Data set on hydroxycinnamic acid ester analysis from the cell walls of apples and grapes

Kévin Vidot a, b, Sylvain Guyot c, Chantal Maury b, René Siret b, Marc Lahaye a, *

a UR 1268 Biopolymères Interactions Assemblages, équipe Paroi Végétale et Polysaccharides Pariétaux (PVPP), INRA, 44300, Nantes, France
b USC 1422 GRAPPE, INRA, Ecole Supérieure d’Agricultures, SFR 4207 QUASAV, 49100, Angers, France
c UR 1268 Biopolymères Interactions Assemblages, équipe Polyphénols, Réactivité, Procédés (PRP), INRA, 35653, Le Rheu, France

ABSTRACT

Data on the esters of hydroxycinnamic acids (HCAs) from the cell walls of wine grapes (Cabernet franc) and cider apples (Douce Moen and Guillevic) were acquired. Caffeic acid, p-coumaric acid (pCA) and ferulic acid (FA) monomers were identified by HPLC-UV/MS. Means to limit the oxidative degradation during cell wall preparation were assessed by the yield of HCA recovered after alkaline extraction. Following the optimum cell wall preparation, the pCA content varied between 2.3 and 32.5 mg kg⁻¹ dry cell wall and that of FA varied between 0.3 and 17.2 mg kg⁻¹ dry cell wall. Higher HCA quantities were found in the peels compared to the flesh and in apples compared to grapes. The Douce Moen apple was richer in HCAs than the Guillevic apple. pCA was localized in the cell wall as observed by TEM after labeling with the INRA-COU1 antibody that recognizes pCA linked to O-5 of arabinose. The anti-FerAra antibody targeting FA on O-5 of arabinose failed to locate FA esters in the apple and grape cell walls.

© 2019 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
Specifications Table

| Subject       | Food Science |
|---------------|--------------|
| Specific subject area | Identification, quantification and location of hydroxycinnamic esters in fleshy fruit cell walls |
| Type of data  | Figure |
| How the data were acquired | Fig. 1 was obtained by HPLC-UV coupled to a mass spectrometry (MS) system composed of: |
|                | - Thermostated autosampler (model Surveyor, Thermo Finnigan, San Jose, CA, USA). |
|                | - Binary high-pressure pump (model 1100, Agilent Technologies, Palo Alto, CA, USA). |
|                | - UV–vis diode array detector (model UV6000 LP, Thermo Finnigan) set at 320 nm. |
|                | - Ion trap mass spectrometry detector equipped with an electrospray ionization source (model LCQ Deca, Thermo Finnigan) set in negative ion mode by deprotonation. |
|                | - Column was a Purospher Star RP-18 end-capped (3 μm) Hibar HR (Merck, 2.1 × 150 mm), thermostated at 30 °C. |
|                | - Precolumn was an Eclipse XDB-C8 (Agilent Technologies, 2.1 × 12.5 mm, 5 μm). |
|                | - Solvent degasser SCM1000 vacuum membrane degasser (Thermo Fisher Scientific Inc). |
|                | - Solvent A: acidified pure water (0.1% formic acid) |
|                | - Solvent B: acidified acetonitrile (0.1% formic acid) |
|                | - Flow rate: 0.2 mL min⁻¹ |
|                | - Elution gradient: 0 min (97% A; 3% B), 3 min (93% A; 7% B), 21 min (87% A; 13% B), 27 min (87% A; 13% B), 41 min (80% A; 20% B), 51 min (55% A; 45% B), 53 min (10% A, 90% B), 56 min (10% A, 90% B), 58 min (97% A; 3% B) and 76 min (97% A; 3% B). |
|                | - Data collection by Xcalibur software (version 1.2, Thermo Finnigan). |
|                | Figs. 3, 4 and 6 were obtained by an HPLC-UV system composed of: |
|                | - C18 reversed-phase column (Vision HT C18 HL 5 μL, 250 mm × 4.6 mm, Grace, Germany) thermostated at 25 °C |
|                | - UV–vis diode array detector (Dionex UltiMate 3000, Thermo Fisher Scientific, USA) set at 320 nm. |
|                | - Solvent A: acetonitrile |
|                | - Solvent B: acetate buffer (4.5 g of sodium acetate trihydrate dissolved in 1 L of distilled water containing 2.2 mL of acetic acid). |
|                | - Binary pump (Dionex UltiMate 3000 pump, Thermo Fisher Scientific, USA) |
|                | - Flow rate: 1 mL min⁻¹ |
|                | - Elution gradient: 0–5 min (15% A; 85% B), 20 min (25% A; 75% B) and 25 min (25% A; 75% B) |
|                | - Data collection by Chromeleon software (version 6.8, Thermo Scientific, USA). |
| Data format    | Raw: (TEM images) |
|                | Analyzed |
| Parameters for data collection | HPLC-UV and HPLC-UV-MS were performed to identify and quantify hydroxycinnamic acids in saponified fractions of apples and grape cell wall material prepared under different limited oxidation conditions. Immunohistochemistry was performed to visualize the esters of p-coumaric and ferulic acids in the cell wall of Douce Moen apple sections labeled by FerAra and INRA-COU1 antibodies. |
| Description of data collection | HPLC-UV chromatograms and MS ion profiles of the alkaline hydrolyzates of apple cell walls were prepared by three different methods. |
| Data source location | TEM micrographs of cell walls labeled by FerAra and INRA-COU1 antibodies. |
|                | UR 1268 Biopolymères Interactions Assemblages, équipe Paroi Végétale et Polysaccharides Pariétaux (PVPP), INRA Nantes/Pays de la Loire France. |
|                | Cabernet franc (CF) wine grape fruits were harvested in September 2016, 2017 and 2018 were provided by ESA-GRAPPE, Angers-France. |
|                | Douce Moen (DM) and Guillevic (GU) apples were harvested in October 2017 and 2018 from a commercial orchard (IFPC, Sees, France). |
| Data accessibility | For the article and raw data for Figs. 3, 4 and 6 at https://doi.org/10.15454/YPCRML. |
**Value of the Data**

