**Quantitative Composition of Carotenoids (Maloideae, Sunflower).** As summarized in Table 3, amounts of carotenoids occurring in apple seed oils significantly differed. Total contents ranged from 0.95 (cv. 'Gewürzluike') to 15.80 mg/kg (cv. 'Brettacher'). This was in accordance with


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Table 4. Contents of Individual Carotenoids (mg/kg Oil) of Quince and Sunflower Seed Oils[a]

| | quince I | quince II | quince III | quince IV | sunflower |
|---|---|---|---|---|---|
| all-*trans*-neoxanthin | ND | tr | 0.36 ± 0.01 b | tr | ND |
| 9-*cis*-neoxanthin | ND | 0.17 ± 0.04 b | 0.34 ± 0.03 a | 0.08 ± 0.03 c | ND |
| all-*trans*-violaxanthin | 0.07 ± 0.01 d | 0.07 ± 0.00 d | 0.58 ± 0.02 c | 0.06 ± 0.03 d | ND |
| 9-*cis*-violaxanthin | ND | 0.43 ± 0.06 g | 0.96 ± 0.00 f | 0.39 ± 0.02 g | ND |
| all-*trans*-lutein | 0.23 ± 0.03 e | 0.87 ± 0.05 b | 0.97 ± 0.04 b | 0.53 ± 0.02 c | 0.4 ± 0.02 cd |
| all-*trans*-zeaxanthin | 0.13 ± 0.01 c | 1.13 ± 0.10 a | 1.22 ± 0.09 a | 1.16 ± 0.00 a | 0.48 ± 0.01 b |
| all-*trans*-$\beta$-cryptoxanthin | ND | 0.06 ± 0.00 de | 0.08 ± 0.01 de | 0.20 ± 0.03 bc | ND |
| all-*trans*-$\beta$-carotene | 0.05 ± 0.01 g | 0.87 ± 0.05 c | 0.31 ± 0.01 def | 0.33 ± 0.01 de | 0.19 ± 0.03 defg |
| 9-*cis*-$\beta$-carotene | ND | tr | ND | ND | ND |
| total | 0.48 ± 0.04 e | 3.60 ± 0.15 d | 4.80 ± 0.10 c | 2.74 ± 0.06 d | 1.08 ± 0.04 e |

[a]Results are expressed as means ± standard deviations ($n = 2$). Identical letters within the corresponding lines in Tables 3 and 4 indicate that samples did not significantly differ.

the visual appearance of the extracted oils of the apple cultivars 'Gewürzluike', 'Pinova', and 'Royal Gala', each of them exhibiting a faint yellow tint. For most of the apple samples, major carotenoids were all-*trans*-violaxanthin and 9-*cis*-violaxanthin, accounting for 15% (cv. 'Gewürzluike') to 34% (cv. 'Royal Gala') and for 24% (cv. 'Gewürzluike') and even for 54% (cvs. 'Royal Gala' and 'Pinova') of total carotenoids, respectively. All-*trans*-$\beta$-carotene contents varied from 0.07 mg/kg (cv. 'Pinova') to 3.10 mg/kg (cv. 'Brettacher'). Hence, all-*trans*-$\beta$-carotene accounted for 2 up to 27% of the total contents, indicating seed oils from cider apple cultivars to be richer in $\beta$-carotene than those from dessert apple cultivars, for example, cv. 'Pinova'.

