Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

69451 Weinheim, Germany

Colorless Tetrapyrrolic Chlorophyll Catabolites Found in Ripening Fruit Are Effective Antioxidants

Thomas Müller[1,2], Markus Ulrich[1,2], Karl-Hans Ongania[1] & Bernhard Kräutler[1,2*]

[1]Institute of Organic Chemistry and [2]Center of Molecular Biosciences, LFU-Innsbruck

Supporting Information - Methods

Materials. Reagents used were reagent grade commercials and solvents were distilled before use. Reagents and HPLC-solvents were from Fluka (Buchs, Switzerland) and from Merck (Darmstadt, Germany). Sep-Pak-C18-cartridges were from Waters Corp., Milford (MA). The fruit, apples (*Malus sylvestris*, brand name: "Golden Delicious") and pears (*Pyrus communis*, brand name: "Williamine"), were obtained from the farmers market in Innsbruck. Green and yellow leaves from "Williamine" and "Golden Delicious" trees were collected in early August and early October, respectively. **High performance liquid chromatography (HPLC), NMR and UV/Vis-spectroscopy, mass spectrometry:** HPLC: Gynkotek HPLC System with vacuum on-line degasser; HP 1100 HPLC System(Agilent) or Ultimate Nano-HPLC System (with LC Packings); solvents were degassed by sonication; detectors: Gynkotek diode array detector UVD 340, Hitachi SPD-6AV UV/Vis detector. All analytical and preparative chromatograms were taken at RT and data were processed by Chromeleon 6.5 software; a) analytical column: Hypersil ODS 5 µm, 250 x 4.6 mm i.d. (pump: Gynkotek M480G); b) preparative column: Hypersil ODS 5 µm, 250 x 21.2 mm i.d. (pump: Gynkotek M300). Nuclear magnetic resonance (NMR)-Spectra: Varian Unity$_{plus}$ 500 ($\delta(C^1HD_2OD)$ = 3.31 ppm and $\delta(^{13}CD_3OD)$ = 49.0 ppm). UV/Vis-Spectra (in methanol): U-3000 spectrophotometer (Hitachi); λ_{max}(nm)/(log ε), concentrations were calculated following Lambert-Beer's Law. CD-Spectra: Jasco-J715 spectra-polarimeter; λ_{max}(nm) and λ_{min}(nm)/(Δε). Mass-Spectra: Finnigan MAT 95-S, (+)-ion mode; high resolution "Fast atom bombardment" (HR-FAB-MS): cesium gun, 20 keV, matrix: glycerine; "Electro spray ionization" (ESI-MS): 3.2 kV, solvents: water/ MeOH mixtures.

Isolation and spectroscopic characterization of "fruit NCCs".

a) From pear peels (*Pyrus communis*): $\mathbf{1}^{Pc}$ and $\mathbf{2}^{Pc}$. 450 g (fresh weight) peels of ripe "Williamine" pears were thoroughly blended with 2.25 l of acetone/methanol 1:1 (v/v) by a Braun hand blender Model MR 5000 in a 5 l stainless steel beaker. The resulting slurry was filtered through a layer of cellite (1 cm thick). The yellowish residue was transferred back to the beaker, was mixed with 500 ml of acetone/methanol 1:1 (v/v) and filtered (this procedure was repeated three more times until the residue was nearly colorless). The combined filtrates (about 4.25 l) were concentrated to 500 ml by evaporation in vacuo at r.t. Water (200 ml) was added to the mixture, which was concentrated to 300 ml and transferred to a separatory funnel. The aqueous mixture was extracted twice with (400 ml/200 ml of) petrol ether and then five times with (200 ml/ 100 ml/ 100 ml/ 100 ml/ 100 ml of) dichloromethane. The combined dichloromethane extracts were filtered through a plug of cotton wool, the solvents were evaporated in vacuo at r.t. to give 0.82 g of a greenish-yellow powder as a residue. The crude isolate was stored at –20°C over night and was then extracted eight times with 6 ml portions of 100 mM potassium phosphate (pH 7.0)/methanol 40:60 (v/v). The suspension was centrifuged at 11000 x g for 10 min and the supernatant was collected and separated by preparative HPLC (6 ml injection volume; isocratic elution with 100 mM potassium phosphate buffer (pH 7.0) / methanol 40:60 (v/v), flow rate: 5 ml.min^{-1}). Two fractions were collected at 15.5 min and 19 min and subjected to "desalting", as follows: The volumes were reduced to 40 % by evaporation in vacuo at r.t. The concentrated fractions were then each diluted with 2 volumes of water, applied to a Sep-Pak-C18 cartridge, which was washed with 20 ml of water; the crude NCC fractions were eluted with 5 ml of methanol and the solvents were removed by evaporation in vacuo at r.t. The samples were re-dissolved in 1 ml of 100 mM potassium phosphate (pH 7.0)/methanol 55:45 (v/v) and subjected to HPLC with 100 mM potassium phosphate buffer (pH 7.0)/ methanol 55:45 (v/v). After "desalting" the sample (see above), it was subjected again to HPLC with 100 mM potassium phosphate buffer (pH 6.0)/methanol 55:45 (v/v). After "desalting" (see above) and drying, pure samples of the NCCs $\mathbf{1}^{Pc}$ (312 µg) and $\mathbf{2}^{Pc}$ (190 µg) were obtained, which were identified with $\mathbf{1}^{Cj}$ [1, 2] and $\mathbf{2}^{Nr}$ [3] by HPLC (Figure S1) and by spectroscopic analysis:

