Analytical methods for extraction and identification of primary and secondary metabolites of apple (*Malus domestica*) fruits: a review

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Supplementary Material

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Table S1. Overview of the analytical techniques used in the last 10 years (reported in Chronological order) for the extraction and the analysis of secondary metabolites occurring in *Malus Domestica*.

Apple cultivar	Extraction method and Analytical technique used	Instrumental conditions		Compounds detected and major important results	Reference
- **Crimson Crisp** - **Golden Delicious** - **Fuji** - **Gala Royal** - **Pink Lady**	80 mL of water MilliQ, 0.5 g of gluconolacton and 50 µL of internal standard n-heptanol (100 mg/L) were added to 30 g of deep-frozen sample powder. After homogenization, centrifugation and filtration through rapid paper filter, Solid phase extraction (SPE) was performed GC-MS/MS	**GC conditions** **Column**: 30 m VF-WAXms capillary column with an internal diameter of 0.25 mm and a film thickness of 0.25 µm **Temperature:** - 40 °C hold for 2 min after injection, 10 °C/min up to 50 °C, 1.4 °C/min up to 60 °C, hold for 2 min, 1.6 °C/min up to 70 °C, hold for 1 min, 2.2 °C/min up to 100 °C, hold for 0.5 min, 3.1 °C/min up to 140 °C, 4.4 °C/min up to 200 °C, 12 °C/min up to 250 °C, hold for 6 min. **Injection parameters** were: splitless injection, splitless time: 0.8 min, inlet temperature 250 °C, carrier gas was helium 5.5, programmed flow: 0.8 mL/min hold for 62.50 min, 0.8 mL/min up to 1.2 mL/min in 0.5 min, hold for 7 min.	**MS conditions** **Electron ionisation** source a 70 eV **Transfer line temperature**: 220 °C **The filament current** was 50 µA	VOC's The method proposed provided excellent selectivity and sensitivity and was successfully used to determine 69 compounds in apples. Moreover, can be easily extended to volatile compounds in other fruits and can be therefore be widely used for quantification/profiling studies in the field of fruit aroma.	[88]
***Malus* Domestica (4 cultivars):** **- Golden Delicious** **- Nicoter Kanzi** **- Shinano Gold yello** **- Luresweet Redlove**	Extraction of powdered pulp at 5°C with 80% methanol-20% H_2O, acidified with H_3PO_4 (1% w/v); addition of NaF solution. HPLC-UV-FL	**HPLC conditions** **Column**: Kinetex F5 (150 x 4.6 mm, 2.6 µm) equipped with F5 UHPLC pre-column **Mobile phase**: 0.1% formic acid in water (A), 0.1% formic acid in methanol (B) **Flow rate**: 1.0 mL/min **Elution**: 0 - 18 min → from 10% to 18% B 18 - 20 min → from 18% to 35% B 20 - 32.5 min → from 35% to 45% B 32.5 - 33 min → from 45% to 80% B 33 - 35 min → 80% B 35 - 36 min → from 80% to 10% B 36 - 40 min → 10% B **Injection volume**: 5 µL	**Fluorescence detector** (for (+)-catechin, (-)-epicatechin, procyanidin B1, procyanidin B2, procyanidin C1) **λ excitation**: 280 nm **λ emission**: 320 nm **Diode array detector** **λ of acquisition**: 254 nm (quercetin-3-rhamnoside), 280 nm (phloridzin, gallic acid), 320 nm (chlorogenic acid), 360 nm (quercetin glycosides)	Gallic acid - Procyanidin B1 - (+)-Catechin - Procyanidin B2 - Chlorogenic acid - (−)-Epicatechin - Procyanidin C1 - Quercetin-3-*O*-galactoside - Quercetin-3-*O*-glucoside - Quercetin-3-*O*-xyloside - Phloridzin - Quercetin-3-*O*-arabinoside - Quercetin-3-*O*-rhamnoside The analytical method was fully validated and allowed to underline some important differences in the phenolic composition of apple samples.	[110]

***Malus* Domestica (3 varieties):** **- Ozark Gold** **- Starkinson** **- Kosztela**	Extraction with 80% of aqueous methanol with 1% of HCl and sonication at 4°C HPLC-ESI-MS (for identification)	**HPLC conditions** **Column**: Symmetry C18 column (150 x 4.6 mm, 5 µm) **Mobile phase**: 1.0% formic acid in water (A), 100% acetonitrile (B) **Flow rate**: 0.5 mL/min **Elution**: 0 -1 min → 95% A 1 - 41 min → from 95% to 0% A 42 - 51 min → 0% A Reconditioning for the next 9 min	**MS conditions** **Source voltage**: 0.1 kV for positive ionization **Source voltage**: 3 kV for negative ionization **Capillary temperature**: 300 °C **Sheath gas pressure**: 50 units **Auxiliary pressure**: 5 units	**Hydroxycinnamic acid** *p*-coumarylquinic acid - 5-*O*-caffeoylquinic acid - caffeic acid hexose conjugate **Flavanols and procyanidins** Procyanidin B1 - (+)-catechin - procyanidin B2 - (-)-epicatechin - procyanidin C1 **Dihydrochalcones** Phloretin-2'-*O*-xyloglucoside - phloretin-2'-*O*-glucoside **Flavonols** Quercetin-3-*O*-rutinoside - quercetin-3-*O*-galactoside - quercetin-3-*O*-glucoside - quercitin-3-*O*-arabinoside - quercitin-3-*O*-xyloside - quercetin-3-*O*-rhamnoside By using these HPLC methods 16 polyphenolic compounds belonging to five major polyphenolic groups were identified and quantified	[111]
	Extraction with 80% of aqueous methanol with 1% of HCl and sonication at 4°C HPLC-DAD (for quantification)	**HPLC conditions** **Column**: Synergi Fusion RP-80A (150 x 4.6 mm, 4 µm) **Mobile phase**: 2.5% formic acid in water (A), 100% acetonitrile (B) **Flow rate**: 1.0 mL/min **Elution**: 0 -1 min → 95% A 1 - 41 min → from 95% to 0% A 42 - 51 min → 0% A Reconditioning for the next 9 min	**λ of acquisition**: 280 nm (flavan-3-ols and dihydrochalcones), 320 nm (phenolic acid), 360 nm (flavonols), 520 nm (anthocyanin)		
***Malus* domestica (10 scab-resistant apple cultivars)** **- GoldRush** **- Florina** **- Discovery** **- William's Pride** **- Prima** **- Champion** **- Rewena** **- Remura** **- Topaz** **- Idared**	Apple juice was obtained from a whole fruit by using an electric juicer. Arbutin in methanol was added as internal standard HPLC-MS	**HPLC conditions** **Mobile phase**: water (A), acetonitrile (B), 1% formic acid in water (C) **Flow rate**: 0.5 mL/min **Elution**: 0 - 13.3 min → 5-16% B and 10% C 13.3 - 16 min → 16-28% B and 10% C 16 - 22 min → 28-40% B and 10% C 22 - 23 min → 40-100% B and 10% C 23 - 25 min → 100% B 25 - 25.1 min → 100-5% B and 0-10% C 25.1 - 33 min → 5% B and 10% C **Injection volume**: 1.5 µL	**MS conditions** **ESI + -** **MRM mode** **Source temperature**: 150 °C **Capillary voltage**: 3500 V **Cone voltage**: 30 V **Drying gas flow (N_2)**: 800 L/h **Drying gas temperature**: 400 °C	**Hydroxycinnamic acids** Chlorogenic acid **Flavan-3-ols** Epicatechin **Flavonols** Quercetin glycosides **Hydrochalcones** Phloridzin **Anthocyanins** Cyanidin-3-galactoside Various phenolic compounds were tentatively identified by LC-MS analyses	[100,127]

