Impact of air-drying on polyphenol extractability from apple pomace

Simona Birtic-Pindat, Sylvaine Regis, Carine Le Bourvellec, Catherine M.G.C. Renard

Impact of air-drying on polyphenol extractability from apple pomace

Simona Birtic[1,2],Sylvaine Régis[1], Carine Le Bourvellec[1]& Catherine M.G.C. Renard[1,#]

1 UMR408 Sécurité et Qualité des Produits d'Origine Végétale, INRA, Avignon University, F-84000 Avignon, France

2 Naturex SA, Site d'Agroparc BP 1218, 84911 Avignon Cedex 9, France

: corresponding author

Postal address :C. Renard,
UMR408 SQPOV
INRA,
Domaine St Paul,
CS40509
84914 Avignon cédex 09
France

Email : catherine.renard@inra.fr

Tel: +33 4 32 72 25 28

Simona Birtic :s.birtic@naturex.com

Sylvaine Régis:sylvaine.regis@inra.fr

Carine Le Bourvellec : carine.le-bourvellec@inra.fr

Abstract

Little data are available on the impact of pomace pre-treatment, notably drying, on the nature and yield of polyphenols. Pomace from two apple varieties ('Avrolles' and 'Kermerrien'), pressed with and without oxidation, were air-dried to different degrees. Drying led to the loss of native molecules, notably 5-O-caffeoylquinic acid and flavan-3-ols. Total polyphenol yields, after sequential pressurized liquid extraction (water 10 MPa, 70°C, then ethanol 48%, 10 MPa, 70°C), varied between 5 and 15 g/kg dry weight but showed no marked trend with drying. Extracts from dried pomace contained few native polyphenols. Water extracts from 'Kermerrien' contained flavonols, flavanols and phloridzin and those from 'Avrolles' contained phloridzin. Water:ethanol extracts were rich in procyanidins, especially from 'Avrolles', where they represented > 80% of analysable polyphenols. Presence of polyphenol molecules with modified structures in the extracts of dried pomaces might lead to different biological properties than those with native molecules.

Keywords: *Malus domestica* Borkh, by-product, procyanidin, extraction, quercetin glycoside, flvonol, dihydrochalcone, chlorogenic acid

1- Introduction

The food industry produces large volumes of apple pomaces during processing. When apples are juiced, ca. 20%-25% w/w pomace is also formed. Historically, pectins are extracted from pomace, provided the juice is produced without the addition of enzymes, as pressing aids (May, 1990). Otherwise, these pomaces were considered to be waste and used for animal feed or as soil improvers, both with low added-value. If discarded, valuable biomass and nutrients that are often more abundant in the pomace than in the juice are lost. However, for economic reasons, and the need to conserve energy and new materials, new methods and policies for edible and non-edible food waste have been introduced, including recovery, bioconversion, and utilization of valuable constituents. Apple pomaces are typical by-products of the fruit food industry that can be recovered and, often, upgraded to higher value, useful products or even raw material for other products (e.g. cosmetic or pharmaceutics, food or feed/fodder) after, if necessary, biological treatment (Kennedy et al., 1999).

Recently, there has been a renewed interest in apple pomaces as a rich-source of functional components and extracts (fibres, pectins, antioxidant polyphenols), as summarized in a recent review by Perussello, Zhang, Marzocchella, & Tiwari (2017). Still, few articles report actual polyphenolic compositions of these extracts, while most use antioxidant assays or “total polyphenols”using the Folin-Ciocalteu assay to quantify yields. Solvents, water and water-ethanol mixtures have been demonstrated to extract polyphenols efficiently from apple pomace (Plaza, Abrahamsson, & Turner, 2013; Wijngaard & Brunton, 2009, 2010) in pressurized liquid extraction (PLE). However, there a range of extractions conditions have been used; Reis, Rai, & Abu-Ghannam (2012) reported that water at room temperature extracted 67% of total polyphenols from an apple pomace and Plaza et al.(2013) that PLE at 120°C for 3 min gave the highest yield of flavonols. Wijngaard & Brunton (2010) reported 56% ethanol at 80°C for 31 min or 65% acetone at 25°C for 60 min as optimal conditions for

67 extracting antioxidants from apple pomaces, while the same authors (Wijngaard & Brunton, 2009)
68 optimized extraction with 60% ethanol at 102°C using pressurized liquid extraction. In comparison,
69 Virot, Tomao, Le Bourvellec, Renard, & Chemat (2010), using ultrasound assisted extraction, found
70 50% ethanol to be the optimal solvent for polyphenol extraction.

71 As apple pomaces are enriched with peels and seeds, they are particularly abundant in polyphenolic
72 substances. Polyphenols include diverse classes of compounds ranging from phenolic acids, coloured
73 anthocyanins, and simple and complex flavonoids. Polyphenols in apple pomace (Lu & Foo, 1997) are
74 primarily procyanidins, retained due to their interactions with cell walls (Le Bourvellec, Guyot, &
75 Renard, 2004), but there are also dihydrochalcones, initially concentrated in the seeds (Fromm,
76 Bayha, Carle, & Kammerer, 2012; Guyot, Marnet, Laraba, Sanoner, & Drilleau, 1998) and flavonols,
77 initially present in the peel (Guyot, et al., 1998; Kolodziejczyk et al., 2009), and minor amounts of
78 hydroxycinnamic acids.

79 These are valuable compounds for two main reasons. Firstly, they play a significant role in overall
80 organoleptic properties of foods, as they are major contributors to the bitterness and astringency of
81 the fruit (Symoneaux, Baron, Marnet, Bauduin, & Chollet, 2014) and they confer yellow, red or brown
82 colouring to food products (Nicolas, Richard-Forget, Goupy, Amiot, & Aubert, 1994). Secondly,
83 consumption has been associated with a decreased incidence of cardiovascular diseases and cancers
84 (Scalbert, Manach, Morand, Remesy, & Jimenez, 2005). This putative health benefit of polyphenols
85 has been ascribed to their ability to scavenge free radicals responsible for oxidative damage, but
86 more recent data indicate that these effects might be due, for a large part, to specific activities of
87 their colonic fermentation products (Del Rio, Costa, Lean, & Crozier, 2010).