1^{Pc}: UV/vis (λ_{max}, relative absorbance): 310.5 (1.00), 235s (1.61). ^{1}H-NMR (500 MHz, CD_3OD):δ = 1.92 = 1.92 (s, 3H), 1.98 (s, 3H), 2.13 (s, 3H), 2.23 (s, 3H), 2.29-2.36 (m, 2H), 2.45 (dd, *J*=8.8, 14.6, 1H), 2.61-2.75 (m, 3H), 2.88 (dd, *J*=4.9, 14.6, 1H), 3.39-3.48 (m, 2H), 3.75 (s, 3H), 3.98-4.03 (m, 3H), 5.35 (dd, *J*=1.9, 11.7, 1H), 6.10 (dd, *J*=1.9, 17.6, 1H), 6.45 (dd, *J*=11.7, 17.6, 1H), 9.31 (s, 1H). MS (HR-FAB, *m/z*): $[M+H]^+$ calcd for $C_{35}H_{41}N_4O_8$, 645.292; found, 645.292). MS (ESI, *m/z*): 645.3 (100, $[M+H]^+$), 612.7 (55, $[M-CH_4O+H]^+$), 522.2 (12, [M-ring A+H]$^+$). **2**^{Pc}: UV/vis (λ_{max}, relative absorbance): 310.5 (1.00), 235s. ^{1}H-NMR (500 MHz, CD_3OD):δ = 1.92 (s, 3H), 1.98 (s, 3H), 2.13 (s, 3H), 2.23 (s, 3H), 2.23-2.38 (m, 2H), 2.45 (dd, *J*=8.8, 14.6, 1H), 2.61-2.77 (m, 4H), 2.88 (dd, *J*=4.9, 14.6, 1H), 3.16 (m, 1H), 3.25 (m, 2H), 3.34 (m, 1H), 3.44 (dd, *J*=6.8, 15.8, 1H), 3.64 (dd, *J*=4.9, 11.7, 1H), 3.76 (s, 3H), 3.78-3.88 (m, 2H), 3.94-4.05 (m, 3H), 4.19 (d, *J*=7.8, 1H), 5.36 (br. d, *J*=11.7, 1H), 6.10 (dd, *J*=1.9, 17.6, 1H), 6.45 (dd, *J*=11.7, 17.6, 1H), 9.31 (s, 1H). MS (HR-FAB, *m/z*): $[M+H]^+$ calcd for $C_{41}H_{51}N_4O_{13}$, 807.345; found, 807.348). MS (ESI, *m/z*): 845.2 (100, $[M+K]^+$), 829.2 (55, $[M+Na]^+$), 807.2 (93, $[M+H]^+$), 683.2 (20, $[M-C_6H_{10}O_5+K]^+$), 667.2 (12, $[M-C_6H_{10}O_5+Na]^+$).