***Malus* Domestica (3 varieties):** **- Golden Delicious** **- Red Delicious** **- Tetovka**	After lyophilization apple flesh/peel, and dried leaves samples, previously ground, were extracted twice with methanol–water (90:10, V/V) using ultrasound for 1 h UHPLC-DAD-HESI-MS	**HPLC conditions** **Column**: Zorbax Eclipse Plus C18 Rapid Resolution HD column (50 x 2.1 mm, 1.8 µm) **Mobile phase**: 1% formic acid in water (A), 1% formic acid in methanol (B) **Flow rate**: 0.7 mL/min **Elution**: 0 - 1.5 min → 10% B 4.5 - 5.5 min → 35% B 8.5 min → 40% B 9.0 - 10.0 min → 45% B 10.5 - 14.5 min → 80% B **λ of acquisition**: 260 nm (flavanols), 280 nm (dihydrochalcones and hydroxybenzoic acid derivatives), 320 nm (hydroxycinnamic acids), 330 nm (flavones), 350 nm (flavonols)	**MS conditions** **ESI -** **Heater temperature**: 285 °C **Capillary temperature**: 275 °C **Sheet gas flow (N_2)**: 42 psi **Auxiliary gas flow (N_2)**: 11 psi **Source voltage**: 4 kV **Capillary voltage**: -40 V **Tube lens voltage**: -80 V **Normalized collision energy**: 35 eV	**Phenolic acids** Gallic acid - Gallic acid derivatives - Chlorogenic acid - *p*-coumaric acid - *p*-coumaric acid derivatives - Caffeic acid **Flavan-3-ols** Procyanidin B3 - Procyanidin B2 - Catechin - Procyanidin B1 - Epicatechin **Dihydrochalcones** Phloridzin - Rhamnosyl phloridzin - 3-hydroxyphloridzin - Phloretin penthosylhexoside - Phloretin **Flavonols** Rutin - Isoquercetin - Quercetin pentoside - Kaempferol hexoside - Kaempferol pentoside - Quercetin - Kaempferol - Myricetin **Flavones** Luteolin - Apigenin - Apigenin-7-*O*-glucoside This validated method was used for identification and quantification of 27 polyphenolic compounds, showing analytical characteristics of MS method comparable to UV-DAD with regard to recovery, precision and robustness, and superior for its linear range, sensitivity and selectivity.	[71,101]

***Malus* Domestica (9 varieties):** **- Di Corone A2** **- Rosso invernale B1** **- Limoncello antiche C1** **- Pomacia D2** **- De la rosa E1** **- Belladonna F1** **- Regina G1** **- Del Mieli H1** **- Di Giulio I1**	Ultrasound assisted extraction was performed at room temperature on dried pulp and peel samples by adding methanol–water (50%) solution HPLC-DAD-ESI-MS	**HPLC conditions** **<u>Polyphenols separation</u>** **Column**: Eclipse XDC C-18 (3.0 x 150 mm, 3.5 µm) **Mobile phase**: acetonitrile (A), 0.1% formic acid in water (B) **Flow rate**: 500 µL/min **Elution**: 0 - 15 min → A:B (5:95) - A:B (15:85) 15 - 35 min → A:B (85:15) - A:B (100:0) 48 - 53 min → A:B (100:0) - A:B (5:95) **λ of acquisition**: 280, 330, 350 nm	**MS conditions** **ESI –** **Range: 50-2000 m/z**	Catechin - Chlorogenic acid - Procyanidin dimer B1 - Caffeic acid derivative - Procyanidin trimer B - Procyanidin pentamer B - Procyanidin tetramer B - Flavan-3-ol derivative - Quercetin-3-O-galactoside - Quercetin-3-O-glucoside - Rutin - Quercetin-3-O-xyloside - Phloretin-2-O-xyloglucoside - Quercetin-3-O-arabynoside - Quercetin-3-O-rhamnoside - Phloretin-2-O-glucoside - Rhamnetin-3-O-glucoside - Cuneataol - Pomaceic acid - Euscaphyc acid - Annurcoic acid - Pomolic acid - Maslinic acid - Corosolic acid - Euscaphic acid derivative - Betulinic acid - Oleanolic acid - Ursolic acid	[59,151]
	Ultrasound assisted extraction was performed at room temperature on dried pulp and peel samples by adding methanol–water (50%) solution HPLC-(APCI)-MS	**HPLC conditions** **<u>Triterpenes separation</u>** **Column**: Eclipse XDB C-18 (3.0 x 150 mm, 3.5 µm) **Mobile phase**: methanol (A), water (B) **Flow rate**: 500 µL/min **Elution**: 0 - 12 min → A:B (45:55) - A:B (80:20) 12 - 48 min → A:B (80:20) - A:B (80:20) 48 - 49 min → A:B (80:20) - A:B (45:55) 49 - 55 min → A:B (45:55) - A:B (45:55)	**MS conditions** **APCI -** **Range: 50-2000 m/z** **Shield**: 600 V **Nebulizer gas flow (N_2)**: 35 psi **Drying gas flow (N_2)**: 25 psi **Corona disharge**: ±5 amp **Capillary voltage**: 100 V **Drying gas flow temperature ramp:** from 350°C to 290°C in 25 minutes and then kept constant at 290°C.	This method offers the possibility to rapidly identify the triterpene acids in samples and can be useful for assessing differences of the various apples related to these compounds. In addition, the opportunity to have structural information on such compounds and the selectivity of the MS detector represents an improvement in the triterpene acids identification compared to other methods	

***Malus* Domestica (1 variety):** **- Anna cultivar from Costa Rica**	Freeze-dried samples were extracted using acetone:water (70:30) at 40 ◦C. Next, the extract was evaporated under vacuum to eliminate the acetone and the aqueous phase was washed with ethyl acetate and chloroform to remove less-polar compounds. UPLC-DAD-ESI-MS	**UPLC conditions** **Column**: Hypersil Gold AQ RP-C18 column (200 x 2.1 mm, 1.9 µm) with an UltraShield pre-column filter **Mobile phase**: 0.1% formic acid in water (A), 0.1% formic acid in acetonitrile (B) **Flow rate**: 0.3 mL/min **Elution**: 0 - 20 min → from 4% to 20% B 20 - 30 min → from 20% to 35% B 30 - 31 min → from 35% to 100% B 31 - 35 min → held at 100% B **λ range of acquisition**: 200 - 700 nm	**MS conditions** **ESI -** **Spray voltage**: 4.8 kV **Capillary temperature**: 300 °C **Capillary voltage**: 15 V **Tube lens**: 70 V **Sheath gas**: 70 a.u. **Auxiliary and sweep gas**: 15 a.u.	**<u>Proanthocyanidins</u>** Procyanidin B-type dimer - (epi)catechin 3-*O*-gallate - Procyanidin B-type dimer - Catechin - Procyanidin B-type dimer - Epicatechin - Procyanidin B-type trimer - Procyanidin B-type trimer - Procyanidin B-type tetramer - Procyanidin B-type pentamer - Procyanidin B-type trimer - Procyanidin B-type pentamer - Procyanidin B-type trimer - Procyanidin A-type dimer - Procyanidin B-type dimer **<u>Glycosylated flavonols</u>** Kaempferol-hexoside - Naringenin-hexoside - Quercetin-pentosylhexoside - Quercetin-hexoside - Quercetin-rutinoside - Quercetin-pentoside - Quercetin-deoxyhexoside **<u>Acids and derivatives</u>** Protocatechuic acid - *p*-coumaroyl-hexoside - *p*-coumaroyl-hexoside - Caffeoylquinic acid isomer - *p*-coumaroylquinic acid **<u>Chalcones</u>** 3-hydroxyphloretin-pentosylhexoside - 3-hydroxyphloretin - Phloretin-pentosilhexoside - Phloretin **<u>Other compounds</u>** Vomifoliol-pentosilhexoside By using this UPLC-DAD-ESI-MS method 52 compounds were characterized, distributed as 21 proanthocyanidins, 15 flavonoids, including kaempferol, quercetin and naringenin derivatives, and eight phenolic acid derivatives; as well as chalcones and isoprenoid glycosides	[105]