- The data reports on methods to improve the cell wall preparation from cider apples and wine grape for the analysis of cell wall hydroxycinnamic acid esters (HCAs; p-coumaric and ferulic acids) with limited contamination by noncell wall phenolic compounds.
- Additional data on the immunolocalization by transmission electron microscopy of p-coumaric acid ester on the O-5 of arabinose supported the presence of HCA in the cider apple cell wall.
- The methods and data reported provide grounds for future works on the nature, structure and function of HCAs in the cell walls of plant organs rich in phenolic compounds.
- The methods allowing for the limited oxidation of cell walls to open the way for further developments aimed at discriminating phenolic compounds according to their oxidative susceptibility and their affinity to cell wall materials.
- The methods and data reported provide grounds for future works on the nature, structure and function of HCAs in the mechanical properties and enzymatic processing during the development and processing of fleshy fruit tissue in relation to their organoleptic properties and nutritional characteristics.

1. **Data**

The phenolic esters found in the saponified fraction of the peel and fleshy cell wall of Cabernet Franc grapes (CF), Douce Moen and Guillevic apples (DM, GU) were identified by HPLC coupled to mass spectrometry. The results showed that caffeic acid, p-coumaric acid (pCA) and ferulic acid (FA) eluted at 19.8, 38.5 and 31.2 min on the HPLC chromatogram. These HCAs were identified by comparing their elution time with that of reference compounds and by their molecular weights of 164 and 194 Da measured by MS for pCA and FA (Fig. 1). Other peaks that did not correspond to common phenolic standards were also present on the HPLC chromatogram and were in amounts that were too low to be identified by mass spectrometry. None of the compounds observed had retention times of known FA dimers.