b) From apple peels (*Malus sylvestris*): **1**^{Ms} and **2**^{MS}. "Golden delicious" apples were bought at a local market in Innsbruck. Peelings of six ripe apples were immersed into liquid N_2 and ground in a mortar and extracted with 50 ml of methanol. The resulting slurry was filtered through a layer of cellite (0.5 cm thick). The light yellowish residue was extracted three more times (until the residue was nearly colourless). The combined filtrates (about 200 ml) were evaporated in vacuo at r.t. The solid crude extract was extracted three times with 60ml portions of 100 mM potassium phosphate (pH 7.0) and the aqueous extract was applied to a Sep-Pak-C18 cartridge. After washing with 40 ml of water the crude product was eluted with 10 ml of methanol and the solvents were evaporated in vacuo at r.t. The solid crude product was re-dissolved in 2ml of 100 mM potassium phosphate (pH 7.0)/methanol 55:45 (v/v) and separated by HPLC (2ml injection volume, 21.2 mm i.d. Hypersil ODS 5μm column; elution with 100 mM potassium phosphate (pH 7.0)/methanol 55:45 (v/v), flow rate 5 ml.min^{-1}). The fractions of **1**^{Ms} and **2**^{Ms} (more polar) were collected and reduced to 2 ml by evaporation in vacuo at r.t. The concentrated fractions were desalted by applying to HPLC (1 ml injection volume, 4.6 mm i.d. Hypersil ODS 5µm column, flow rate 0.5 ml.min^{-1}, desalting at 100% water, elution by increasing methanol to 100% within 10 min). The eluted salt-free NCC fractions were collected and identified by co-injection with HPLC (with **1**^{Cj} [1, 2] and **2**^{Nr} [3]) and by using ESI mass spectrometry.

For further identification of **1**^{Ms} by ^{1}H-NMR, 200 g (fresh weight) of peelings of ripe fruits were treated as described in the section "Isolation of **1**^{Pc} and **2**^{Pc}", however, the volumes of the solvents were smaller by a factor of 2.25. 120 mg of a light greenish-yellow crude product was obtained; after several preparative HPLC runs 22.4 μg of salt-free **1**^{Ms} were collected and analyzed by 500 MHz-^{1}H-NMR spectroscopy (Figure 3).

1^{Ms}: UV/vis (λ_{max}, relative absorbance, methanol): 310.5 (1.00), 235 (1.27). ^{1}H-NMR (500 MHz, CD_3OD): δ1.92 (s, 3H), 1.95 (s, 3H), 2.10 (s, 3H), 2.25 (s, 3H), 2.30-2.36 (m, 2H), 2.48 (dd, *J*=8.8, 14.6, 1H), 2.58-2.66 (m, 2H), 2.67-2.75 (m, 1H), 2.85 (dd, *J*=4.8, 14.6, 1H), 3.43-3.53 (m, 2H), 3.75 (s, 3H), 3.94 (s,2H), 3.95-3.99 (m, 1H), 4.87 (s, 1H), 5.35 (dd, *J*=1.9, 11.7, ca. 1H), 6.09 (dd, *J*=1.9, 17.6, 1H), 6.44 (dd, *J*=11.7, 17.6, 1H), 9.34 (s, 1H). MS (HR-FAB, *m/z*): $[M+H]^+$ calcd for $C_{35}H_{41}N_4O_8$, 645.292; found, 645.294. MS (ESI, *m/z*): 683.3 (40, $[M+K]^+$), 645.3 (100, $[M+H]^+$), 613.2 (12, $[M-CH_4O+H]^+$), 522.2 (6, [M-ring A+H]$^+$). **2**^{Ms}: UV/vis (λ_{max}, relative absorbance, 100 mM potassium phosphate buffer (pH 7.0)/ methanol 40:60 (v/v)): 320 (1.00), 235 (1.58). MS (ESI, *m/z*): 883.3 (40, $[M-H+2K]^+$), 867.3 (35, $[M-H+K+Na]^+$), 845.4 (100, $[M+K]^+$), 829.3 (30, $[M+Na]^+$), 807.3 (20, $[M+H]^+$). MS (ESI negative ions, methanol/water): *m/z* = 805.5 (100, $[M-H]^-$).

c) NCCs from fruit flesh of the pear (*Pyrus communis*): Fruit flesh of a ripe "Williamine" pear (4 g) was mixed and ground in a mortar with 0.5 g sea sand and 3.4 ml of methanol. The slurry was centrifuged (1 min, 11000 x g), and 0.5 ml of the supernatant were diluted with 0.5 ml 100 mM potassium phosphate (pH 7.0). After further centrifugation (10 min, 11000 x g), a sample of the supernatant was injected for analytical HPLC. NCCs were identified and quantified by standard HPLC-

analysis and comparison to authentic reference material: a) outer fruit flesh near the peel: $\mathbf{2}^{Pc}$ at 25.4 min, 0.12 µg.g^{-1} $\mathbf{2}^{Pc}$; $\mathbf{1}^{Pc}$ at 27.1 min, 1.08 µg.g^{-1} $\mathbf{1}^{Pc}$. a) Inner fruit flesh, near the core: $\mathbf{2}^{Pc}$ not detected; $\mathbf{1}^{Pc}$ at 27.1 min, 0.13 µg.g^{-1} $\mathbf{1}^{Pc}$.