***Malus* Domestica (5 clones):** **- Gala Baigent** **- Fuji Mishima** **- Fuji Select** **- Fuji Suprema** **- Maxi Gala**	Small pieces of apple peel were homogenized with acetone and extracted in an ultrasonic bath at room temperature for 60 minutes. HPLC-MS	**HPLC conditions** **Column**: Column C18 RP (150 x 4.6 mm, 5 µm) **Mobile phase**: acetonitrile (A), ammonium acetate 10 mM pH 6 (B) **Flow rate**: 1.0 mL/min **Elution**: isocratic (80:20 A:B) **Injection volume**: 10 µL	**MS conditions** **ESI +** **SIM mode** **Capillary voltage**: 4000 V **Drying gas flow**: 12 L/min **Nebulizer gas pressure**: 50 psi **Drying gas temperature**: 350 °C	Betulinic acid (BA) - Ursolic acid (UA) This is a validated method efficient, sensitive and rapid that combines techniques of extraction, detection, and quantification for analysis of UA and BA with the advantage of requiring less than 4 h for the total process and yields compatible with those reported in the literature.	[149]
***Malus* domestica (5 varieties):** **- Royal Gala** **- Golden Delicious** **- Red Delicious** **- Fuji** **- Honeycrisp**	Frozen sample powder was ground in 50% methanol containing 2% formic acid at 0–4 °C, and centrifuged at 12,000 g for 20 min at 4 °C .HPLC-DAD	**HPLC conditions** **Column**: Inertsil ODS-3 column (250 x 4.6 mm, 5 µm) with an Inertsil ODS-3 guard column (10 x 4.0 mm, 5 µm) **Mobile phase**: 10% formic acid in water (A), 10% formic acid and 1.36% water in acetonitrile (A) **Flow rate**: 1.0 mL/min **Elution**: 0 min → 95% A 25 min → 85% A 42 min → 78% A 60 min → 64% A 65 min → 95% A	**λ of acquisition**: 365 nm	Quercetin - Quercetin-3-galactoside - Quercetin-3-rutinoside - Quercetin-3-glucoside - Quercetin-3-xyloside - Quercetin-3-arabinoside - Quercetin-3-rhamnoside	[35,121]

	HPLC-MS	**HPLC conditions** **Column**: Inertsil ODS-3 column (250 x 4.6 mm, 5 µm) with an Inertsil ODS-3 guard column (10 x 4.0 mm, 5 µm) **Mobile phase**: 10% formic acid in water (A), 10% formic acid and 1.36% water in acetonitrile (A) **Flow rate**: 0.6 mL/min **Elution**: 0 min → 95% A 40 min → 70% A 55 min → 70% A	**MS conditions** **Positive mode** **Spray voltage**: 4.5 kV **Capillary temperature**: 300 °C **Sheath gas pressure (N_2)**: 30 a.u. **Auxiliary gas pressure (N_2)**: 55 a.u.	Quercetin and its glycoside derivatives were identified and quantified in the leaves, flowers, and fruits of 22 Malus genotypes. Results obtained showed that, in general, the concentration of quercetin and its glycoside derivatives depended on the species and tissue type.	
***Malus* domestica (4 varieties):** **- Fuji** **- Golden Delicious** **- Granny Smith** **- Pink Lady**	A sequential extraction was performed. UHPLC-ESI-MS	**UHPLC conditions** **Column**: Poroshell C18 (100 x 2.1 mm, 2.7 µm) with a C18 guard column (5 x 2.1 mm, 1.8 µm) **Mobile phase**: 0.1% acetic acid in water (A), 0.1% acetic acid in acetonitrile (B) **Flow rate**: 0.4 mL/min **Elution**: 0 - 5 min → from 5% to 10% B 5 - 8 min → from 10% to 12% B 8 - 10 min → from 12% to 15% B 10 - 15 min → 15% B 15 - 18 min → from 15% to 55% B 18 - 20 min → from 55% to 90% B Re-equilibration for 4 min **Injection volume**: 5 µL	**MS conditions** **ESI-** **Capillary voltage**: 2.5 kV **Drying gas temperature**: 225 °C **Drying gas flow rate**: 8.0 L/min **Sheath gas temperature**: 300 °C **Sheath gas flow rate**: 10.0 L/min **Nebulizer gas pressure**: 45 psi **Skimmer voltage**: 65 V **Octopole RF**: 750 V **Fragmentor voltage**: 125 V	**Phenolic acids** Methyl gallate - Protocatechuic acid - Syringic acid - Dicaffeoylquinic acid - 4-Hydroxy benzoic acid - Ethyl gallate - Chlorogenic acid - Hydroxy phenyl acetic acid - Vanillic acid - Benzoic acid - Phenylacetic acid - Caffeic acid - Rosmarinic acid - Coumaroylquinic acid - 3-(4-Hydroxyphenyl) propionic acid - *p*-Coumaric acid - Salicylic acid - Rosmarinic acid - *t*-Ferulic acid - Sinapic acid - Phenylacetic acid - Homoveratric acid - Phenylacetic acid By using this validated method twenty-five phenolic acids were identified with: LOD ranged between 1 and 10 ng/mL with the linear dynamic ranges between 10–1000 and 30–1000 ng/mL; r^2 ranged between 0.9963 and 1.0000; recoveries ranged between 87.8 and 110.2%.	[130]

***Malus* domestica (6 varieties):** **- Aldas** **- Auksis** **- Connel Red** **- Ligol** **- Lodel** **- Rajka**	The lyophilized samples were extracted with 70% ethanol (1:30, v/v), in an ultrasonic bath for 20 m UPLC-PDA-ESI-MS	**UPLC conditions** **Column**: Waters Aquity HSS T3 **Mobile phase**: 2% acetic acid in water (A), acetonitrile (B) **Elution**: 0 - 1 min → 88% A 1 - 8 min → from 88% to 70% A 8 - 9 min → from 70% to 90% A 9 - 10 min → 90% A	**MS conditions** **ESI-** **Capillary voltage**: 4000 V **Nebulizing gas pressure (N_2)**: 2.5 Bar **Drying gas flow (N_2)**: 10 L/min **Drying gas temperature**: 200 °C	Quinic acid - Proanthocyanidin B1 - (+)-Catechin - Chlorogenic acid - Proanthocyanidin B2 - (–)-Epicatechin - Coumaroylquinic acid - Proanthocyanidin C1 - Proanthocyanidin derivative - Quercetin-3-*O*-rutinoside - Quercetin-3-*O*-galactoside - Quercetin-3-*O*-glucoside - Quercetin pentoside - Quercetin 3-*O*-arabinoside - Quercetin-3-*O*-rhamnoside - Phloretin xyloglucoside – Phloridzin The identification and structure elucidation of 19 bioactive compounds possessing reducing and radical scavenging properties were performed in less than 10 minutes by using an UPLC-PDA-ESI-MS method	[107]
***Malus* domestica (4 cultivars):** **- Golden Delicious** **- Jonagold** **- Elstar** **- Ligol** **- Mutsu**	Different extraction solvents (ethyl acetate, methanol, water) were used to obtain leaf extracts with high, average and low amounts of phenolic compounds in a multi-step procedure. SPE procedure was employed to remove chlorophyll and other ballast hydrophobic components HPLC-PDA	**HPLC conditions** **Column**: Aquasil C18 (250 x 4.6 mm, 5 µm) **Mobile phase**: acetonitrile (A), 1mM phosphoric acid (B) **Flow rate**: 1.0 mL/min **Elution**: 0 min → 15% (A in B) 15 min → 15% 25 min → 20% 35 min → 20% 50 min → 50% 60 min → 90% (A in B) 15 min post time for re-equilibration	**λ range of acquisition**: 190 - 400 nm	Chlorogenic acid - *p*-hydroxybenzoic acid - Hyperoside - Isoquercitrin - Quercitrin - Phloridzin – Rutoside Phenolic profile of ***Malus* domestica** leaves was elucidated in different extracts. In the richest phenolic extracts main phenolic fraction was flavonoids and phenolic acids were present in traces.	[118]