88 Apple pomaces represent large volumes of co-products for the industry, with an estimated 5.8
89 million tonnes of apples processed in the world in 2016 (Heuzé, Tran, Hassoun, & Lebas, 2018), a 20-
90 25% yield in pomace means circa 1.4 million tonnes of apple pomace produced annually. Apple
91 pomaces are rich in water (water content ca. 700 g/kg fresh weight) meaning that, if they are to be

stored for polyphenol production, they need to be dried to avoid microbial spoilage, otherwise they require a lot of storage space at sub-zero temperatures. Air-drying down to 100 g water per kg increases considerably pomace stability while decreasing the volume rapidly at a reasonable cost (Lavelli & Corti, 2011). However, drying can impact strongly the properties of pomace, such as polyphenol extractability and integrity, and is costly in terms of energy. Few articles, however, have dealt with the impact of pomace drying pre-treatments on polyphenol extraction. Lavelli & Corti (2011) reported that air-drying at 60°C was better than vacuum drying at 40°C for retention of anthocyanins and flavanols in apple pomace during nine months storage, with the maximum stability at the lowest water activity. Heras-Ramirez et al., (2012) indicated that drying led to significant reductions in antioxidant activity, while dry unblanched pomace reduced the concentrations of epicatechin and caffeic acid compared with dry blanched pomace. However, the pomaces were first dipped in an antioxidant solution. Ferrentino, Morozova, Mosibo, Ramezani, & Scampicchio (2018), comparing extraction of polyphenols from freeze-dried, oven-dried (50°C, 4 days) and frozen apple pomace, found higher yields from freeze-dried than oven-dried pomaces (comparison with “frozen” cannot be done as their results were not normalized for dry matter content). Yan & Kerr (2013), comparing vacuum-belt dried to freeze-dried pomace, reported higher total polyphenol contents (extracted with 80% acetone) after vacuum-belt drying. To our knowledge, no article has examined systematically the impact of drying on polyphenolic composition and extractability from apple pomace.

Here, we intended to evaluate at what point in the drying curve polyphenols became significantly less extractable. The second approach aimed at producing polyphenol extracts directly from (dried) apple pomaces using non-toxic solvents to avoid any subsequent biological treatment. To elaborate this approach, we determined the extractability of polyphenols from pomaces with different water contents, using a new method based on pressurized liquid extraction (PLE), and extraction solvents, such as water and ethanol 48% (ethanol:water 48:52; mL/mL), which do not leave any harmful residues. Finally, the polyphenol composition and patterns of differentially air-dried apple pomaces

and their polyphenol extracts were determined. The work involved apple varieties with contrasting polyphenol compositions, namely 'Kermerrien' and 'Avrolles'.

2- Materials and methods

2.1 Pomace material

Apple fruits from the cultivars 'Kermerrien' and 'Avrolles' (circa 40 kg each) were harvested at commercial maturity during the 2007 season in the experimental orchard of the Institut Français des Productions Cidricoles (Sées, France). For production of pomace, apples were ground and pressed as described in Renard et al. (2011). Apples were pressed at 14°C in a Speidel-90 ''hydropress" pneumatic press (Speidel Tank- und Behaelterbau GmbH, Ofterdingen, Germany), modified by addition of an inox steel tube around the press to allow inerting with a heavier-than-air gas. The apples (ca. 15 kg/pressing) were left at 14°C for 24 h to equilibrate prior to pressing.

For pressing without oxidation, the press was flushed with water first. Inerting was carried out by flushing the water out of the press with CO_2 and connecting the press to the exit of the grinder (Stossier, Malters, Switzerland) via plastic tubing. Crushing started when a burning match was inserted into the entrance of the grinder and combustion could not maintained. A standing time of 20 min was observed between crushing and pressing of the apples. For pressing with oxidation, apples were pressed as above, except crushed apples were dropped into a 30 L-drum that was closed and agitated ''head-over-tail" for 20 min at room temperature prior to pressing.

2.2 Standards and chemicals

Chlorogenic acid (5-O-caffeoylquinic acid), (+)-catechin, (-)-epicatechin were obtained from Sigma-Aldrich (Deisenhofen, Germany). *P*-coumaric acid and quercetin were obtained from Extrasynthese

(Genay, France). Phloridzin was obtained from Fluka (Buchs, Switzerland). Sugar standards were from Fluka (Buchs, Switzerland). D_3-methanol was from Acros organics (Geel, Belgium). Sodium borohydride, N-methyl imidazole, acetic anhydride, toluene-α-thiol, Folin-Ciocalteu's phenol reagent were from Sigma-Aldrich (Deisenhofen, Germany). Acetonitrile, ethanol and methanol were analytical grade and from Fisher Scientific (Fair Lawn, New Jersey, USA).

2.3 Drying treatment

Samples with differential water contents ranging from 72%fresh weight down to 0% (no detectable water by drying to constant weight)were prepared by air-drying pomaces in a ventilated oven (Memmert, Schwabach, Germany) at 70 °C with the pomace spread to a monolayer (depth < 0.5 cm) of apple chips. All samples were subsequently freeze-dried then ground in a Warring blender and were hermetically sealed in polyethylene tetraphtalate / aluminium bags (Amcor Flexibles, Montreuil, France) before being stored at –20 °C.

Initially, to evaluate pomace water content (WC), samples of 5 g (n=6) were dried until they achieved a constant weight (117°C, 17h). Water content is expressed on a fresh weight basis. Pomace samples from 'Kermerrien' (OX and NOX) and 'Avrolles' (OX and NOX) were prepared in triplicate by drying down to 72%, 62%, 34%, 12%, 4%, 2% and 0% (no detectable weight loss)(using drying durations calculated from the drying kinetics) and used, subsequently, for polyphenol extractions and analyzes.

Actual water contents are summarized in Supplementary Table 1.

2.4 Preparation of alcohol insoluble solids

For analysis of constituent cell walls, alcohol insoluble solids (AIS) were prepared from the apple pomaces as described in Renard (2005). Briefly, this consisted of rinsing in 70% ethanol

(ethanol:water 700:300 mL/mL) until the filtrates were sugar-free (as assessed by the phenol sulphuric test), followed by solvent exchange drying (rinsing 3 times with 96% ethanol and three times with acetone, followed by evaporation of the acetone in a ventilated oven at 40°C overnight).