d) NCCs from senescent leaves of the pear tree: One senescent leaf (area: about 25 cm^2) was ground in a mortar with 0.5 g sea sand and 4 ml of methanol. The slurry was centrifuged (1 min, 11000 x g). A sample (0.5 ml) of the supernatant was diluted with 0.5 ml 100 mM potassium phosphate (pH 7.0) and centrifuged (5 min at 11000 x g). A sample of 0.5 ml of the resulting supernatant was further diluted with 0.5 ml 100 mM potassium phosphate (pH 7.0) and centrifuged for 10 min at 11000 x g. A sample (100 µl) of the supernatant was injected for analytical HPLC (at r.t., flow rate: 0.5 ml.min^{-1}, solvent A: 100 mM potassium phosphate (pH 7.0), solvent B: methanol; starting at 20% B and increasing solvent B to 60% within 10 min). Identification ($\mathbf{2}^{Pc}$ at 25.4 min, $\mathbf{1}^{Pc}$ at 27.1 min) and quantification ($\mathbf{2}^{Pc}$: 4.9 µg/cm^2; $\mathbf{1}^{Pc}$: 29 µg/cm^2) by standard analytical HPLC-procedure.

For FAB-mass spectrometry, the fractions (0.5 ml each) of $\mathbf{2}^{Pc}$ and $\mathbf{1}^{Pc}$ from senescent leaves were collected by HPLC and were acidified to pH < 5.2 by adding 4 µl of 85% formic acid. The acidified $\mathbf{2}^{Pc}$ solution was saturated with NaCl, and $\mathbf{2}^{Pc}$ was extracted with 0.5 ml of methyl acetate. The organic phase was separated and evaporated at r.t. in vacuo. The acidified $\mathbf{1}^{Pc}$ solution was extracted with 0.5 ml of dichloromethane. The organic phase was separated and evaporated in vacuo and analyzed (Figure S3).

e) NCCs from senescent leaves of the apple tree: One senescent leaf (area: about 25 cm^2) was ground in a mortar with 0.5 g sea sand and 10 ml of methanol. The slurry was filtered through a sintered glass filter, and the residue was again ground in a mortar with 10 ml of methanol. The procedure was repeated until the residue was colourless. A sample (1 ml) of the combined methanolic extracts (100 ml) was diluted with 2 ml 100 mM potassium phosphate (pH 7.0) and 1.36 ml were injected to analytical HPLC (at r.t., flow rate: 0.5 ml.min^{-1}, solvent A: 100 mM potassium phosphate (pH 7.0), solvent B: methanol; starting at 50% B and increasing solvent B after 7 min to 60% within 18 min). Identification ($\mathbf{2}^{Ms}$ at 19.75 min, $\mathbf{1}^{Ms}$ at 22.87 min) and quantification ($\mathbf{2}^{Ms}$: 0.42 µg.cm^{-2}; $\mathbf{1}^{Ms}$: 22.8 µg.cm^{-2}) by standard analytical HPLC-procedure.

Isolation of $\mathbf{1}^{Cj}$ of *Cercidiphyllum japonicum*: For experimental details, see ref.[1].

Identification of $\mathbf{1}^{Pc}$ and $\mathbf{1}^{Ms}$ with $\mathbf{1}^{Cj}$ by HPLC: NCC stock solutions were prepared by dissolving isolated $\mathbf{1}^{Cj}$ (from *Cercidiphyllum japonicum,* see ref.[1, 2], $\mathbf{1}^{Ms}$ and $\mathbf{1}^{Pc}$ in 100 mM potassium phosphate (pH 7.0)/methanol 80:20 (v/v). The stock solutions were stored at –20 °C. Analytical HPLC: 250 mm x 4.6 mm i.d. Hypersil ODS 5 µm column, at r.t., flow rate: 0.5 ml.min^{-1}, solvent A: 100 mM potassium phosphate (pH 7.0), solvent B: methanol; starting at 20% B and increasing solvent B to 60% within 15 min (Figure S1).