***Malus* domestica (3 cultivars):** **- Ariane** **- Melrose** **- Smoothee (mutant of Golden Delicious)**	Freeze-dried samples were submitted to the thiolysis reaction in methanol according to Guyot 2001 HPLC-DAD	**HPLC conditions** **Column**: Licrospher PR-18 (250 x 4 mm, 5 µm) **Mobile phase**: 2.5% acetic acid in water (A), acetonitrile (B) **Flow rate**: 1 mL/min **Elution**: 0 - 5 min → from 3% to 9% B 5 - 15 min → from 9% to 16% B 15 - 45 min → from 16% to 50% B 45 - 48 min → from 50% to 90% B 48 - 52 min → 90% B 52 - 55 min → from 90% to 3% B 55 - 60 min → 3% B	**λ of acquisition**: 280 nm ((+)-catechin, (-)-epicatechin, phloretin, phloridzin, (-)-epicatechin benzyl tioether), 320 nm (5-*O*-caffeoylquinic acid, *p*-coumaric acid), 350 nm (quercetin glycosides), 540 nm (cyanidin glicosides)	(+)-catechin - (-)-epicatechin - phloretin - phloridzin - (-)-epicatechin benzyl tioether - 5-*O*-caffeoylquinic acid - *p*-coumaric acid - quercetin - cyanidin-3-*O*-galactoside Five phenolic classes with a total of sixteen identified individual compounds were quantified in the pulp and in the skin of apple	[119,120]
***Malus* domestica (2 cultivars):** **- Szampion** **- OzarkGold**	Freeze-dried leaves or fruits were extracted by 10 mL of mixture containing methanol (30 mL/100 mL), ascorbic acid (2.0 g/100 mL) and acetic acid in an amount of 1.0 mL/100 mL of reagent. The extracts were sonicated for 15 min left for 24 h at 4 °C without light and then sonicated again for 15 min. Then, the samples were centrifuged for 10 min at 4 °C and 20,000 rpm. UPLC-PDA	**UPLC conditions** **Polyphenols** **Column**: BEH Shield C18 analytical column (50 x 2.1 mm, 1.7 µm) **Mobile phase**: 4.5% formic acid in water (A), acetonitrile (B) **Flow rate**: 0.45 mL/min **Elution**: Initial conditions → 1% B 0 - 5 min → from 1% to 75% B 5.0 - 6.5 min → from 75% to 100% B 6.5 - 7.5 min → 100% B 7.5 - 8.5 min → reconditioning to initial conditions **Injection volume**: 5 µL	**λ of acquisition**: 280 nm (flavan-3-ols), 320 nm (phenolic acid), 360 nm (flavonol glycosides)	**Flavan-3-ols** (+)-Catechin - Procyanidin B1 - (-)-Epicatechin - Procyanidin B2 - Procyanidin C1 **Dihydrochalcones** Phloretin-2'-xylo-glucoside - Phloridzin **Phenolic acids** Neochlorogenic acid - *p*-coumaric-quinic acid - Chlorogenic acid - Cryptochlorogenic acid **Flavonols** Quercetin-3-*O*-galactoside - Quercetin-3-*O*-rutinoside - Quercetin-	[106]

	UPLC-FL	**UPLC conditions** **<u>Proanthocyanidins</u>** **Column**: BEH Shield C18 analytical column (50 x 2.1 mm, 1.7 µm) **Mobile phase**: 2.5% acetic acid in water (A), acetonitrile (B) **Elution**: Initial conditions → 2% B 0.6 - 2.17 min → from 2% to 3% B 2.17 - 3.22 min → from 3% to 10% B 3.22 - 5.00 min → from 10% to 15% B 5.00 - 6.00 min → washing and reconditioning (1.50 min)	**λ excitation**: 278 nm **λ emission**: 360 nm	3-*O*-glucoside - Quercetin-3-*O*-rhamnoside LC-MS and UPLC-PDA-FL methods were used to elucidate and compare polyphenolic profiles in leaves and fruits of 2 selected cultivars of Malus domestica. The leaves showed a significantly higher polyphenol amount than the fruits.	
	UPLC-MS	**UPLC conditions** **<u>Polyphenols</u>** **Column**: UPLC BEH C18 column (50 x 2.1 mm, 1.7 µm) **Mobile phase**: 4.5% formic acid in water (A), acetonitrile (B) **Flow rate**: 0.45 mL/min **Elution**: 0 -1 min → 99% A 1 - 12.5 min → from 99% to 0% A 12.5 - 13.5 min → return to initial conditions and held constant to re-equilibrate the column **Injection volume**: 10 µL	**MS conditions** **ESI -** **Source temperature**: 130°C **Desolvation temperature**: 350 °C **Capillary voltage**: 2.5 kV **Cone voltage**: 30 V **Desolvation gas flow (N_2)**: 300 L/h		

***Malus* domestica (4 varieties):** **- Aldas** **- Auksis** **- Ligol** **- Lodel**	Lyophilized apple leaf powder was extracted with ethanol (70%, v/v), and extracted in a ultrasonic bath for 40 minutes at 60◦ and centrifuged for 7 minutes at 6000 rpm. HPLC-DAD	**HPLC conditions** **Column**: YMC-Pack ODS-A C18 column (250 x 4.6 mm, 5 µm) equipped with a C18 YMC-Triart precolumn (10 x 3.0 mm, 5 µm) **Mobile phase**: 1 mL/min **Flow rate**: 2% acetic acid in water (A), acetonitrile (B) **Elution**: 0 - 30 min → from 3% to 15% B 30 - 45 min → from 15% to 25% B 45 - 50 min → from 25% to 50% B 50 - 55 min → from 50% to 95% B **Injection volume**: 10 µL	**λ of acquisition**: 280 nm (dihydrochalcones, catechins), 320 nm (phenolic acids), 360 nm (flavonols)	(+)-catechin - Chlorogenic acid - Caffeic acid - (-)-Epicatechin - Rutin - Hyperoside - Isoquercitrin - Avicularin - Quercitrin - Phloridzin – Phloretin Phenolic compounds of various groups were separated with a good resolution and quantified by HPLC-DAD. The results of this study will provide more information about the composition and content of phenolic compounds in apple leaves extracts, showing that they can be employ as the source of phenolic compounds.	[117]
Red-fleshed apple hybrid cultivar	HPLC-UV & HPLC-MS	**HPLC conditions** **Column**: ZORBAX Eclipse XDB-C18 (150 x 2.1 mm, 3.5 µm) equipped with a precolumn (12.5 x 2.1 mm) of the same material **Mobile phase**: 0.1% formic acid in water (A), 0.1% formic acid in acetonitrile (B) **Flow rate**: 0.2 mL/min **Elution**: Initial → 3% B 0 - 40 min → 20% B 40 - 55 min → 35% B Washing and equilibrating **λ of acquisition**: 280 nm (flavanols and dihydrochalcones), 320 nm (hydroxycynnamates), 350 nm (flavonols), 510 nm (anthocyanins)	**MS conditions** **ESI -** **Capillary temperature**: 240 °C **Spray voltage**: -5 kV **Capillary voltage**: -70 V **Sheath gas**: 67 a.u. **Auxiliary gas**: 4 a.u. **Collision energy**: 25 - 35 % a.u.	Procyanidin B1 - (+)-Catechin - 5-caffeoylquinic acid - Cyanidin-3-galactoside - Procyanidin B2 - (−)-Epicatechin - 4-*p*-coumaroylquinic acid - Cyanidin pentoside - Flavanol trimer - Flavanol tetramer - Quercetin 3-*O*-galactoside - Procyanidin B5 - Quercetin 3-*O*-glucoside - Quercetin 3-*O*-arabinoside - Phloretin-2-xyloglucoside - Phloridzin -Quercetin 3-*O*-xyloside - Quercetin 3-*O*-rhamnoside - Anthocyanin and flavanol adduct - 5-carboxypyranocyanidinglucoside	[132]