2.5 Extraction of polyphenols

Apple pomaces with differential moisture contents were extracted using an ASE 200 system (Dionex, Sunnyvale, CA). As polyphenols were extracted from samples of a large size (equivalent to 4 g dry weight), the largest ASE (accelerated solvent extraction) cell (33 mL) was used. Each sample was mixed with 24 g of sand for better dispersion and assure better contact between the samples and the solvent as well as preventing the ASE cell from clogging. A complete extraction cycle consisted of the following steps. The ASE cell, containing the sample and sand, was filled with solvent and a pressure of 10 MPa applied to the cell. The cell was heated to 70 °C for 5 min and kept at this temperature for a further 5 min. At this point, the extract was recovered in the reception vial in two steps: in a first step fresh solvent (60%) was injected, displacing the extract, and in a second step , a nitrogen purge of 2 min displaced the residual solvent.

Each sample was extracted twice with water and twice with ethanol:water (48:52; mL/mL, hereafter abbreviated as 48% EtOH). Each extract was purged in a separate vial. Hence, one sample yielded four separate extracts (two water and two 48% EtOH extracts). All samples were extracted in three replicates. Preliminary trials showed no significant difference in polyphenol yield as a function of static time (durations tested: 5, 10, 15 min). A second extraction using the same solvent yielded about 15% of the first with water, and 40% for 48% EtOH, and a third less than 5% for both. Thus, 5 min static time and 2 extractions per solvent were chosen as optimal.

2.6 Measurement of “total polyphenols”

188 “Total polyphenols”i.e. reducing compounds were evaluated using spectrophotometric analysis with
189 Folin-Ciocalteu’s phenol reagent. A major advantage of this Folin–Ciocalteu’s procedure is that it has
190 an equivalent response to different polyphenolic substances in biological materials, making it
191 suitable for estimating concentrations of total polyphenolic substances in a series of related samples
192 (Vrhovsek, Mattivi, & Waterhouse, 2001). Also, evolved polyphenols still react to the Folin-Ciocalteu
193 assay (De Beer et al., 2004).

194 Briefly, an aliquot (100 µL) of extract was mixed with100 µL of ultra-pure water (dilution 1:2) or
195 standard solutions of 5-O-caffeoylquinic acid (0 [blank] to 260 mg/L). Diluted Folin-Ciocalteu’s phenol
196 reagent (dilution 1:5) and 2 mL Na_2CO_3 (0.4 mol/L) were added to the extracts and standards. After
197 incubation for 30 min at room temperature, the absorbance of samples versus a prepared blank
198 were measured at 730 nm. “Total polyphenolic” contents of pomace extracts are expressed as mg of
199 5-O-caffeoylquinic acidequivalents per g of dry sample. Both water and both ethanol extracts of all
200 three sample replicates were analyzed separately. After the analysis of total polyphenols, water and
201 ethanol extracts from each sample replicate were pooled and freeze dried.

202

203 **2.7Individual polyphenol analysis**

204 Polyphenols were measured by HPLC after thioacidolysis, as described by Guyot, Marnet, Sanoner, &
205 Drilleau(2001). For typical chromatograms, see Supplementary Figure 1. The average degree of
206 procyanidin polymerization was calculated as the molar ratio of all flavan-3-ol units (thioether
207 adducts plus terminal units) to (-)-epicatechin and (+)-catechin, which correspond to terminal units.
208 The HPLC apparatus was an Agilent 1050 series (Palo Alto, CA, USA). The column was a Purospher
209 RP18 endcapped, 5µm (Merck, Darmstadt, Germany). The solvent system was a gradient of solvent A
210 (aqueous acetic acid, 25 mL/L) and solvent B (acetonitrile): initial composition 3% B; linear gradient
211 to 9% B from 0-5 min.; linear gradient to 16% B from 5 to 15 min.; linear gradient to 50% B from 15 to

45 min; followed by washing and reconditioning the column. Catechins and their thioesters were quantified at 280 nm against an epicatechin standard. Chlorogenic acid (5-O-caffeoylquinic acid), 4-*p*-coumaroylquinic acid and their methyl derivatives were quantified at 320 nm using as standards 5-O-caffeoylquinic acid and *p*-coumaric acid, respectively. All flavonols were quantified at 350 nm against a quercetin standard. Phloridzin and phloretin xyloglucoside were quantified at 280 nm against a phloridzin standard.

2.8 Polysaccharide analysis

All AIS were analyzed in duplicate for neutral sugars, uronic acids and methanol content. Neutral sugars were analyzed using gas chromatography (GC) with flame ionization detector (FID) as alditol acetates after acid hydrolysis: samples (c.a. 10 mg of AIS) were subjected to pre-hydrolysis with 250 µL sulphuric acid (12 mol/L) for 1 hour at room temperature (Saeman, Moore, Mitchell, & Millett, 1954), which was diluted to 1 mol/L sulphuric acid with the addition of water and the internal standard (inositol). All samples were placed in oven at 100 °C for 3 hours for hydrolysis. Afterwards, they were derivatized to volatile alditol acetates by reduction with sodium borohydride and, then, acetylation in acetyl anhydride catalysed by N-methyl imidazole, and extracted in dichloromethane (Englyst, Wiggins, & Cummings, 1982). Extracts were injected onto a GC-FID HP 5890 Serie II (Agilent, Inc, Palo Alto, USA) with a capillary column of 30 m x 0.25 mm i.d. coated with DB225 MS, 0.25 µm film thickness (J&W Scientific, Agilent, Inc, Palo Alto, USA). The conditions were: temperature of injection 250°C in split mode (ratio 1:25); hydrogen as carrier gas at 45 cm/s (at 215 °C), column flow was 1.3 ml/min; the oven temperature was isothermal at 215 °C.

Uronic acids were measured spectrophotometrically using the m-hydroxydiphenyl assay, as described by Blumenkrantz & Asboe-Hansen (1973) with galacturonic acid as the external standard, after Saeman hydrolysis, and are expressed as anhydrouronic acids (AUA).