Identification of $\mathbf{2}^{Pc}$ and $\mathbf{2}^{Ms}$ and $\mathbf{2}^{Nr}$ by HPLC: NCC stock solutions were prepared by dissolving isolated $\mathbf{2}^{Nr}$ (from tobacco *(Nicotiana rustica)* see ref.[3], $\mathbf{2}^{Ms}$ and $\mathbf{2}^{Pc}$ in 100 mM potassium phosphate (pH 7.0)/methanol 80:20 (v/v). The stock solutions were stored at –20 °C. Analytical HPLC: at r.t., flow rate 0.5 ml.min^{-1}, solvent A: 100 mM potassium phosphate (pH 7.0), solvent B: methanol. For the analysis of $\mathbf{2}^{Pc}$/$\mathbf{2}^{Nr}$, the content of B increased from 20% B to 60% B within 15 min. For analysis of $\mathbf{2}^{Ms}$/$\mathbf{2}^{Nr}$, the content of B increased from 20% B to 60% B within 10 min (Figure S1).

Determination of NCCs in fruit peelings by analytical HPLC. a) Standard procedure: The area of the fruit peelings (around 30 cm^2) was determined by photographical integration. NCC extraction and HPLC analysis were done as described in the previous section. For quantification of the NCCs by HPLC an NCC standard was prepared by dissolving $\mathbf{1}^{Cj}$ (c = 2.14 x 10.4 mol.l^{-1}) in 100 mM potassium phosphate (pH 7.0)/methanol 80:20 (v/v).

b) Analysis of peelings of green unripe pear p3 (Figure 1): NCCs were not detected; analysis of peelings of green pear p2 from the market (Figure 1): 0.45 µg.cm^{-2} (area 28.16 cm^2; 7.04 µg $\mathbf{1}^{Pc}$ and 5.63 µg $\mathbf{2}^{Pc}$ were found); analysis of peelings of yellow pear p1 from the market (Figure 1 and Figure S2): 0.55 µgcm^{-2} (area 37.19 cm^2; 11.2 µg $\mathbf{1}^{Pc}$ and 9.3 µg $\mathbf{2}^{Pc}$ were found);

analysis of peelings of green unripe apple a3 (Figure 1): NCCs were not detected; analysis of peelings of green apple a2 from the market (Figure 1): 0.07 µgcm^{-2} (area 28.32 cm^2; 0.57 µg **1**^{Ms} and 0.28 µg **2**^{Ms} were found); analysis of peelings of yellow apple a1 from the market (Figure 1 and Figure S2): 0.03 µg.cm^{-2} (area 28.55 cm^2; 1.71 µg **1**^{Ms} and 0.29 µg **2**^{Ms} were found).

Determination of residual chlorophyll in fruit peelings by UV/vis spectroscopy: a) Standard procedure: 15-70 cm^2 of a fruit peel (area was determined by photographical integration) was ground in a mortar with 0.5 g sea sand and 10 ml of methanol. The slurry was filtered through a sintered glass filter, and the residue was ground in a mortar with 10 ml of methanol. The procedure was repeated until the residue was colourless. The combined methanolic extracts were analyzed by UV/vis spectrometry. Data analysis is based ref.[4].

b) Analysis of peelings of green unripe pear p3 (Figure 1): 14.3 µg.cm^{-2} (632.6 µg chlorophyll *a* / *b* on 44,13 cm^2); analysis of peelings of green pear p2 from the market (Figure 1): 8.4 µg.cm^{-2} (128.8 µg chlorophyll *a* / *b* on 15.42 cm^2); analysis of peelings of yellow pear p1 from the market (Figure 1): 2.5 µg.cm^{-2} (40.35 µg chlorophyll *a* / *b* on 16.12 cm^2); analysis of peelings of a green unripe apple a3 (Figure 1): 5.57 µg.cm^{-2} (370.9 µg chlorophyll *a* / *b* on 66.6 cm^2); analysis of peelings of a green apple a2 from the market (Figure 1): 5.0 µg.cm^{-2} (89.05 µg chlorophyll *a* / *b* on 17.67 cm^2); analysis of peelings of yellow apple a1 from the market (Figure 1): 0.9 µg.cm^{-2} (22.98 µg chlorophyll *a* / *b* on 25.53 cm^2).