***Malus* domestica (4 varieties):** **- Kolacara** **- Budimka** **- Sumatovka** **- Kozara**	Apple juice was obtained from a whole fruit by using an electric juicer. Arbutin in methanol was added as internal standard HPLC-MS/MS	**HPLC conditions** **Column**: not specified **Mobile phase**: water (A), acetonitrile (B), 1% formic acid in water (C) **Flow rate**: 0.5 mL/min **Elution**: 0 - 13.3 min → 5 - 16% B and 10% C 13.3 - 16 min → 16 - 28% B and 10% C 16 - 22 min → 28 - 40% B and 10% C 22 - 23 min → 40 - 100% B and 10% C 23 - 25 min → 100% B 25 - 25.1 min → 100 - 5% B and 0 - 10% C 25.1 - 33 min → 5% B and 10% C **Injection volume**: 1.5 µL	**MS conditions** **ESI + -** **MRM mode** **Source temperature**: 150 °C **Capillary voltage**: 3500 V **Cone voltage**: 30 V **Drying gas flow (N_2)**: 800 L/h **Drying gas temperature**: 400 °C	Gallic acid - Protocatechuic acid - Chlorogenic acid - Catechin - Caffeic acid - Epicatechin - *p*-Coumaric acid - Rutin - Hyperoside - Isoquercitrin - Quercitrin - Phloridzin – Phloretin Phenolic composition of apple peel and pulp samples was determined by LC–MS/MS for the first time in cultivars Budimka, Kolacara, Kozara and Sumatovka. Chlorogenic acid was the dominant phenolic acid in all studied samples.	[127]
***Malus* domestica (1 cultivar):** **- Braeburn**	Fresh mass was homogenized in 50 mL of bidistilled water, left for 30 min at room temperature and centrifugated at 10 000 rpm for 7 min at 4 °C. HPLC-RID	**HPLC conditions** **Column**: Rezex RCM-monosaccharide column (300 x 7.8 mm) **Mobile phase**: bidistilled water **Flow rate**: 0.6 mL/min **Total run time**: 30 min **Injection volume**: 20 µL		**Sugars** Sucrose - Glucose - Fructose - Sorbitol **Organic acids** Citric acid - Malic acid	[137]

	Apple peel was extracted methanol containing 3% (v/v) formic acid and 1% (w/v) 2.6-ditert-butyl-4-methylphenol (BHT) in a cooled ultrasonic bath for 1 h. After extraction, the treated samples were centrifuged for at 10 000 rpm. HPLC-DAD MS	**HPLC conditions** **Column**: Phenomenex Gemini C18 column (150 x 4.6 mm, 3 µm) protected with a Phenomenex security guard column **Mobile phase**: 1% formic acid and 5% acetonitrile in water (A), 100% acetonitrile (B) **Flow rate**: 1 mL/min **Elution**: 0 - 5 min → from 3% to 9% B 5 - 15 min → from 9% to 16% B 15 - 45 min → from 16% to 50% B 45 - 50 min → 50% B Washing and reconditioning **Injection volume**: 20 µL	**λ of acquisition**: 280 nm (hydroxycinnamic acids, dihydrochalcones, flavanols), 350 nm (flavonols), 530 nm (anthocyanins) **MS conditions** **ESI -** **Data dependent MS, scanning from m/z 115 to 2000 was used.**	**<u>Flavanols</u>** Procyanidin B1 - Catechin - Procyanidin B2 -Epicatechin **<u>Hydroxycinnamic acids</u>** Chlorogenic acid - Caffeic acid - *p*-coumaric acid **<u>Flavonols</u>** Quercetin-3-*O*-rutinoside - quercetin-3-*O*-galactoside - quercetin-3-*O*-glucoside - quercetin-3-*O*-xyloside - quercetin-3-*O*-arabinofuranoside - quercetin-3-*O*-arabinopyranoside quercetin-3-*O*-rhamnoside **<u>Dihydrochalcones</u>** Phloridzin - Phloretin-2'-*O*-xylosylglucoside **<u>Anthocyanins</u>** Cyanidin-3-galactoside - Cyanidin-3-glucoside - Cyanidin-3-arabinoside - Cyanidin-7-arabinoside - Cyanidin-3-xyloside In this study the changes of the concentrations of sugars, organic acids and a wide range of polyphenols as well as total phenolic compounds in the "Braeburn" apple peel during the advanced maturation of apples in two growing seasons are reported.	
***Malus* domestica (13 varieties):** **- Ljubenicarka** **- Astrahan** **- Crvenka** **- Kardinal** **- Kraljevina** **- Ruzica** **- Pisanica** **- Petrovka** **- Slavonska Srcika** **- Bjelicnik** **- Ledenara** **- Stegerova** **- Jaje**	Lyophilised samples were extracted in 80% methanol by vortexing and by ultrasonic bath for 15 min. The same quantity of sample was again weighed and extracted using 80% methanol acidified with 1% HCl. Both extracts were separately centrifuged at 10,621g for 10 min HPLC-UV-ITMS	**HPLC conditions** **Column**: LiChroCART C-18 column (250 x 4 mm, 4.5 µm) **Mobile phase**: 1% formic acid in water (A), acetonitrile (B) **Flow rate**: 0.8 mL/min **Elution**: 0 min → 5% B 0 - 10 min → 5 - 10% B 10 - 45 min → 10 - 36% B 45 - 52 min → 36 - 95% B 52 - 55 min → 95% B 55 - 58 min → 95 - 5% B 58 - 65 min → 5% B	**MS conditions** **ESI -** **Capillary voltage**: 4 kV **Nebuliser pressure**: 65 psi **Dry gas flow**: 11 L/min **Dry gas temperature**: 350 °C	**<u>Flavan-3-ols and proanthocyanidins</u>** Catechin - Epicatechin - Dimer - Trimer - Tetramer - Pentamer - Hexamer - Heptamer **<u>Flavonols</u>** Quercetin-rutinoside - Quercetin-hexoside - Quercetin-pentoside - Quercetin-rhamnoside - Rhamnetin-hexoside - Kaempferol-pentoside - Rhamnetin-pentoside - Kaempferol-rhamnoside - Rhamnetin-rhamnoside **<u>Dihydrochalcones</u>**	[103]