pCA can result from p-coumaroylquinic or p-coumaroyltartric acid degradation and caffeic acid can originate from caffeoylquinic acid contaminating the cell wall preparation. Among the identified HCAs, the current experiments focused on pCA and FA because they have been reported to be esterified in other plant cell walls [1,2]. The quantification of pCA and FA was performed following three cell wall preparation methods (A, B and C) for CF grapes and two methods (B and C) for DM and GU apples. The sum of the pCA and FA contents showed important variations according to the cell wall preparation method (Fig. 2). This value reached 1.532 g kg⁻¹ in CF cell walls when oxidation was not prevented (method A, Fig. 3). The sum was drastically reduced to 0.136 g kg⁻¹ when oxidation by ambient oxygen and oxidative enzyme activities were limited (method B, Fig. 3). Moreover, removing the water-soluble component (method C, Fig. 3) further significantly reduced the amount of extracted pCA and FA to 29 mg kg⁻¹. For DM apples, removing air from the intercellular spaces and water-soluble components (method C) significantly reduced the amount of extracted pCA and FA from 97 mg kg⁻¹ (method B) to 62 mg kg⁻¹. The efficiency of method C over method B was more obvious for CF and DM than for GU; for GU, both methods yielded approximately 41–42 mg kg⁻¹ of the two esters (Fig. 3). Assuming that method C allowed measuring HCA content in the saponified fraction of the cell wall with limited oxidation artifacts, the contents of pCA and FA ranked from the highest to the lowest as follows: Douce Moen > Guillevic > Cabernet franc (Fig. 3).

The mean pCA and FA contents in the saponified fraction of the cell wall of the peels and fleshy of grapes and apples prepared according to method C are presented in Fig. 4. For the three fruits, the content of the two HCAs was significantly higher in the cell wall from the peel than in the cell wall from the flesh, and the two HCAs also showed a significantly higher proportion of pCA compared to FA. The pCA content from the peel of Douce Moen was significantly higher than that of Guillevic, while the difference in FA content between the two apple varieties was not significant. In the flesh, the higher amount of pCA and FA in Douce Moen compared to Guillevic was significant. The content of the two HCAs in the peel of Cabernet franc grapes was significantly lower than that of the two apple varieties (Fig. 4). However, in the saponified fraction of the cell wall from the flesh of Cabernet franc, the pCA and FA contents were similar to those of Douce Moen, with approximately 10 mg kg⁻¹ pCA and 2.4 mg kg⁻¹ FA. These contents were significantly higher than those found for Guillevic.
CA and FA were examined from the cuticle, subcuticle and parenchyma tissue of Douce Moen by transmission electron microscopy following labeling by INRA-COU1 and anti-FerAra antibodies recognizing pCA and FA linked to O-5 of arabinose. pCA labeling was low and distributed in the cell wall of the cuticle and the subcuticle areas without specific localization (Fig. 5). Labeling by anti-FerAra was too faint to identify the presence of FA linked to O-5 of arabinose in the apple cell wall (Fig. 5). Immunostaining was only performed in Douce Moen apples because the antibody availability was limited.

2. Experimental design, materials, and methods

2.1. Materials

2.1.1. Chemicals

Paraformaldehyde (OH(CH2O)nH), sodium phosphate, London Resin White (LRW) acrylic resin, bovine serum albumin (BSA), sodium fluoride, sodium dodecyl sulfate, sodium chloride, sodium hydroxide, 3-4-5-trimethoxy-trans-cinnamic acid (TMCA), FA, pCA, hydrochloric acid (HCl, 37%), formic acid (HCOOH), hydrazine hydrate, sodium borohydride (NaBH4), 1,4-dithiothreitol (DTT), 1,2-ethanedithiol (EDT), 2-mercaptoethanol (ME), 3-mercapto-1-propanol (3MP).
acid (95%), sodium acetate trihydrate, acetic acid, acetone, diethyl ether, ethanol absolute, methanol (MeOH) and acetonitrile were from Sigma-Aldrich, France. Glutaraldehyde 25% (R1012), carbon-coated nickel TEM grids and uranyl acetate were obtained from Agar Oxford Instruments, UK. Unless otherwise stated, all chemicals were of far UV quality HPLC grade purity.

2.1.2. Fruit

Phenolic compound-rich fruit models were selected according to their interest in the cider- and wine-making industries in the western regions of France. The fruit varieties selected were Cabernet Franc grapes, Douce Moen and Guillevic apples, which are rich in phenolic compounds, including hydroxycinnamate derivatives.

CF wine grape fruits were harvested in September 2016, 2017 and 2018 and provided by ESA-GRAPPE, Angers-France.

Douce Moen (DM) and Guillevic (GU) apples were harvested in October 2017 and 2018 from a commercial orchard (IFPC, Sees, France).