Methanol was determined by stable isotope dilution assay against D_3-methanol by headspace-GC-MS (mass spectrometry) after saponification, as described by Renard & Ginies (2009). The GC apparatus was a GC-MS QP2010 Shimadzu with capillary column (Cp_wax_52cb 30m x 0.32mm x 0.5 µm; Varian, Inc, Palo Alto, USA) equipped with auto sampler AOC5000. Sealed vials were placed at 50 °C for 15 min and then 0.5 mL of head-space was injected in split injector (ratio 1:10). GC conditions were: helium as carrier gas at 45 cm/s, oven temperature isothermal at 40°C. Mass detector conditions were: electronic impact ionization mode (70eV), temperature of source 200°C with data collected using selected ions (m/z 31; 32; 35) at 5 scans/s. The degree of methylation (DM) was calculated as molar ratio of methanol to galacturonic acid.

2.9 Statistical analysis

Results are presented as mean values, and the reproducibility of the results is expressed as pooled standard deviation. Pooled standard deviations were calculated for each series of replicates using the sum of individual variances weighted by the sum of the individual degrees of freedom (Box,Hunter &Hunter, 1978).Two-way analysis of variance (ANOVA) by Fisher 's test (F) was used to compare the “total polyphenol” yields,as a function of drying and oxidation, and performed using Excelstat. Differences were considered significant at $P < 0.05$.

3 Results and discussion

3.1 Reduction of apple pomace water contents by air-drying

Upon air-drying, the water loss in the monolayer-displayed apple pomaces followed a sigmoidal shape, until complete desiccation, which occurred in less than 10 h (Supplementary Figure 2). Water

contents and drying curves did not differ significantly, irrespective of apple variety (not shown) or different oxygen conditions used for production.

Only six hours were required to dry apple pomaces down to 12% and decrease pomace weights by more than three-fold. This considerable weight decrease resulted in a significant reduction in volume. While drying in non-freezing conditions induces oxidation processes, polyphenol conservation following drying is improved in material where oxidation and degradation are slowed down (Lavelli & Corti, 2011). Further drying of the pomace was much slower, which meant that reaching lower water contents would require additional time, money and energy inputs. Additionally, during further drying, polyphenol concentrations and patterns remained unchanged (see the following sections) while the pomace volume was not reduced considerably.

3.2 Dried pomaces composition

3.2.1 Polysaccharide composition

Polysaccharides, arising from the cell walls of the fruits, constituted most of the pomace dry matter (> 500 g/kg), compared with circa 150 g/kg of the fresh fruit dry matter. Similar polysaccharides or dietary fibre contents in apple pomace have been reported previously (Perussello, et al., 2017): recent examples include Yan & Kerr (2013) who reported 442 - 495 g/kg total dietary fibre (TDF) in dried apple pomaces versus 124 mg/g in freeze-dried apple, while Kolodziejczyk et al. (2009) reported an average TDF content of 524 g/kg for pomace from clear juice production. There were no significant differences in polysaccharide yield and composition as a function of drying or oxidation.

Table 1 shows the yields and composition recorded for the two extreme water contents for non-oxidised and oxidised pomaces from both cultivars.

280 Compositions were similar to those reported earlier for AIS from apple pomaces (Kolodziejczyk, et al.,
281 2009; Renard & Thibault, 1991), i.e. a large predominance of glucose (from cellulose) and lower
282 amounts of uronic acids than in fresh fruit AIS, followed by arabinose and galactose, xylose,
283 mannose, and very low amounts of rhamnose, fucose and mannose. As these pomaces were
284 obtained without enzymes, methanol contents and degrees of methylation remained high. There
285 were differences between the cultivars, most noticeably with 'Kermerrien' being poorer in arabinose
286 and galactose, and richer in glucose than 'Avrolles'.

287 **3.2.2. Polyphenol composition and patterns during drying of apple pomaces**

288 'Kermerrien' and 'Avrolles' apples were chosen due to their contrasting polyphenolic compositions
289 (Sanoner, Guyot, Marnet, Mollé, &Drilleau, 1999), with 'Avrolles' being particularly rich in highly
290 polymerized procyanidins. This contrast carried over into the original pomaces (Table 2).

291 The main polyphenols in both pomaces were procyanidins, especially in 'Avrolles' pomace, followed
292 by dihydrochalcones, 5-O-caffeoylquinic acid (in 'Kerrmerrien') and flavonols. Small amounts of 4-*p*-
293 coumayolquinic acid and phloretin xyloglucoside were also present in the 'Avrolles' pomace. The
294 degrees of procyanidins polymerization were also typical for these two varieties, about 7 for
295 'Kerrmerrien' and very high (> 20) for 'Avrolles'. Oxidation during pressing had only a limited impact
296 on pomace compositions, probably due to oxidation after recovery of the press cake and its handling.
297 However, lower concentrations of flavanols and 5-O-caffeoylquinic acid were detected in OX
298 pomaces, while apparent degrees of procyanidins polymerization decreased. 'Kermerrien' pomaces
299 produced in OX conditions displayed browning compared with those produced under NOX. 'Avrolles'
300 pomaces did not show any particular differences in colour between OX and NOX treatments.

301 Concentrations (per dry matter) of native polyphenols were low relative to the fresh apples (Sanoner,
302 et al., 1999) in both NOX and OX pomace. Two factors appeared to be at play: one was extraction of
303 some polyphenols with the juice whilst the other was oxidation, after pressing, during handling of the
304 pomaces, explaining why NOX and OX samples were quite similar. Higher residual concentrations in

305 ‘Avrolles’, while ‘Kermerrien’ apples are richer in polyphenols (Sanoner, et al., 1999), might be due to
306 inhibition of polyphenol oxidase by the high molecular weight tannins in ‘Avrolles’ (Le Bourvellec, Le
307 Quéré, Sanoner, Drilleau, & Guyot, 2004). The dominance of procyanidins was even more marked in
308 the pomace than in the fruits, especially for ‘Avrolles’. This is linked to differences in procyanidins
309 transfer rates, depending on their degree of polymerization, as shown by Le Bourvellec, Le Quéré, &
310 Renard (2007). Kolodziejczyk et al. (2009) also report lower polyphenolic concentrations in pomaces
311 than in fruits, with a relative increase in flavonols. The predominance of procyanidins in apple
312 pomace has been reported previously (Garcia, Valles, & Lobo, 2009; Lavelli & Corti, 2011; Lu & Foo,
313 1997), but many articles fail to quantify these polymers and, thus, identify dihydrochalcones or
314 flavonols as the dominant polyphenols (Ferrentino, et al., 2018; Suarez et al., 2010; Wijngaard &
315 Brunton, 2009, 2010).