Determination of the antioxidant activity of the NCC 1 from fruit[5]. Stock solutions of linoleic acid, azoisobutyronitrile (AIBN), **1** (isolated as **1**^{Cj} [1, 2]) and bilirubin were prepared by dissolving 470 µl linoleic acid in 1530 µl of chloroform, 6.9 mg AIBN in 2030 µl of chloroform, 0.5 mg **1** in 300 µl of chloroform and 0.4 mg bilirubin in 550 µl of chloroform. The concentrations of **1** and bilirubin in the stock solutions were determined by UV/vis spectroscopy (50 µl of the stock solution is diluted with 3 ml methanol; ε (**1**, methanol) at 312 nm is 14500[2]; ε (bilirubin, chloroform) at 450 nm is 55000[6]. Reaction mixtures were prepared by mixing aliquots of the stock solutions to obtain the following final concentrations: 0.15 M linoleic acid, 2 mM AIBN and 0 - 200 µM of **1** or bilirubin. The air saturated reaction mixture was kept at 37 °C, the formation of linoleic acid hydro-peroxide was determined by UV/vis spectroscopy and HPLC analysis. UV/vis spectroscopy: 40 µl of the reaction mixture were diluted with 3 ml of methanol. Absorbance was measured against methanol, the overall absorbance at 234 nm was used for monitoring. For analytical HPLC isocratic separation was achieved with methanol as eluent (flow rate of 1 ml.min^{-1}). 40 µl of the reaction mixture were diluted with 1.5 ml of methanol, 20 µl of this solution were applied to the HPLC.

References:

[1] M. Oberhuber, J. Berghold, K. Breuker, S. Hörtensteiner, B. Kräutler, *Proc. Nat. Acad. Sci. USA* **2003**, *100*, 6910.
[2] C. Curty, N. Engel, *Phytochemistry* **1996**, *42*, 1531.
[3] J. Berghold, C. Eichmüller, S. Hörtensteiner, B. Kräutler, *Chem. Biodiv.* **2004**, *1*, 657.
[4] Porra., R. J., Thompson, W. A., Kriedemann, P. E. Biochim. Biophys. Acta 975, 384 (1989).
[5] R. Stocker, Y. Yamamoto, A. F. Mcdonagh, A. N. Glazer, B. N. Ames, *Science* **1987**, *235*, 1043.
[6] G. Agati, F. Fusi, *Journal of Photochemistry and Photobiology B-Biology* **1990**, *7*, 1.

Supporting Information - Figures

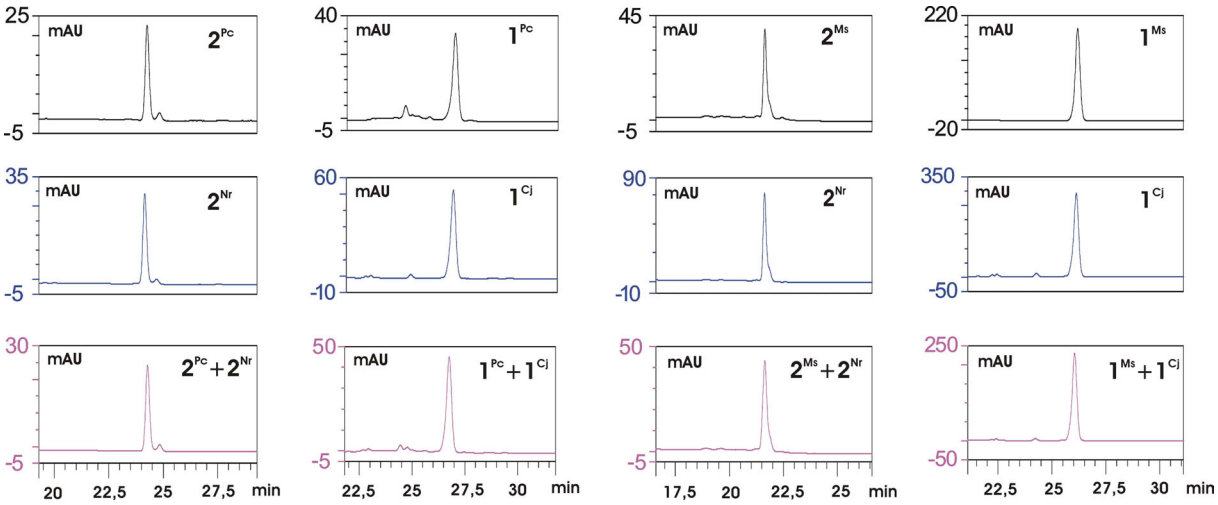

Figure S1. Identification of "fruit NCCs" by HPLC-experiments. NCCs from "Williamine" pears ($\mathbf{1}^{Pc}$ and $\mathbf{2}^{Pc}$) and "Golden delicious" apples ($\mathbf{1}^{Ms}$ and $\mathbf{2}^{Ms}$) (top) were identified with $\mathbf{1}^{Cj}$ from *Cercidiphyllum japonicum* and $\mathbf{2}^{Nr}$ from *Nicotiana rustica* (middle and bottom). HPLC-traces. First column: $\mathbf{2}^{Pc}$, $\mathbf{2}^{Nr}$ and their 1:1 mixture. Second column: $\mathbf{1}^{Pc}$, $\mathbf{1}^{Cj}$ and their 1:1 mixture. Third column: $\mathbf{2}^{Ms}$, $\mathbf{2}^{Nr}$ and a 1:1 mixture of $\mathbf{2}^{Ms}$/$\mathbf{2}^{Nr}$. Fourth column: $\mathbf{1}^{Ms}$, $\mathbf{1}^{Cj}$ and their 1:1 mixture.