According to Table 4, the total carotenoid contents of quince seed oils were relatively low coming close to those of the apple cultivars with the lowest amounts such as 'Gewürzluike' and 'Royal Gala'. The total carotenoid content of quince seed oils ranged from 0.48 (quince I) to 4.80 mg/kg (quince III). Seed oil recovered from sample quince I showed the lowest contents of individual carotenoids. This might be ascribed to differing drying conditions causing oxidative losses of carotenoids as compared to the remaining quince samples. In contrast to the seed oils recovered from apple, all-*trans*-zeaxanthin and all-*trans*-lutein were the major carotenoids of quince seed oils. With their contents varying from 0.13 (quince I) to 1.22 mg/kg (quince III) and 0.23 (quince I) to 0.97 mg/kg (quince III), all-*trans*-zeaxanthin and all-*trans*-lutein accounted for 25−42 and 19−48% of total carotenoids, respectively. Moreover, quince seed oils were found to contain minor contents of 9-*cis*-neoxanthin, ranging between 0.08 (quince IV) and 0.34 mg/kg (quince III). Thus, their differing carotenoid profiles appear to be suitable tools to distinguish between oils originating from apple and quince seeds, respectively. In quince, all-*trans*-$\beta$-carotene was also present in appreciable amounts ranging from 0.05 (quince I) to 0.87 mg/kg (quince II), while 9-*cis*-violaxanthin contents varied between 0.39 (quince IV) and 0.96 mg/kg (quince III). Both all-*trans*-$\beta$-carotene and 9-*cis*-violaxanthin reached a maximum of 24 and 20% of total carotenoid contents for the samples quince II and III, respectively.

The carotenoid profile of sunflower seed oil recovered under identical conditions was restricted to the all-*trans* isomers of zeaxanthin, lutein, and $\beta$-carotene, yielding a total carotenoid content of 1.08 mg/kg. Analogous to quince seed oils, all-*trans*-zeaxanthin and all-*trans*-lutein were the major carotenoids accounting for 44 and 37% of total carotenoid contents, respectively. Thus, besides the tocopherol profile, the carotenoid profile of sunflower seed oil may serve as a further criterion for the authentication of apple and sunflower seed oils.

**Carotenoid Profile (Rosoideae).** Figure 3 displays the chromatogram of the saponified rose hip seed oil. Retention times, UV/vis, and mass spectrometric characteristics are listed in Table 5. In contrast to apple and quince, rose hip seed oil is characterized by a broader diversity of carotenoids. Because the rose hip seeds were isolated from the pulp, residual carotenoids originating from the edible parts of the fruit, for example, lycopene, could not be totally avoided.

In subsequent MS/MS experiments, compound **1** exhibited a protonated molecular ion at $m/z$ 601 and fragment ions typical of xanthophylls (Table 5). Consequently, considering its UV/vis characteristics, compound **1** was assumed to be all-*trans*-violaxanthin, which again was corroborated by coelution with the authentic reference compound. Because of the similarity of the UV/vis and mass spectra features with that of all-*trans*-violaxanthin, peak **3** was assigned to the 9-*cis*-isomer. This assignment is in accordance with the presence of an additional "*cis*-peak" at 328 nm and the observed elution order on the column used for separation.

Compound **2** showed UV/vis and mass spectra being in accordance with literature[27,36] recorded for the all-*trans* isomers of neochrome and luteoxanthin. Because of the lack of authentic standards, their distinction by cochromatography was not feasible. However, compound **2** was tentatively identified as all-*trans*-neochrome according to reports on its elution behavior using the same column and mobile phase, thus demonstrating all-*trans*-neochrome to elute closely to all-*trans*-violaxanthin, and therefore somewhat earlier than all-*trans*-luteoxanthin.[23,33] On the basis of these findings and because of similar mass spectrometric and UV/vis data indicating the presence of a *cis* bond (% $A_B/A_{II}$, $\lambda_{max}$), compound **4** was tentatively identified as a *cis*-neochrome isomer. According to the aforementioned study on the chromatographic behavior of various epoxycarotenoid isomers,[33] compound **4** was tentatively identified as 9-*cis*-neochrome, since the *cis*-isomers were shown to elute after the all-*trans*-isomers.

Compounds **5** and **6** were identified as all-*trans*-lutein and all-*trans*-zeaxanthin, respectively, based on their UV/vis and mass spectra as described earlier. Their identification was corroborated by comparison of their chromatographic properties with that of authentic standards.