316 NOX pomaces were enriched with flavonols and dihydrochalcones, due to the concentration of these
317 compounds in the skins and pips (Fromm, et al., 2012; Guyot, et al., 1998), respectively. They were
318 also depleted of phenolic acids and monomeric catechins, which are the primary substrate of apple
319 polyphenoloxidase (5-O-caffeoylquinic acid) and the main reactive species during oxidation transfer
320 (catechins), respectively (Guyot, Bernillon, Poupard, & Renard, 2008; Nicolas, et al., 1994).

321 Levels of naturally occurring forms of all polyphenols decreased upon drying, irrespective of apple
322 variety and the presence or absence of oxygen during production (Figure 1). Different patterns could
323 be observed regarding the various classes of polyphenolic compounds: high molecular weight
324 procyanidins of ‘Avrolles’ only decreased by half and a high concentration of native molecules still
325 persisted after drying. Apparent degrees of polymerization increased, which might be a marker of
326 covalent bond formation with the cell walls via the terminal units. As far as hydroxycinnamic acids
327 were concerned, the decrease during drying was rapid and these compounds were nearly all
328 converted in the pomace. Flavonols decreased rather more in ‘Kermerrien’ than in ‘Avrolles’, while
329 dihydrochalcones (data not shown) varied widely without any specific tendency. This was probably

due to their preferential location in the seeds and irregular distribution of seeds in the samples used for analysis. Drying at 70 °C induced and enhanced pomace browning in both varieties, irrespective of treatment with OX or NOX. Again, drying-provoked browning was more significant in 'Kermerrien' pomaces. After drying (Table 2), pomaces were poorer in native polyphenols and, in particular, did not contain monomeric flavanols and were poor in hydroxycinnamic acids.

Upon preparation of OX 'Kermerrien' pomaces,, enzymatic browning occurred, involving the conversion of polyphenolic compounds first to quinones and then to brown polymers under the catalytic influence of the polyphenol oxidase (PPO) (Nicolas, et al., 1994). In 'Avrolles', low amounts of PPO substrates (5-O-caffeoylquinic acid), the absence of catechins, which have been identified as the main cause of browning in apple through secondary oxidation reactions, and high concentrations of high molecular-weight procyanidins, known PPO inhibitors (Le Bourvellec, et al., 2004), all contributed to the decreased impact of oxidation. Heras-Ramirez, et al. (2012), comparing drying (between 50°C and 80°C) of blanched and unblanched pomaces, found significant decreases in polyphenol content in both, but much more marked in unblanched pomaces. Lavelli & Corti (2011), comparing apple pomaces air-dried at 60°C and vacuum-dried at 40°C, found only limited differences, as a function of drying method, but their pomaces were recovered from blanched apples. This further underlines the role of PPO in polyphenol evolution.

3.3 Impact of drying on polyphenol extractability

Polyphenols were extracted from the pomaces with different degrees of drying using two "green" solvents, namely water and 48% EtOH (Supplementary Figure 3). The ethanol concentration chosen corresponded to the highest yields reported by Virot et al. (2010). Wijngaard & Brunton (2009) also reported the highest extraction rate with intermediate ethanol concentrations. As a high proportion of polyphenols from the pomaces were oxidised, they were quantified first using a global method

(Folin-Ciocalteu or "total polyphenols"). Interference from other reductants, such as ascorbic acid, was not likely in these samples, as no ascorbic acid was added during pressing and apples are naturally low in ascorbic acid, which is consumed by oxidation during pressing and pomace handling (Varming, Petersen, & Toldam-Andersen, 2013). There were no significant differences or clear trends in amounts of extracted Folin-Ciocalteu-reactive species ("total polyphenols") with drying or oxidation (Supplementary Figure 2).

Table 3 shows means ± standard deviations of extract yields calculated on a dry weight basis after extraction using either water or 48 % EtOH for samples (n=18) within one apple variety and within OX/ NOX treatment as well as the proportion (%) of polyphenols in each extract. Water extracts contained only 2 % polyphenols, because water extracted mainly carbohydrates from apple pomaces. Ethanol (48% EtOH) extracts contained higher proportions of polyphenols (~15 %) compared with the water extracts.

Other compounds, including sugars, proteins and nucleic acids, composed the bulk of the extracts, but studies on these (macro)molecules lay outside the framework of this paper.

3.4 Composition of the extracts

Individual native polyphenols were analyzed in water and 48% EtOH extracts from both varieties and OX/NOX treatments, for initial water contents of 72, 34 and 0% (Supplementary table). The sum of polyphenols detected was noticeably lower than the amounts of "total polyphenols", i.e. Folin-reactive substances detected in the same extracts. The Folin-Ciocalteu reagent reported polyphenol average contents of ca. 20 mg/g and 150 mg/g in water and 48% EtOH extracts, respectively, and only 2-5 mg/g and 18 to 67 mg/g, respectively, were identified as specific polyphenols originally present in the apples. This was due to the presence of oxidised polyphenols, which are not quantified even by thioacidolysis.

Native polyphenol compositions in the extracts were noticeably different from those of the pomaces (Figure 2) with, in particular, very low recoveries of 5-O-caffeoylquinic acid, even from 'Kermerrien' pomaces. Native polyphenols detected in the extract were procyanidins, phloridzin and flavonols; traces of phloretin xyloglucoside or 4-*p*-coumaroylquinic acid were also detected.

Water extracts contained higher proportions of monomeric polyphenols while the 48% EtOH extracts contained mostly procyanidins, notably in extracts from 'Avrolles'. Water extracts from 'Avrolles' pomace showed a distinctly higher proportion of phloridzin while in 'Kermerrien' extracts flavonols were present in relatively high proportions, again more in the water extracts. Virotet al. (2010) also found, as main constituents of apple pomace extracts, procyanidins followed by dihydrochalcones, flavonols (specifically quercetin glycosides), and some hydroxycinnamic acids. Plaza et al.(2013) did not analyse procyanidins in their water extracts and reported, as main constituents, quercetin glycosides (notably hyperoside), phloridzin, and 5-O-caffeoylquinic acid in varying proportions, depending on extraction temperature.