	UPLC-QTOF	**Injection volume**: 20 µL **λ of acquisition**: 280 nm (flavan-3-ol monomers, proanthocyanidins, dihydrochalcones), 360 nm (flavonols), 320 nm (phenolic acids), 520 nm (anthocyanins)	**MS conditions** **ESI -** **Gas temperature**: 300 °C **Drying gas flow**: 11 L/min **Nebuliser pressure**: 65 psi **Sheath gas temperature**: 400 °C **Sheath gas flow**: 12 L/min **Fragmentor voltage**: 100 V **Skimmer voltage**: 65 V	Hydroxyphloretin-xyloglucoside - Phloretin-xyloglucoside - Phloretin-hexoside **<u>Phenolic acids</u>** Chlorogenic acid - Chlorogenic acid isomer - *p*-coumaroyl quinic acid **<u>Anthocyanins</u>** Cyanidin-hexoside - Cyanidin-pentoside - Unknown cyaniding derivative	
	HPLC-UV-ITMS (specific for proanthocyanidins)	**HPLC conditions** **Column**: Atlantis dC-18 column (250 x 4.6 mm, 5 µm) **Mobile phase**: 2.5% acetic acid in water (A), acetonitrile (B) **Flow rate**: 1 mL/min **Elution**: 0 min → 3% B 0 - 5 min → 3 - 9% B 5 - 15 min → 9 - 16% B 15 - 45 min → 16 - 50% B 45 - 52 min → 50 - 3% B 52 - 57 min → 3% B **Injection volume**: 10 µL **λ of acquisition**: 280 nm (proanthocyanidins)	**MS conditions** **ESI -** **Nebuliser pressure**: 65 psi **Dry gas flow**: 11 L/min **Dry gas temperature**: 325 °C	In this paper, the phenolic profile in the flesh and peel of thirteen old apple varieties from the Balkan region was studied. 26 compounds were identified Proanthocyanidins were detected as dimers to heptamers and also after acid-catalysis in the presence of phloroglucinol. Phloroglucinolysis, followed by reverse-phase HPLC demonstrated to be an efficient method to characterise and quantify proanthocyanidins in apple varieties.	

***Malus* domestica (14 varieties) - apple juice:** **- Arlet** **- Idared** **- Jonafree** **- Jonatan** **- Koral** **- Ligol** **- Ozark Gold** **- Pepine Linneusza** **- Rajka** **- Red Elstar** **- Rubin** **- Shampion** **- Szara Reneta** **- Topaz**	Apples were ground with and without ascorbic acid and pressed in a laboratory hydraulic press. After pressing, the obtained juices were heated in Thermomix up to 90 °C during 4 min, put in glass jars, cooled at 20°C and then centrifugated at 20,8789g for 10 min. UPLC-MS	**UPLC conditions** **Column**: UPLC BEH C18 column (50 x 2.1 mm, 1.7 µm) **Mobile phase**: 4.5% formic acid in water (A), acetonitrile (B) **Flow rate**: 0.45 mL/min **Elution**: 0 - 1 min → 99% A 1 - 12.5 min → 99 - 0% A 12.5 - 13.5 min → 0% - 99% A **Injection volume**: 10 µL	**MS conditions** **ESI + -** **Source block temperature**: 130 °C **Desolvation temperature**: 350 °C **Capillary voltage**: 2.5 kV **Cone voltage**: 30 V **Desolvation gas flow (N_2)**: 300 L/h	**Hydroxycinnamic acids** Chlorogenic acid - Cryptochlorogenic acid - *p*-Coumaroylquinic acid **Flavanols and procyanidins** Procyanidin B1 - (+)-Catechin - Procyanidin B2 - (-)-Epicatechin - Procyanidin C1 **Dihydrochalcones** Phloretin 2'-*O*-xyloglucose - Phloretin 2'-*O*-glucose **Flavanols** Quercetin-3-*O*-galactoside - Quercetin-3-*O*-glucoside - Quercetin-3-*O*-xyloside - Quercetin-3-*O*-arabinoside - Quercetin-3-*O*-rhamnoside **Anthocyanins** Cyanidin-3-*O*-glucoside - Cyanidin-3-*O*-galactoside A total of 17 kinds of polyphenolic compounds were identified in apple tissues by HPLC with DAD and LC–MS. content of bioactive compounds in apple juices is closely related to a variety, but also strictly connected with ascorbic acid content.	[108,129]

***Malus* domestica (cider apple juice)**	Raw apple juice and apple juice after dilution with an equal volume of MeOH with 1% acetic acid were injected in UHPLC without any other pre-treatment UHPLC-UV & UHPLC-MS/MS	**UHPLC conditions** **Column**: ZORBAX Eclipse Plus C18 column (50 x 2.1 mm, 1.8 µm) equipped with an in-line filter (0.2 µm) **Mobile phase**: 0.1% formic acid in water (A), methanol (B) **Flow rate**: 250 µL/min **Elution**: 0 min → 10% B 1 min → 10% B 3 min → 18% B 11 min → 18.5% B 13 min → 21.5% B 17 min → 25.5% B 21 min → 29% B 23 min → 32% B 35 min → 50% B **λ of acquisition**: 320 nm (hydroxycinnamic acids), 280 nm (dihydrochalcones, flavonols, flavanols)	**MS conditions** **ESI -** **SRM mode** **Spray voltage**: 3500 V **Vaporizer temperature**: 350 °C **Capillary temperature**: 200 °C **Sheath gas pressure**: 48 a.u. **Auxiliary gas pressure**: 13 a.u. **Collision gas (Ar)**: 1.5 mTorr	Procyanidin B1 - (+)-Catechin - Chlorogenid acid - Procyanidin B2 - 4-Caffeoylquinic acid - (-)-Epicatechin - Procyanidin C1 - 4-*p*-coumaroylquinic acid - Procyanidin B5 - Hyperin - Phloretin xyloglucoside - Rutin - Phloridzin - Avicularin – Quercitrin In this paper the performances of the two validated analytical methods were compared for the quantification of the major polyphenols in apple juices, both showing an excellent correlation for major compounds quantified in 120 different samples.	[128]
***Malus* domestica (7 cultivars):** **- Braeburn** **- Granny Smith** **- Fuji** **- Scilate** **- Cripps Pink** **- Sciroc** **- Red Field**	HPLC-HRMS	**HPLC conditions** **Column**: Zorbax SB-C18 (100 x 2.1 mm, 1.8 µm) **Mobile phase**: 0.5% formic acid in water (A), acetonitrile (B) **Flow rate**: 400 µL/min **Elution**: 0 - 0.5 min → 70% A and 30% B 0.5 - 25 min → linear gradient to 45% A and 55% B 25 - 45 min → linear gradient to 2% A and 98% B 45 - 50 min → 2% A and 98% B 50 - 50.2 min → linear gradient 70% A and 30% B 50.2 - 54 min → 70% A and 30% B **Injection volume**: 2 µL	**MS conditions** **ESI +** **Temperature**: 200 °C **Capillary voltage**: -4000 V **Drying gas flow (N_2)**: 8 L/min **Nebulizer gas (N_2) pressure**: 4.0 bar **End plate offset**: -500 V	Triterpene acids identified as ursenoic (or oleanoic) acid derivatives containing hydroxyl, oxo, and coumaroyloxy groups	[153]

Apple pomace	HPLC-ESI/MS	**HPLC conditions** **Column**: Atlantis T3 C18 column (100 x 2.1 mm, 3 µm) **Mobile phase**: 0.5% formic acid in water (A), 0.5% formic acid in 50:50 acetonitrile:methanol (B) **Flow rate**: 0.2 mL/min **Elution**: Stepwise gradient from 10 to 95% solvent B for 30 min	**MS conditions** **ESI -** **Capillary voltage**: 3 kV **Cone voltage**: 30 V **Collision energy**: 10 - 30 eV	**Hydroxycinnami acids** Chlorogenic acid - Feruloylquinic acid **Flavonols** Quercetin - Isorhamnetin - Quercetin 3-*O*-arabinoside - Quercetin 3-*O*-glucoside - Quercetin 3-*O*-rhamnoside - Quercetin 3-*O*-galactoside - Quercetin 3-*O*-rutinoside (rutin) **Flavanols** Epicatechin - Procyanidin dimer A2 - Procyanidin dimer B1 or B2 - Procyanidin trimer C - Procyanidin tetramer D **Dihydrochalcones** Phloretin - Phloridzin - Phloretin 2'-*O*-xylosil-glucoside **Flavones** Kaempferol	[131]
***Malus* domestica**	HPLC-UV-MS	**HPLC conditions** **Column**: Zorbax Eclipse XDB-C18 (150 x 2.1 mm, 3.5 µm) **Mobile phase**: 0.1% formic acid in water (A), 0.1% formic acid in acetonitrile (B) **Flow rate**: 0.2 mL/min **Elution**: Initial conditions → 3% B 0 - 5 min → 9% B linear 5 - 15 min → 16% B linear 15 - 45 min → 50% B linear Washing and reconditioning **λ of acquisition**: 280 nm (dihydrochalcones), 320 nm (hydroxycinnamic acids), 350 nm (flavonols)	**MS conditions** **ESI -** **Spray voltage**: 4.2 kV **Capillary temperature**: 275 °C **Sheath gas**: 60 a.u. **Auxiliary gas**: 20 a.u.	**Hydroxycinnamic acids** Caffeoylquinic acid - *p*-coumaroyl glucose **Flavonols** Hyperoside - Isoquercitrin - Reynoutrin - Kaempferol glycoside - Avicularin - Quercitrin - Isorhamnetin glycoside **Dihydrochalcones** Sieboldin - Phloridzin - Trilobatin - 3-hydroxyphloretin dihexoside - Phloretin	[138]