Peak **8** showed an absorption spectrum with $\lambda_{max}$ at 425, 451, and 476 nm, being devoid of an additional "*cis*-peak", and mass spectrometric data were in accordance with literature data.[36,38] Consequently, compound **8** was identified as the all-*trans*-

**Table 5. Chromatographic, UV/vis, and Mass Spectrometric Characteristics of Carotenoids Detected in Rose Hip Seed Oil**

| no.[a] | carotenoid | $t_R$ (min) | $\lambda_{max}$ (nm) | % III/II | % $A_B/A_{II}$ | $[M + H]^+$ ($m/z$) | MS/MS fragment ions ($m/z$) |
|---|---|---|---|---|---|---|---|
| **1** | all-*trans*-violaxanthin[b] | 11.1 | 328/416/439/469 | 81 | | 601 | 583 [M + H − 18], 565 [M + H − 18−18], 509 [M + H − 92], 491 [M + H − 18−92] |
| **2** | all-*trans*-neochrome | 12.9 | 399/422/448 | 95 | | 601 | 583 [M + H − 18], 565 [M + H − 18−18], 509 [M + H − 92] |
| **3** | 9-*cis*-violaxanthin | 15.5 | 328/415/437/464 | 65 | 7 | 601 | 583 [M + H − 18], 565 [M + H − 18−18], 509 [M + H − 92], 491 [M + H − 18−92] |
| **4** | 9-*cis*-neochrome | 16.8 | 312/396/417/443 | 93 | 15 | 601 | 583 [M + H − 18], 565 [M + H − 18−18], 509 [M + H − 92], 491 [M + H − 18−92] |
| **5** | all-*trans*-lutein | 18.3 | 420/445/472 | 50 | | 569 | 551 [M + H − 18], 495 [M + H − 18−56],[c] 430 [M + H − 139][c] |
| **6** | all-*trans*-zeaxanthin | 20.7 | 424/450/476 | 25 | | 569 | 551 [M + H − 18], 533 [M + H − 18−18] |
| **7** | not identified | 25.2 | 420/445/473 | 58 | | 601 | 583 [M + H − 18], 491 [M + H − 18−92] |
| **8** | all-*trans*-β-cryptoxanthin | 28.0 | 425/451/476 | 25 | | 553 | 535 [M + H − 18], 461 [M − 92] |
| **9** | not identified | 30.1 | 346/433/453/477 | 5 | 39 | 537 | 444 [M − 92] |
| **10** | 13-*cis*-β-carotene | 31.3 | 338/422/444/470 | 6 | 33 | 537 | 444 [M − 92] |
| **11** | all-*trans*-β-carotene | 35.7 | 426/452/478 | 20 | | 537 | 444 [M − 92] |
| **12** | 9-*cis*-β-carotene | 37.2 | 342/422/445/472 | 21 | 9 | 537 | 444 [M − 92] |
| **13** | all-*trans*-rubixanthin I | 40.6 | 439/462/491 | 50 | | 553 | 535 [M + H − 18], 461 [M − 92] |
| **14** | all-*trans*-rubixanthin II | 41.2 | 439/462/491 | 50 | | 553 | 535 [M + H − 18], 461 [M − 92] |
| **15** | all-*trans*-γ-carotene | 47.3 | 432/462/492 | 48 | | 537 | 467 [M − 69], 444 [M − 92] |
| **16** | 15- or 13-*cis*-lycopene | 47.7 | 360/440/465/496 | 47 | 55 | 537 | 467 [M − 69], 444 [M − 92] |
| **17** | 9-*cis*-lycopene | 52.9 | 361/442/467/498 | 60 | 12 | 537 | 467 [M − 69], 444 [M − 92] |
| **18** | all-*trans*-lycopene | 59.0 | 362/447/472/503 | 71 | | 537 | 467 [M − 69], 444 [M − 92], 430 [M − 106] |
| **19** | 5-*cis*-lycopene | 59.5 | 362/447/472/503 | 73 | 5 | 537 | 467 [M − 69], 444 [M − 92] |

[a]Peak number in Figure 3. [b]Cochromatography with the corresponding reference compound. [c]According to ref 19.