Polyphenols that could be identified in the extracts reflected those that remained in native form in dried pomaces. As extensive oxidation occurred during air-drying, it can be speculated that these native molecules are preserved because they are not easily degraded by the coupled oxidation reactions starting with polyphenoloxidase oxidation of 5-O-caffeoylqunic acid in the apple (Guyot et al., 2008). This could be due to chemical properties (i.e. redox potential, as is known for *p*-coumaroylquinic acid) or topological effects, i.e. sequestration either in the pips (for dihydrochalcones) or the peels (for flavonols) (Guyot et al., 1998, Fromm et al., 2012). Further work is needed to identify the newly formed molecules in apple pomace and, notably, formation of inter- or intramolecular bonds between polyphenols or polyphenols and other macromolecules in the pomaces.

A neoformed molecule was detected by HPLC with a maximum absorbance at 270 nm, which increased with drying. Its spectrum was close to that reported by Garcia et al.(2009). Purification was attempted, but the compound degraded during evaporation of the (acidic) HPLC solvent. NMR analysis (not described) indicated the presence of tyrosine and a sugar moiety. Presumably, this molecule is a tyrosine glycoside, formed during drying from free aminoacids and residual sugars in the pomace.

4 Conclusion

Functionality but not structure of polyphenols were preserved upon drying of apple pomace. Drying had little influence on the amounts of Folin-Ciocalteu-reactive species ("total polyphenols") extracted from apple pomace. However, the composition of polyphenols in the extracts was modified: the greater the drying, the lower the amounts of native molecules. This was particularly marked for the monomeric flavanols and hydroxycinnamic acids, and least obvious for dihydrochalcones. Most of the differences in composition occurred during the first steps in air-drying, with limited modifications observed between 34% residual water and total dryness. Though extracts from apple pomaces might still have high antioxidant capacities, the structures of molecules involved remain unknown and different from those present in apple. This is particularly relevant for their physiological properties, which need to be studied specifically. Extraction of native polyphenols from apple pomace would necessitate a blanching step soon after pressing, as proposed by Heras-Ramirez et al. (2012) or freeze-drying immediately after pressing, but both are likely to involve additional energy requirements.

Acknowledgements

425 The authors thank MM G. Le Bail, R. Bauduin & S. Hinguant for production of apple pomace, Mrs L.
426 Touloumet and Mr. C. Ginies for their excellent technical help.

427 This work is part of the ISAFRUIT project funded by the European Commission under the Thematic
428 Priority 5–Food Quality and Safety of the 6th Framework Programme of RTD (Contract no. FP6-
429 FOOD–CT-2006-016279).

430 Disclaimer: The views and opinions expressed in this publication are purely those of the writers and
431 may not in any circumstances be regarded as stating an official position of the European Commission.

432 The authors declare no conflict of interest

433

References

Blumenkrantz, N., & Asboe-Hansen, G. (1973). New method for quantitative determination of uronic acids. *Analytical Biochemistry, 54*, 484-489.

Box, G. E., Hunter, W. G., Hunter, J. S. (1978). Statistics for experimenters, an introduction to design, data analysis and model building. New York: Wiley and Sons., 352 pp.

De Beer, D., Harbertson, J. F., Kilmartin, P. A., Roginsky, V., Barsukova, T., Adams, D. O., et al. (2004). Phenolics: A comparison of diverse analytical methods. *American Journal of Enology and Viticulture, 55*(4), 389-400.

Del Rio, D., Costa, L. G., Lean, M. E. J., & Crozier, A. (2010). Polyphenols and health: What compounds are involved? *Nutrition Metabolism and Cardiovascular Diseases, 20*(1), 1-6.

Englyst, H., Wiggins, H. S., & Cummings, J. H. (1982). Determination of the non-starch polysaccharides in plant foods by gas-liquid chromatography of constituent sugars as alditol acetates. *Analyst, 107*, 307-318.

Ferrentino, G., Morozova, K., Mosibo, O. K., Ramezani, M., & Scampicchio, M. (2018). Biorecovery of antioxidants from apple pomace by supercritical fluid extraction. *Journal of Cleaner Production, 186*, 253-261.

Fromm, M., Bayha, S., Carle, R., & Kammerer, D. R. (2012). Characterization and Quantitation of Low and High Molecular Weight Phenolic Compounds in Apple Seeds. *Journal of Agricultural and Food Chemistry, 60*(5), 1232-1242.

Garcia, Y. D., Valles, B. S., & Lobo, A. P. (2009). Phenolic and antioxidant composition of by-products from the cider industry: Apple pomace. *Food Chemistry, 117*(4), 731-738.

Guyot, S., Bernillon, S., Poupard, P., & Renard, C. (2008). *Multiplicity of Phenolic Oxidation Products in Apple Juices and Ciders, from Synthetic Medium to Commercial Products* (Vol. 1).

Guyot, S., Marnet, N., Laraba, D., Sanoner, P., & Drilleau, J. F. (1998). Reversed-phase HPLC following thiolysis for quantitative estimation and characterization of the four main classes of phenolic

compounds in different tissue zones of a french cider apple variety (Malus domestica var. Kermerrien). *Journal of Agricultural and Food Chemistry, 46*, 1698-1705.

Guyot, S., Marnet, N., Sanoner, P., & Drilleau, J. F. (2001). Direct thiolysis on crude apple materials for high-performance liquid chromatography characterization and quantification of polyphenols in cider apple tissues and juices. In L. Packer (Ed.), *Methods in Enzymology - Flavonoïds and other polyphenols* (Vol. 335, pp. 57-70): Academic Press.

Heras-Ramirez, M. E., Quintero-Ramos, A., Camacho-Davila, A. A., Barnard, J., Talamas-Abbud, R., Torres-Munoz, J. V., et al. (2012). Effect of Blanching and Drying Temperature on Polyphenolic Compound Stability and Antioxidant Capacity of Apple Pomace. *Food and Bioprocess Technology, 5*(6), 2201-2210.

Heuzé, V., Tran, G., Hassoun, P., & Lebas, F. (2018, July 10, 2018, 16:28). Apple pomace and culled apples. . *Feedipedia, a programme by INRA, CIRAD, AFZ and FAO.* Retrieved July 20th, from https://www.feedipedia.org/node/20703

Kennedy, M., List, D., Lu, Y., Foo, L. Y., Newman, R. H., Sims, L. M., et al. (1999). Apple pomace and products derived from apple pomace : uses, composition and analysis *Modern methods of plant analyses. Analysis of plant waste materials* (Vol. 20, pp. 75-119). Berlin, Heidelberg: Springer Verlag.