Apple juices	HPLC-UV	**HPLC conditions** **Column**: Cadenza CD C18 (75 x 4.6 mm, 5 µm) **Mobile phase**: 4.5% formic acid in water (A), acetonitrile (B) **Flow rate**: 1.0 mL/min **Elution**: 0 - 36 min → from 0% to 25% B Washing and reconditioning	**λ of acquisition**: 280 nm (flavan-3-ols, dihydrochalcones), 320 nm (hydroxycinnamates), 360 nm (flavonol glycosides)	Chlorogenic acid - Cryptochlorogenic acid - *p*-Coumarylquinic acid - (-)-Epicatechin - Procyanidin B2 - Procyanidin C1 - (+)-Catechin - Procyanidin B1 - Phloretin 2'-*O*-xyloglucose - Phloretin 2'-*O*-glucose - Quercetin 3-*O*-galactoside - Quercetin 3-*O*-glucoside - Quercetin 3-*O*-arabinoside - Quercetin 3-*O*-xyloside - Quercetin 3-*O*-rhamnoside - Cyanidin 3-*O*-galactoside - Polymeric procyanidins	[108]
***Malus* domestica (2 cultivars):** **- Jonagold** **- Golden Delicious**	Sugars were analyzed from the whole edible part of the fruit, homogenized and centrifugated at 12000 rpm for 7 min at 10 ◦C. HPLC-RID	**HPLC conditions** **Column**: Rezex RCM-monosaccharide column (300 x 7.8 mm) **Mobile phase**: bidistilled water **Flow rate**: 0.6 mL/min **Total run time**: 35 min **Injection volume**: 20 µL		**<u>Sugars</u>** Fructose - Glucose - Sucrose - Sorbitol	[102,115]
	Malic acid was analyzed from the whole edible part of the fruit homogenized and centrifugated at 12000 rpm for 7 min at 10 ◦C. For secondary metabolites extraction, peel and pulp were extracted with methanol containing BHT in a cooled water bath using	**HPLC conditions** **Column**: Aminex HPX-87H column (300 x 7.8 mm) **Mobile phase**: 4 mM sulphuric acid in bidistilled water **Flow rate**: 0.6 mL/min **Total run time**: 30 min **Injection volume**: 20 µL	**λ of acquisition**: 210 nm	**<u>Organic acids</u>** Malic acid	

	sonification and centrifugated at 10000 rpm for 10 min at 4 ◦C HPLC-UV	**HPLC conditions** **Column**: Phenomenex Gemini C18 (150 x 4.6 mm, 3 µm) **Mobile phase**: 1% formic acid in water (A), acetonitrile (B) **Flow rate**: 1 mL/min **Elution**: 0 - 5 min → from 3% to 9% B 5 - 15 min → from 9% to 16% B 15 - 45 min → from 16% to 50% B 45 - 50 min → 50% B Washing and reconditioning **Injection volume**: 20 µL	**λ of acquisition**: 280 nm (hydroxycinnamic acids and monomeric flavanols), 350 nm (flavonols), 530 nm (cyanidin galactoside)	**Hydroxycinnamic acid** Chlorogenic acid - Caffeic acid **Flavanols** Catechin - Epicatechin **Flavonols** Quercetin rutinoside - Quercetin rhamnoside - Quercetin glucoside - Quercetin galactoside **Cyanidin derivatives** Cyanidin galactoside In this study, changes of primary and secondary metabolites were evaluated to demonstrate that various parameters react uniquely to a prolonged exposure of fruit to ambient temperatures.	
***Malus* domestica (1 cultivar):** **- Honeycrisp** ***Malus* domestica (3 cultivars):** **- McIntosh** **- Gala** **- Mutsu**	After fractionation of non-polar metabolites into chloroform, the polar phases of each sample were dried under vacuum without heating, and then derivatized with (MSTFA) GC-MS	**GC conditions** **Column**: DB-5MS capillary column (20m x 0.18mm x 0.18 µm) with a 5m Duraguard column in front **Temperature**: - 70 °C for 2.471 min - 10.119 °C/min ramp to 330 °C - Final 2.471 min heating at 330 °C - Cooling and re-equilibration at 70 °C for 5 min **Injection volume**: 1 µL **Carrier gas**: helium at 1.0 mL/min	**MS conditions** Electron ionisation source **Transfer line temperature**: 250 °C **Ion source temperature**: 230 °C	**Carbohydrates** Galactose - Glucose - Fructose - Sucrose - Sorbitol **Organic acids** Fumaric acid - Succinic acid - Citric acid - Malic acid	[65,74]
	Apple flesh sample was ground in 20 mM HCl at 0 C. Norleucine was added as an internal standard for measuring recovery. The extract was thencentrifuged at 13,000g for 10 min HPLC-FL	**HPLC conditions** **Column**: Nova-Pak™ C18 column (150 x 3.9 mm, 4 µm) preceded by a Waters Sentry™ guard column **Mobile phase**: 140 mM sodium acetate and 17 mM triethanolamine (pH 5.05, adjusted with phosphoric acid) (A), 60% acetonitrile in water (B) **Flow rate**: 1.0 mL/min **Elution**: 0 min → 0% B 0.5 min → 2% B 15 min → 7% B 19 min → 10% B 32 - 33 min → 33% B 34 - 40 min → 100% B Post-run time: 10 min **Injection volume**: 5 µL	**λ excitation**: 250 nm **λ emission**: 395 nm	**Aminoacids** Aspartic acid - Asparagine - Glutamic acid - Glutamine - Arginine - Serine - Alanine - Threonine - Histidine - Valine - Glycine - Tyrosine - Isoleucine - Lysine - Methionine - Ornithine - Leucine - γ-aminobutyric acid - Proline - Phenylalanine	