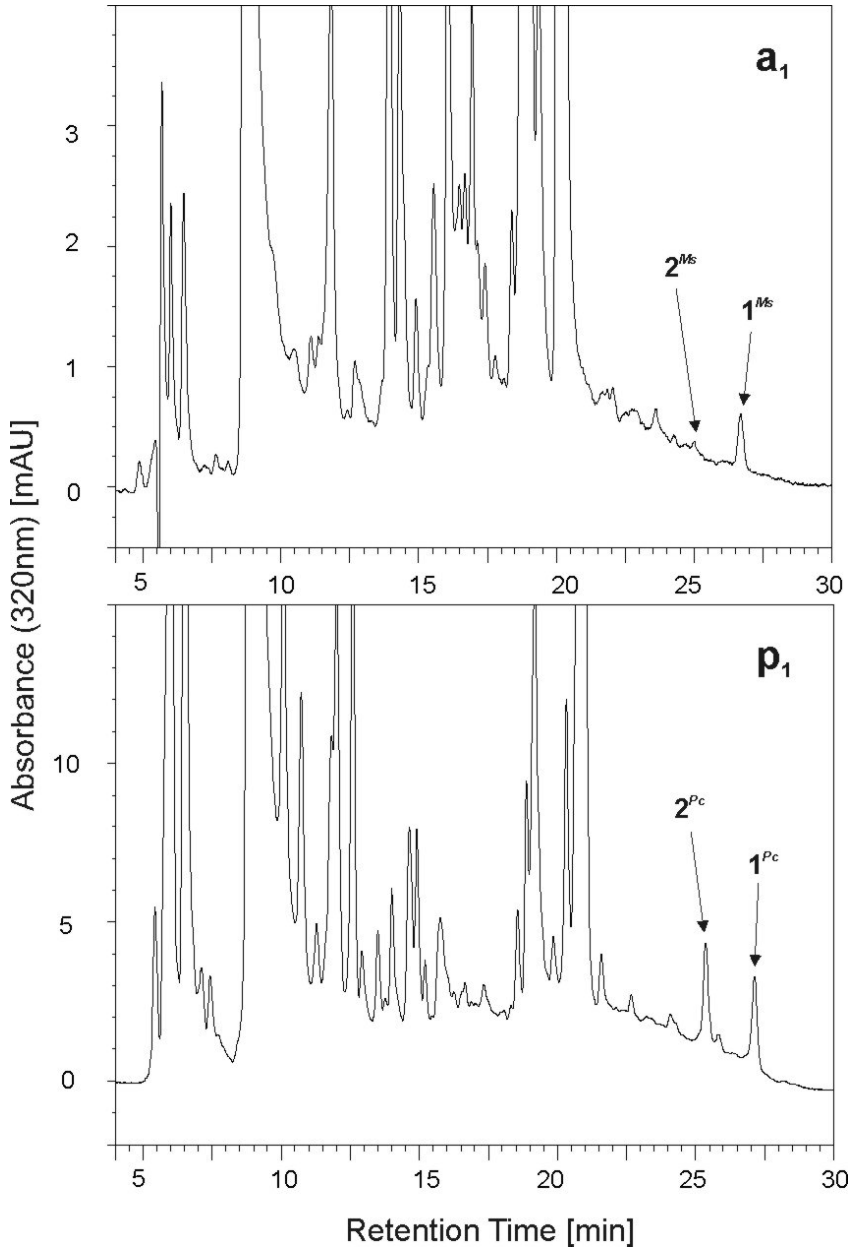

Figure S2. Analysis of NCCs in peels from yellow apple a1 (top) and yellow pear p1 (bottom) from the market by analytical HPLC: at r.t., flow rate 0.5 ml.min^{-1}, solvent A: 100 mM potassium phosphate (pH 7.0), solvent B: methanol. The content of B was increased from 20% B to 60% B within 15 min (the NCC fractions $\mathbf{1}^{Ms}$, $\mathbf{2}^{Ms}$, $\mathbf{1}^{Pc}$ and $\mathbf{2}^{Pc}$ are highlighted).