Kolodziejczyk, K., Kosmala, M., Milala, J., Sojka, M., Uczciwek, M., Krol, B., et al. (2009). Characterisation of the chemical composition of scab-resistant apple pomaces. *Journal of Horticultural Science & Biotechnology*, 89-95.

Lavelli, V., & Corti, S. (2011). Phloridzin and other phytochemicals in apple pomace: Stability evaluation upon dehydration and storage of dried product. *Food Chemistry, 129*(4), 1578-1583.

Le Bourvellec, C., Guyot, S., & Renard, C. (2004). Non-covalent interaction between procyanidins and apple cell wall material Part I. Effect of some environmental parameters. *Biochimica Et Biophysica Acta-General Subjects, 1672*(3), 192-202.

Le Bourvellec, C., Le Quere, J. M., & Renard, C. (2007). Impact of noncovalent interactions between apple condensed tannins and cell walls on their transfer from fruit to juice: Studies in model suspensions and application. *Journal of Agricultural and Food Chemistry, 55*(19), 7896-7904.

Le Bourvellec, C., Le Quere, J. M., Sanoner, P., Drilleau, J. F., & Guyot, S. (2004). Inhibition of apple polyphenol oxidase activity by procyanidins and polyphenol oxidation products. *Journal of Agricultural and Food Chemistry, 52*(1), 122-130.

Lu, Y., & Foo, L. Y. (1997). Identification and quantification of major polyphenols in apple pomace. *Food Chemistry, 59*(2), 187-194.

May, C. D. (1990). Industrial pectins : sources, production and applications. *Carbohydrate Polymers, 12*, 79-99.

Nicolas, J. J., Richard-Forget, F. C., Goupy, P. M., Amiot, M. J., & Aubert, S. Y. (1994). Enzymatic browning reactions in apple and apple products. *Critical Reviews in Food Science and Nutrition, 34*(2), 109-157.

Perussello, C. A., Zhang, Z. H., Marzocchella, A., & Tiwari, B. K. (2017). Valorization of Apple Pomace by Extraction of Valuable Compounds. *Comprehensive Reviews in Food Science and Food Safety, 16*(5), 776-796.

Plaza, M., Abrahamsson, V., & Turner, C. (2013). Extraction and Neoformation of Antioxidant Compounds by Pressurized Hot Water Extraction from Apple Byproducts. *Journal of Agricultural and Food Chemistry, 61*(23), 5500-5510.

Reis, S. F., Rai, D. K., & Abu-Ghannam, N. (2012). Water at room temperature as a solvent for the extraction of apple pomace phenolic compounds. *Food Chemistry, 135*(3), 1991-1998.

Renard, C. M. G. C. (2005). Variability in cell wall preparations: quantification and comparison of common methods. *Carbohydrate Polymers, 60*(4), 515-522.

Renard, C. M. G. C., & Ginies, C. (2009). Comparison of the cell wall composition for flesh and skin from five different plums. *Food Chemistry, 114*(3), 1042-1049.

510 Renard, C. M. G. C., Le Quere, J. M., Bauduin, R., Symoneaux, R., Le Bourvellec, C., & Baron, A. (2011).
511 Modulating polyphenolic composition and organoleptic properties of apple juices by
512 manipulating the pressing conditions. *Food Chemistry, 124*(1), 117-125.
513 Renard, C. M. G. C., & Thibault, J. F. (1991). Composition and physico-chemical properties of apple
514 fibres from fresh fruits and industrial products. *Lebensmittel Wissenschaft und Technologie,*
515 *24*(6), 523-527.
516 Saeman, J. F., Moore, W. E., Mitchell, R. L., & Millett, M. A. (1954). Techniques for the determination
517 of pulp constituents by quantitative paper chromatography. *Tappi, The Journal of the*
518 *Technical Association of the Pulp and Paper Industry, 37*(8), 336-343.
519 Sanoner, P., Guyot, S., Marnet, N., Mollé, D., & Drilleau, J. F. (1999). Polyphenols profiles of french
520 cider apple varieties (Malus domestica sp.). *Journal of Agricultural and Food Chemistry, 47*,
521 4847-4853.
522 Scalbert, A., Manach, C., Morand, C., Remesy, C., & Jimenez, L. (2005). Dietary polyphenols and the
523 prevention of diseases. *Critical Reviews in Food Science and Nutrition, 45*(4), 287-306.
524 Suarez, B., Alvarez, A. L., Garcia, Y. D., del Barrio, G., Lobo, A. P., & Parra, F. (2010). Phenolic profiles,
525 antioxidant activity and in vitro antiviral properties of apple pomace. *Food Chemistry, 120*(1),
526 339-342.
527 Symoneaux, R., Baron, A., Marnet, N., Bauduin, R., & Chollet, S. (2014). Impact of apple procyanidins
528 on sensory perception in model cider (part 1): Polymerisation degree and concentration.
529 *LWT - Food Science and Technology, 57*(1), 22-27.
530 Varming, C., Petersen, M. A., & Toldam-Andersen, T. B. (2013). Ascorbic acid contents in Danish apple
531 cultivars and commercial apple juices. *LWT - Food Science and Technology, 54*(2), 597-599.
532 Virot, M., Tomao, V., Le Bourvellec, C., Renard, C., & Chemat, F. (2010). Towards the industrial
533 production of antioxidants from food processing by-products with ultrasound-assisted
534 extraction. *Ultrasonics Sonochemistry, 17*(6), 1066-1074.

Vrhovsek, U., Mattivi, F., & Waterhouse, A. L. (2001). Analysis of red wine phenolics: Comparison of HPLC and spectrophotometric methods. *Vitis, 40*(2), 87-91.

Wijngaard, H., & Brunton, N. (2009). The Optimization of Extraction of Antioxidants from Apple Pomace by Pressurized Liquids. *Journal of Agricultural and Food Chemistry, 57*(22), 10625-10631.

Wijngaard, H., & Brunton, N. (2010). The optimisation of solid-liquid extraction of antioxidants from apple pomace by response surface methodology. *Journal of Food Engineering, 96*(1), 134-140.