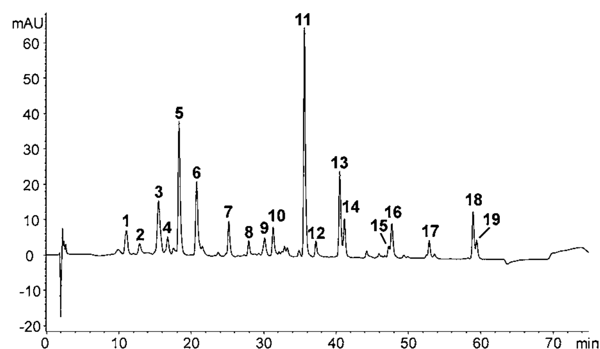


**Figure 3.** Chromatogram recorded at a detection wavelength of 450 nm showing the carotenoid profile of rose hip seed oil. For peak assignment, see Table 5.

isomer of $\beta$-cryptoxanthin. This identification was corroborated by identical elution behavior as compared to the corresponding all-*trans* standard.

Compound **11** was considered to be all-*trans*-$\beta$-carotene due to the similarity of UV/vis and mass spectrometric characteristics available in literature for all-*trans*-$\beta$-carotene.[24,27,36] Analogous to our results for samples other than rose hip, peak **12** was assigned to the 9-*cis*-isomer of $\beta$-carotene, showing reduced spectroscopic fine structure and a further absorbance band at 342 nm. Furthermore, rose hip seed oil contained an additional *cis*-isomer (**10**), most likely 13-*cis*-$\beta$-carotene, showing a marked hypsochromic shift of the absorption maxima (8 nm) with respect to the all-*trans* form and a "*cis*-peak" of high intensity (% $A_B/A_{II}$: 33), thus indicating the *cis* double bond to be located close to the center of the molecule. This identification was corroborated by its mass spectra behavior and by the fact that compound **10** eluted prior to the all-*trans*-isomer.

Compounds **13** and **14** eluting between 40 and 41 min showed similar $\lambda_{max}$ values (439/462/491 nm) and missing "*cis*-peaks". Mass spectrometric analysis revealed protonated molecular ions at $m/z$ 553 and fragments at $m/z$ 535 and $m/z$ 461 resulting from elimination of water and toluene, respectively. These results were indicative of all-*trans*-rubixanthin isomers. However, this assumption seemed to be in disagreement with their expected elution behavior on a $C_{30}$ column. Although belonging to the group of monocyclic monohydroxy carotenoids, they eluted very late, even after all-*trans*-$\beta$-carotene, the latter being devoid of hydroxyl groups. However, this phenomenon has already been reported elsewhere and was attributed to the strong interaction of the unsubstituted acyclic hydrocarbon chain with the $C_{30}$ chains of the column material causing considerable retention of rubixanthin.[39] Moreover, the presence of rubixanthin is in accordance with recently published data on rose hip.[40] Considering these findings, compounds **13** and **14** were identified as diastereoisomers of all-*trans*-rubixanthin.

Compound **15**, partially coeluting with compound **16**, was tentatively identified as all-*trans*-$\gamma$-carotene, showing $\lambda_{max}$ values (432/462/492 nm) and mass spectrometric data already observed.[24,38]

Compound **18** displaying a retention time of 59.0 min showed UV/vis and mass spectrometric data observed for all-*trans*-lycopene. Consistent with previously published data,[41] fragmentation of lycopene yielded MS/MS fragment ions at $m/z$ 467, 444, and 430 resulting from the elimination of a $\psi$ end group, toluene, and *m*-xylene, respectively. Finally, the presence of all-*trans*-lycopene was confirmed by comparison of its retention time with that of an authentic reference compound. Compounds eluting between 43 and 60 min possessed a spectroscopic fine structure being characteristic of lycopene as well. Moreover, they showed increased absorbance in the 360 nm region, a hypsochromatic shift of their maximum absorbance wavelengths, and similar mass spectrometric characteristics as compared to all-*trans*-lycopene. Thus, they were assigned to *cis*-isomers of lycopene with compounds **16**, **17**, and **19** being the most prominent ones. Because maximal intensity of the *cis* band is observed when the double bond is located in or close to the center of the chromophores, compounds **16** and **17** were tentatively identified as 15- or 13-*cis*-lycopene and 9-*cis*-lycopene, respectively. According to a previous report,[42] compound **19** was assumed to be the 5-*cis*-isomer of lycopene. This identification was underpinned by the great similarity of its absorption spectrum with respect to the all-*trans* form, thus indicating the presence of a peripheral *cis* bond, for example, 5-*cis*.