2.1.3. Antibodies

INRA-COU1 monoclonal antibodies specific to para-coumaric acid esterified on O-5 of arabinose [3] and polyclonal anti-FerAra specific to ferulic acid esterified on O-5 of arabinose [4] were obtained from the laboratory collection (INRA-BIA-PVPP, France).

2.2. Methods

2.2.1. Immunohistochemistry (IHC)

IHC was performed on Douce Moen apples harvested in 2018. Cubes of 1 mm³ were sampled from the epidermis of equatorial apple sections. Tissue fixation followed the method of Guillon, Tranquet.
et al. (2004) [5]. Fixation was performed in a mixture of 3% paraformaldehyde and 0.5% glutaraldehyde in 0.1 M phosphate-buffered saline (PBS, pH 7.2) for 4 h at 4 °C. After washing, samples were dehydrated in a graded aqueous ethanol series, progressively infiltrated with LRW acrylic resin and then

Fig. 3. Sum of pCA and FA content in the saponified fraction of the cell wall (mg kg⁻¹ cell wall dry weight) in Cabernet franc (C. franc) grape and Douce Moen and Guillevic apples according to the method of cell wall preparation (A, B or C). Different letters represent significant differences (p-value < 0.05; n ≥ 3).

Fig. 4. Mean content (±SD; n = 4) of pCA and FA in the saponified fraction of the cell wall (mg kg⁻¹ cell wall dry weight) prepared according to method C from the peel and flesh of Cabernet franc grape, Douce Moen and Guillevic cider apples. Different letters present significant differences (p value < 0.05).
embedded in gelatin capsules. The resin was polymerized for 4 days at 55 °C without an accelerator. Ultrathin sections of 80 nm thickness were prepared and mounted on carbon-coated nickel grids. Sections were stained with aqueous uranyl acetate (1%). Immunolabeling was carried out on transverse ultrathin sections treated as follows: 30 min in the blocking solution of PBS-1% BSA, incubation with primary antibodies (diluted 1:1500 and 1:3 for anti-FerAra and INRA-COU1, respectively) in PBS-0.3% BSA for 1 h followed by incubation with a second goat anti-rabbit (anti-FerAra sections) or anti mouse (INRA-COU1 sections) antibodies (1:20 dilution) conjugated to 10 nm gold particles (Aurion, Wageningen, NL). Sections were counterstained with aqueous uranyl acetate (1%) and examined by transmission electron microscopy (JEOL 100S).

2.2.2. Cell wall preparations

Different methods were used to prepare the apple and grape fruit cell wall materials (Fig. 2). The methods differed by their potential to limit the oxidation of phenolic compounds during cell wall preparations. Inspired by Lichtenthaler et al. (1998) [6], the basic process consisted of removing the apple core and grape seeds prior to separating the peel and the flesh. The two fruit parts were mashed, and the cell walls were prepared by the elimination of the 70% ethanol-soluble compounds. Afterward, the insoluble residues were washed with water, aqueous sodium dodecyl sulfate (3%), aqueous sodium chloride (1 M) and acetone prior to drying overnight at 40 °C. In the first experiment, the whole preparation was performed in ambient air (method A). For method B, the fruit parts were first dipped in aqueous sodium fluoride (1%) to inhibit oxidative enzymes [7] before proceeding with method A, which was performed in a glove box filled with nitrogen gas. For the last method (method C), prior to sampling following method B, the fruits were placed in a desiccator in a glove box, and the air from the fruit was removed by vacuum aspiration and nitrogen purge. This gas exchange was only performed on apples. For this method, an extraction of water-soluble compounds was performed prior to 70% ethanol extraction for both apples and grapes. Method A was performed only on CF grapes, and methods B and C were applied on CF, DM and GU. Fruits were separated in pools of 300 g for CF and 500 g for apples. Methods A and B were performed on three pools, and method C was performed on four pools.