	Apple flesh sample was ground in 70% methanol containing 2% formic acid at 0 C. The homogenate was transferred into an Eppendorf tube, and after being shaken at 30 C for 30 min in a thermomixer at 1000 rpm, the combined homogenate was centrifuged at 10,000g for 10 min. HPLC-DAD	**HPLC conditions** **Column**: Inertsil ODS-3 column (250 x 4.6 mm, 5 µm), preceded by an Inertsil ODS-3 Guard column (10.0 x 4.0 mm, 5 µm) **Mobile phase**: 10% formic acid in water (A), 10% formic acid and 1.36% water in acetonitrile (B) **Flow rate**: 1.0 mL/min **Elution**: 0 min → 95% A 25 min → 85% A 42 min → 78% A 60 min → 64% A 65 min → 95% A Post-run time: 10 min **Injection volume**: 25 µL	**λ of acquisition**: 280 nm (flavanols), 320 nm (hydroxycinnamic acids), 365 nm (quercetin derivatives)	Catechin - Epicatechin - Procyanidin B1 - Procyanidin B2 - Phloridzin - Gallic acid - Syringic acid - Chlorogenic acid - Gentisic acid - Caffeic acid - *p*-coumaric acid -Ferulic acid - Quercetin - Hyperin (quercetin-3-galactoside) - Isoquercitrin (quercetin-3-glucoside) - Quercitrin (quercetin-3-rhamnoside) - Rutin (quercetin-3-rutinoside) - Reynoutrin (quercetin-3-xyloside) - Avicularin (quercetin-3-arabinoside) Developmental changes of 12 carbohydrates, 8 organic acids, 20 amino acids, and 18 phenolic compounds in the flesh of apple were determined in this study by HPLC and GC-MS	
Apple	**Extraction**: weighing of a solid sample (100 mg) into a volumetric flask (50 mL) before partially filling with pre-warmed (60°C) aqueous methanol (70%). The dissolution was sonicated in a water bath (60°C; 10 min) to disperse the sample before incubating for 30 min at 60°C. Post incubation, flasks were cooled and then filled to volume with 70% aqueous methanol. Sub-samples of the dissolution were centrifuged (2500g; 5 min) before applying to HPLC. **HPLC-FL**	**HPLC conditions** **Column**: Luna Hilic column (150 x 2.0 mm; 3 µm) **Mobile phase**: 98% acetonitrile and 2% acetic acid (A), 95% methanol, 3% water and 2% acetic acid (B) **Flow rate**: 0.350 mL/min **Elution**: 0 min → 7% B 3 min → 7% B 15 min → 30% B 40 min → 49% B 40.1 min → 7% B 45 min → 7% B **Injection volume**: 2 µL	**λ excitation**: 230 nm **λ emission**: 321 nm	Monomeric and oligomeric procyanidins	[113]

***Malus* domestica (1 cultivar)**: - Kanzi®	Extraction system consists of a consecutive extraction of dry apple peel samples with MeOH:water (20/80, *v/v*) in a first step and 100% MeOH in a second step. UHPLC-DAD/ESI-MS	**UHPLC conditions** **Column**: Waters Acquity UPLC BEH SHIELD RP18 column (150 x 3.0 mm, 1.7 µm) protected with a Acquity BEH RP18 VanGuard precolumn (5 x 2.1 mm, 1.7 µm) **Mobile phase**: 0.1% formic acid in water (solvent A), 0.1% formic acid in acetonitrile (B) **Flow rate**: 500 µL/min **Elution**: 0 - 9.91 min → from 0% to 26% B 9.91 - 18.51 min → from 26% to 65% B 18.51 - 18.76 min → from 65% to 100% B 18.76 - 20.76 min → held at 100% B 20.76 - 20.88 min → from 100% to 0% B 20.88 - 23 min → 0% B **Injection volume**: 5 µL **λ range of acquisition**: 220-400 nm	**MS conditions** **ESI -** **Capillary temperature**: 350 °C **Spray voltage**: -2.5 kV **Sheath gas (N_2)**: 47 a.u. **Auxiliary gas (N_2)**: 15 a.u. **Skimmer voltage**: -25 V **Tube lens voltage**: -110 V	Salicylic acid - Protocatechuic acid - Gallic acid - Propyl gallate - *p*-Coumaric acid - Caffeic acid - Ferulic acid - Sinapinic acid - Chlorogenic acid - Dihydrocaffeic acid - Dihydroferulic acid - 4-*p*-Hydroxyphenyl acetic acid - Apigenin - Apigetrin - Luteolin - Cynaroside - Isorhamnetin - Kaempferol - Astragalin - Quercetin - Isoquercitrin - Hyperin - Rutin - Avicularin - Quercitrin - Galangin - Phloretin - Phlorizin - Naringenin - Naringin - (+)-Catechin - (-)-Epicatechin - (+)-Aromadendrin - (+)-Taxifolin - Cyanidin chloride - Kuromanin chloride - Ideain chloride - Keracyanin chloride - Procyanidin B2 This method represents a valuable tool for characterisation of phenolic components. It was fully validated in terms of model deviation ($r^2 > 0.9990$), range (typically 10–3500 ng g^{-1}), intra/inter-day precision (<6% and <8%, respectively) and accuracy (typically 100 ± 10%). The mass accuracy of each selected phenolic compound was below 1.5 ppm.	[104]
Apple juice (5 varieties): - Meana - Florina - 3 samples from a progeny Meana x Florina	**Extraction**: freeze-dried apple and apple pomace were extracted with methanol–water–acetic acid (30:69:1, v/v/v) in an ultrasonic bath for 10 min. The extract was centrifuged (20 min at 4◦C and 3600 rpm) and the supernatant was filtered twice through a 0.45 µm PTFE filter prior to injection into the UHPLC system UHPLC-DAD–ESI-Q-ToF-MS	**UHPLC conditions** **Column**: Acquity UPLC BEH C18 column (100 x 2.1 mm, 1.7 µm) with a Acquity UPLC BEH C18 1.7 µm VanGuard precolumn **Mobile phase**: 0.1% acetic acid in water (solvent A), 0.1% acetic acid in methanol (solvent B) **Flow rate**: 0.35 mL/min **Elution**: 0 - 0.87 min → 0% B 2.14 min → 15% B 2.14 - 5.04 min → 15% B isocratic 7.63 min → 20% B 9.0 min → 23% B 14.0 min → 35% B 16.0 min → 51% B	**MS conditions** **ESI + -** **Source temperature**: 120°C **Desolvation temperature**: 400°C **Capillary voltage**: 0.7 kV (ESI+), 0.5 kV (ESI-) **Desolvation gas flow (N2)**: 900 L/h **Cone gas flow (N2)**: 10 L/h	**<u>Flavanols</u>** (+)-Catechin - (-)-Epicatechin - Procyanidin B1 - Procyanidin B2 - Procyanidin B3 - Procyanidin B5 - Procyanidin C1 Trimers - Procyanidin C1 Tetramers **<u>Dihydrochalcones</u>** 3-Hydroxyphloretin-2'-*O*-xylosyl-glucoside - 3-Hydroxyphloretin-2'-*O*-glucoside - Phloretin-hexose-hexoside - Phloretin-pentosyl-hexosides - Phloretin-2'-*O*-glucoside - Phloretin **<u>Hydrocycinnamic acids</u>** 5'-Caffeoylquinic acid - Chlorogenic acid - 3'-Caffeoylquinic acid - 4'-	[13]

		17.0 min → 100% B 18.0 min → 100% B 18.0 - 22.0 min → re-equilibration with 0% B **Injection volume**: 5 µL **λ of acquisition**: 280 nm (flavan-3-ols, procyanidins, dihydrochalcones), 320 nm (hydroxycinnamic acids), 370 nm (flavonols), 500 nm (anthocyanins)		Caffeoylquinic acid - *p*-coumaroyl hexose isomers - feruloyl glucose - *p*-coumaroylquinic acid isomers **<u>Flavonols</u>** Quercetin-3-*O*-galactoside - Quercetin-3-*O*-glucoside - Quercetin-3-*O*-rutinoside - Quercetin-3-*O*-arabinopyranoside - Quercetin-3-*O*-xyloside - Quercetin-3-*O*-arabinofuranoside - Quercetin-3-*O*-rhamnoside - Isorhamnetin-3-*O*-galactoside - Isorhamnetin-3-*O*-glucoside - Isorhamnetin-3-*O*-rutinoside - Isorhamnetin-3-*O*-arabinopyranoside - Isorhamnetin-3-*O*-arabinofuranoside - Isorhamnetin-3-*O*-rhamnoside **<u>Anthocyanins</u>** Cyanidin hexoside	