**Quantitative Composition of Carotenoids (Rosoideae).** Table 6 summarizes the contents of individual carotenoids present in rose hip seed oil. As apparent from Table 6, the latter markedly differed from the oils obtained from the representatives of the Maloideae subfamily not only regarding the diversity of carotenoids but also with respect to their quantitative composition. The total carotenoid content of rose hip seed oil (39.15 mg/kg) was approximately twice as high as that of the apple cultivar 'Brettacher' being the richest source within the oils deriving from the Maloideae species under investigation. To some extent, this was probably due to accompanying carotenoids originating from the edible parts of the rose hip fruit. Showing a content of 9.28 mg/kg, all-*trans*-$\beta$-carotene was the most abundant carotenoid accounting for approximately 24% of total carotenoid content. Apart from $\beta$-carotene, the all-*trans*-isomers of lutein, zeaxanthin, and rubixanthin isomers and lycopene were also present in

**Table 6. Contents of Individual Carotenoids (mg/kg Oil) in Rose Hip Seed Oil**[a]

| carotenoid | rose hip |
|---|---|
| all-*trans*-violaxanthin | 1.01 ± 0.08 |
| all-*trans*-neochrome | 0.75 ± 0.28 |
| 9-*cis*-violaxanthin | 2.79 ± 0.25 |
| 9-*cis*-neochrome | 0.56 ± 0.05 |
| all-*trans*-lutein | 5.35 ± 0.33 |
| all-*trans*-zeaxanthin | 3.64 ± 0.19 |
| not identified | 1.35 ± 0.04 |
| all-*trans*-$\beta$-cryptoxanthin | 0.49 ± 0.04 |
| Not identified | 0.89 ± 0.09 |
| 13-*cis*-$\beta$-carotene | 0.96 ± 0.14 |
| all-*trans*-$\beta$-carotene | 9.28 ± 0.24 |
| 9-*cis*-$\beta$-carotene | 0.74 ± 0.09 |
| all-*trans*-rubixanthin I | 3.76 ± 0.22 |
| all-*trans*-rubixanthin II | 2.11 ± 0.08 |
| all-*trans*-$\gamma$-carotene | 0.47 ± 0.02 |
| 13- or 15-*cis*-lycopene | 1.48 ± 0.02 |
| 9-*cis*-lycopene | 0.73 ± 0.12 |
| all-*trans*-lycopene | 1.93 ± 0.14 |
| 5-*cis*-lycopene | 0.84 ± 0.14 |
| total | 39.15 ± 0.71 |

[a]Results represent means ± standard deviations ($n$ = 2).

appreciable amounts accounting for approximately 14, 9, 15, and 5%, respectively. Additionally, the 9-*cis*-violaxanthin content was found to be 2.79 mg/kg, thus adding up to 7% to the total carotenoid content.

The present study demonstrated seed byproducts arising from the fruit processing of certain Rosaceous plants to be promising sources for the recovery of vegetable oils containing appreciable amounts of carotenoids and in particular tocopherols. Our analyses revealed significant differences, for both the qualitative and the quantitative composition of tocopherols and parent carotenoids, not only among the different genera of the Rosaceae subfamilies but also among cultivars of one species. Consequently, apart from being an important source of highly unsaturated and essential fatty acids, the presence of substantial amounts of lipophilic antioxidants having health-beneficial properties makes the recovery of Rosaceous seed oils even more attractive, thus contributing to sustainable food production.

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### Notes

The authors declare no competing financial interest.

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