2.2.3. Saponification of phenolic esters from the cell wall

The saponification of phenolic acid esters was inspired by the method described by Antoine et al. (2007) [8] with some adaptations (Fig. 2). Dry cell wall material (500 mg) was submitted to alkaline
hydrolysis for 30 min at 40 °C in NaOH (2 M, 150 mL) to remove the esterified phenolic compounds from the cell wall. The internal standard (IS, TMCA; 1 g L⁻¹, 15 mL) was then added to the suspension prior to filtration under vacuum on a fritted glass filter (Borosilicate Glass no. 1, Pyrex, ThermoFisher Scientific, France). The alkaline filtrate was slowly acidified in an ice bath to pH < 1 by HCl. Acidification provoked a change in the filtrate color from dark-red to orange. The acidified solution was extracted at least three times with diethyl ether (∼50 mL). The pooled ether phases were then evaporated to dryness, and the dry residue was solubilized by a solution of MeOH-(H₂O, formic acid 1%) (1:1). A standard solution was prepared by dissolving pCA, FA and TMCA at a concentration of 0.1 g L⁻¹ in the MeOH-(H₂O, formic acid 1%) (1:1) solution. The extract and standard solution were then filtered through PTFE (UPTIDISC PTFE filters, 4 mm, 0.45 μm, PP HOUSING Interchrom, France) and transferred to amber vials prior to chromatographic analysis.

2.2.4. Chromatographic analysis

Phenolic compounds were identified by HPLC-UV coupled to mass spectrometry (MS) as described by Malec et al. (2014) [9]. Analysis was carried out using a system composed of a thermostated
autosampler (model Surveyor, Thermo Finnigan, San Jose, CA, USA), a binary high-pressure pump
(model 1100, Agilent Technologies, Palo Alto, CA, USA), a UV–vis diode array detector (model UV6000 LP, Thermo Finnigan), and an ion trap mass spectrometer equipped with an electrospray ionization source (model LCQ Deca, Thermo Finnigan). The column was a Purospher STAR RP-18 end-capped (3 μm) Hibar HR (Merck, 2.1 × 150 mm) equipped with an Eclipse XDB-C8 precolumn (Agilent Technologies, 2.1 × 12.5 mm, 5 μm) and thermostated at 30 °C. Solvents were (A) acidified pure water (0.1% formic acid) and (B) acidified acetonitrile (0.1% formic acid), both degassed with a SCM1000 vacuum membrane degasser (Thermo Fisher Scientific Inc). The flow rate was set at 0.2 mL min⁻¹, and the elution gradient was 0 min (97% A; 3% B), 3 min (93% A; 7% B), 21 min (87% A; 13% B), 27 min (87% A; 13% B), 41 min (80% A; 20% B), 51 min (55% A; 45% B), 53 min (10% A, 90% B), 56 min (10% A, 90% B), 58 min (97% A; 3% B) and 76 min (97% A; 3% B). The UV–vis detection was set to 320 nm, and mass detection was realized by electrospray ionization in the negative ion mode by deprotonation. Data were collected and processed by Xcalibur software (version 1.2, Thermo Finnigan). Examples of a chromatogram and mass spectra are presented in Fig. 1.

The identified HCAs were quantified by HPLC-UV using a C18 reversed-phase column (Vision HT C18 HL 5 μL, 250 mm × 4.6 mm, Grace, Germany) thermostated at 25 °C and eluted with acetonitrile (solvent A) and acetate buffer (solvent B: 4.5 g sodium acetate trihydrate dissolved in 1 L of distilled water containing 2.2 mL of acetic acid). Solvent mixing was performed using a binary pump ( Dionex UltiMate 3000 pump, Thermo Scientific, USA) during elution at a flow rate of 1 mL min⁻¹. Elution was realized by 15% solvent A and 85% solvent B for 5 min, which was then increased to 25% solvent A and 75% solvent B with a linear gradient over 15 min and held for an additional 5 min at the last solvent conditions. Detection was performed using a diode array detector (Dionex UltiMate 3000, Thermo Scientific, USA). Data were collected and processed by Chromeleon software® (version 6.8, Thermo Scientific, USA). The peak area at 320 nm was used for quantification and compared to the IS (TMCA) and known amounts of phenolic acids in a standard solution used for calibration. The standard solution contained pCA, FA and TMCA at 0.1 mg mL⁻¹ in H₂O/MeOH (1:1) as the solvent. The standard solution gave access to the response factor (RF) between pCA or FA against TMCA, determined as:

\[ RF = \frac{TMCA \left(\text{peak area}\right)}{pCA \text{ or } FA \left(\text{peak area}\right)} \times \frac{pCA \text{ or } FA \left(\text{weight}\right)}{TMCA \left(\text{weight}\right)} \]