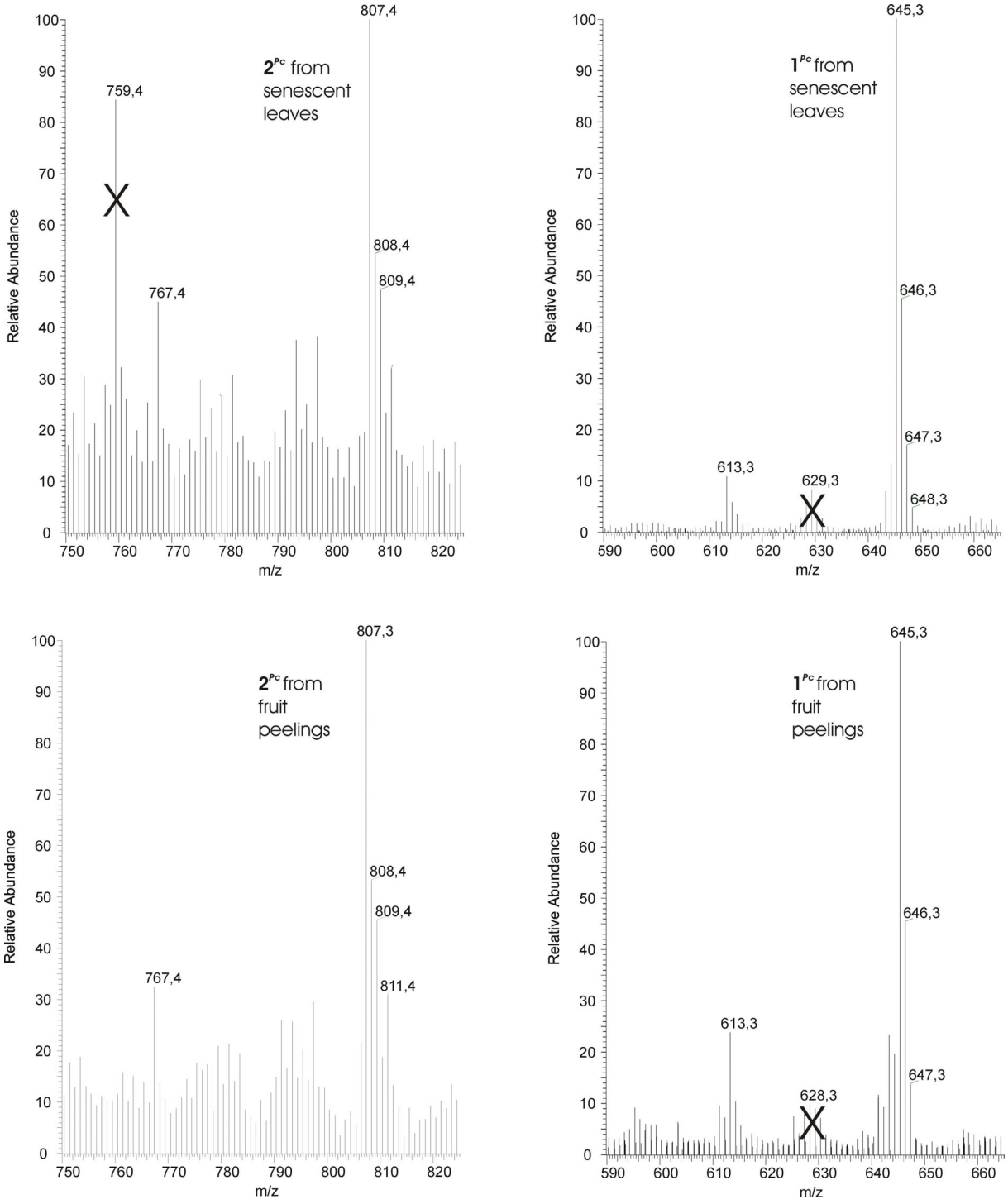

Figure S3. FAB mass spectrometric analysis of the NCCs $\mathbf{1}^{Pc}$ and $\mathbf{2}^{Pc}$ from "Williamine" pears (glycerine matrix, x marks matrix related peaks). Spectra of $\mathbf{1}^{Pc}$ and $\mathbf{2}^{Pc}$ from senescent leaves (top) and from peelings of (ripe) pears (bottom) are compared.