The obtained RFs (six replicates) were constant at 0.50 for pCA and 0.47 for FA. Thus, the pCA and FA amounts in the AIM were determined as follows:

\[ \text{pCA or FA content} = \frac{pCA \text{ or } FA \left(\text{peak area}\right) \times RF \times TMCA \left(\text{weight}\right)}{TMCA \left(\text{peak area}\right) \times AIM \left(\text{weight}\right)} \]

TMCA weight was the weight added to the samples at the saponification step. Thus, calibration curves were not required to quantify pCA and FA content, as we only used the RFs.

Nevertheless, calibration curves were constructed to determine the limit of detection (LOD) and the limit of quantification (LOQ) of the equipment (Fig. 6). Six standard solutions were prepared in H₂O:MeOH (1:1) as the solvent with pCA and FA contents from 1 to 500 mg L⁻¹ and 0.5–10 mg L⁻¹, respectively. The LOD and LOQ corresponded to the concentration at which the signal/noise ratio value was equal to 3 and 10, respectively. The signal/noise ratio is equal to 2H/h, where “H” is the height of the peak compound and “h” is the average of the heights of the baseline over a distance of twenty times of “w” on both sides of the peak compound; “w” equals half the height of the peak compound.

For pCA: LOD <1 mg L⁻¹; and for FA: LOD <0.5 mg L⁻¹ and LOQ <1 mg L⁻¹.

2.2.5. Statistics

Statistical evaluation of means was performed using Student’s t-test with Microsoft Excel® software (2016). Statistically significant differences were taken at a p value ≤ 0.05 with the number of repeats ≥3.
Acknowledgments

This work was supported in part by the Cap Aliment Food for Tomorrow program funded by Région Pays de la Loire. HPLC-MS analysis was performed on the P2M2 analytical platform (Metabolic Profiling and Metabolomic Platform, INRA, Rennes — Le Rheu). The authors thank Dr JM Le Quere (INRA BIA, Rennes) for fruitful suggestions, H Sotin (INRA, BIA Rennes) for carrying out the HPLC-MS analyses, C Alvarado (INRA, BIA Nantes) for microscopy analysis and D Le Meurlay for technical support (ESA, Angers). Part of this work was performed on the platforms BIBS (INRA, BIA Nantes).

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] A.J. Parr, A. Ng, K.W. Waldron, Ester-linked phenolic components of carrot cell walls, J. Agric. Food Chem. 45 (7) (1997) 2468–2471.
[2] M.M.d.O. Buana, Feruloylation in grasses: current and future perspectives, Mol. Plant 2 (5) (2009) 861–872.
[3] O. Tranquet, et al., Monoclonal antibodies to p-coumarate, Phytochemistry 70 (11–12) (2009) 1366–1373.
[4] S. Philippe, et al., Investigation of ferulate deposition in endosperm cell walls of mature and developing wheat grains by using a polyclonal antibody, Planta 225 (5) (2007) 1287–1299.
[5] F. Guillou, et al., Generation of polyclonal and monoclonal antibodies against arabinoxylans and their use for immuno-chemical location of arabinoxylans in cell walls of endosperm of wheat, J. Cereal Sci. 40 (2) (2004) 167–182.
[6] H.K. Lichtenthaler, J. Schweiger, Cell wall bound ferulic acid, the major substance of the blue-green fluorescence emission of plants, J. Plant Physiol. 152 (2) (1998) 272–282.
[7] H. Février, et al., Polyphenol profile, PPO activity and pH variation in relation to colour changes in a series of red-fleshed apple juices, LWT - Food Sci. Technol. (Lebensmittel-Wissenschaft -Technol.) 85 (2017) 353–362.
[8] C. Antoine, et al., Individual contribution of grain outer layers and their cell wall structure to the mechanical properties of wheat bran, J. Agric. Food Chem. 51 (7) (2003) 2026–2033.
[9] M. Malec, et al., Polyphenol profiling of a red-fleshed apple cultivar and evaluation of the color extractability and stability in the juice, J. Agric. Food Chem. 62 (29) (2014) 6944–6954.