Yan, H. T., & Kerr, W. L. (2013). Total phenolics content, anthocyanins, and dietary fiber content of apple pomace powders produced by vacuum-belt drying. *Journal of the Science of Food and Agriculture, 93*(6), 1499-1504.

Captions to figures

Fig 1: Evolution of polyphenol concentrations in the apple pomaces (mg/g d.w.) after different levels of drying. Each point is the average of three replicates, the lines indicate the standard deviations.

□ : 'Avrolles', pressed in absence of oxygen; ■ : 'Avrolles', pressed in presence of oxygen; ◇: 'Kermerrien', not oxidised during pressing; ◆ : 'Kermerrien', oxidised during pressing; :

Fig 2: Proportions (% in weight of identified polyphenols) of the different polyphenols in the extracts from pomaces of Kerrmerien and Avrolle apples by pressurized liquid extraction using water (H_2O) or 48% ethanol (EtOH) at 70°C, after different levels of drying. Pomaces had been generated either in the presence (OX) or absence (NOX) of oxygen.

UD: undried (72% water); ID: intermediate drying level (34% water); AD: air-dried (0% water).

From top to bottom: : flavonols; : phloretin xyloglucoside ; : phloridzin; : 4-*p*-coumaroylquinic acid; : 5-O-caffeoylquinic acid; : procyanidins.

Supplementary Figure 1: HPLC chromatograms at 280 nm for Kerrmerien apple pomaces.

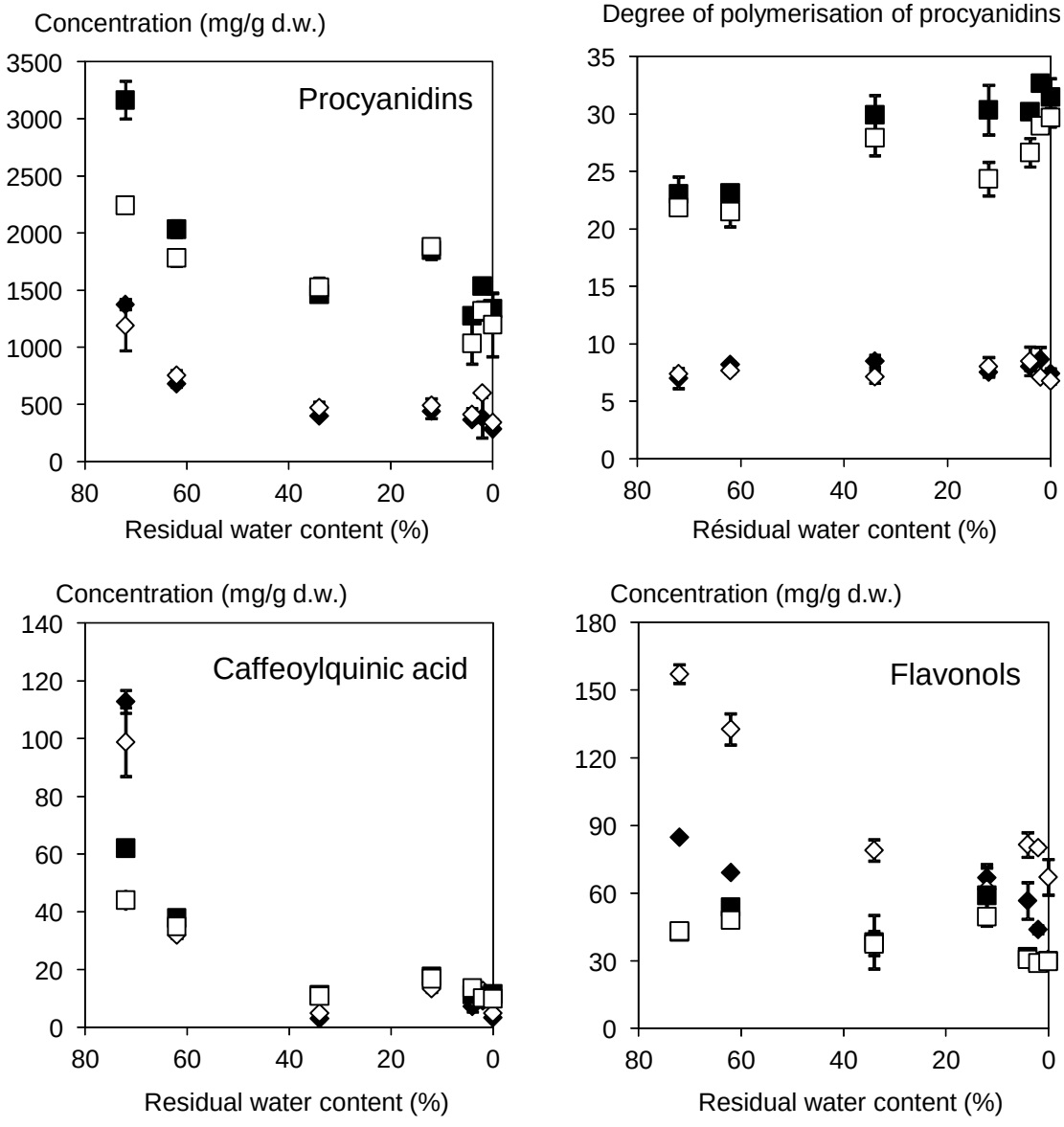
CAT: (+) catechin; 5CQA: 5-O-caffeoylquinic acid; EPI; (-) epicatechin; pCQ: p-coumaroylquinic acid; B2: procyanidin B2; PCA: procyanidin oligomers; PLZ phloridzin.

Supplementary Figure 2: Drying curves of 'Kermerrien' apple pomaces in monolayer (< 0.5 cm) at 70°C in a ventilated oven. Pomaces were generated in the presence (solid circles) or in the absence (empty circles) of oxygen. Each point is the average of three replicates, the lines indicate the standard deviations.

Supplementary Figure 3: Amounts of polyphenols (in mg/g initial dry pomace d.w.) extracted from pomaces of Kerrmerien and 'Avrolles' apples by pressurized liquid extraction using water (black bars) or 48% ethanol (white bars) at 70°C, after different levels of drying. Pomaces had been generated either in the presence (OX) or absence (NOX) of oxygen. Each point is the average of three replicates, the lines indicate the standard deviations.

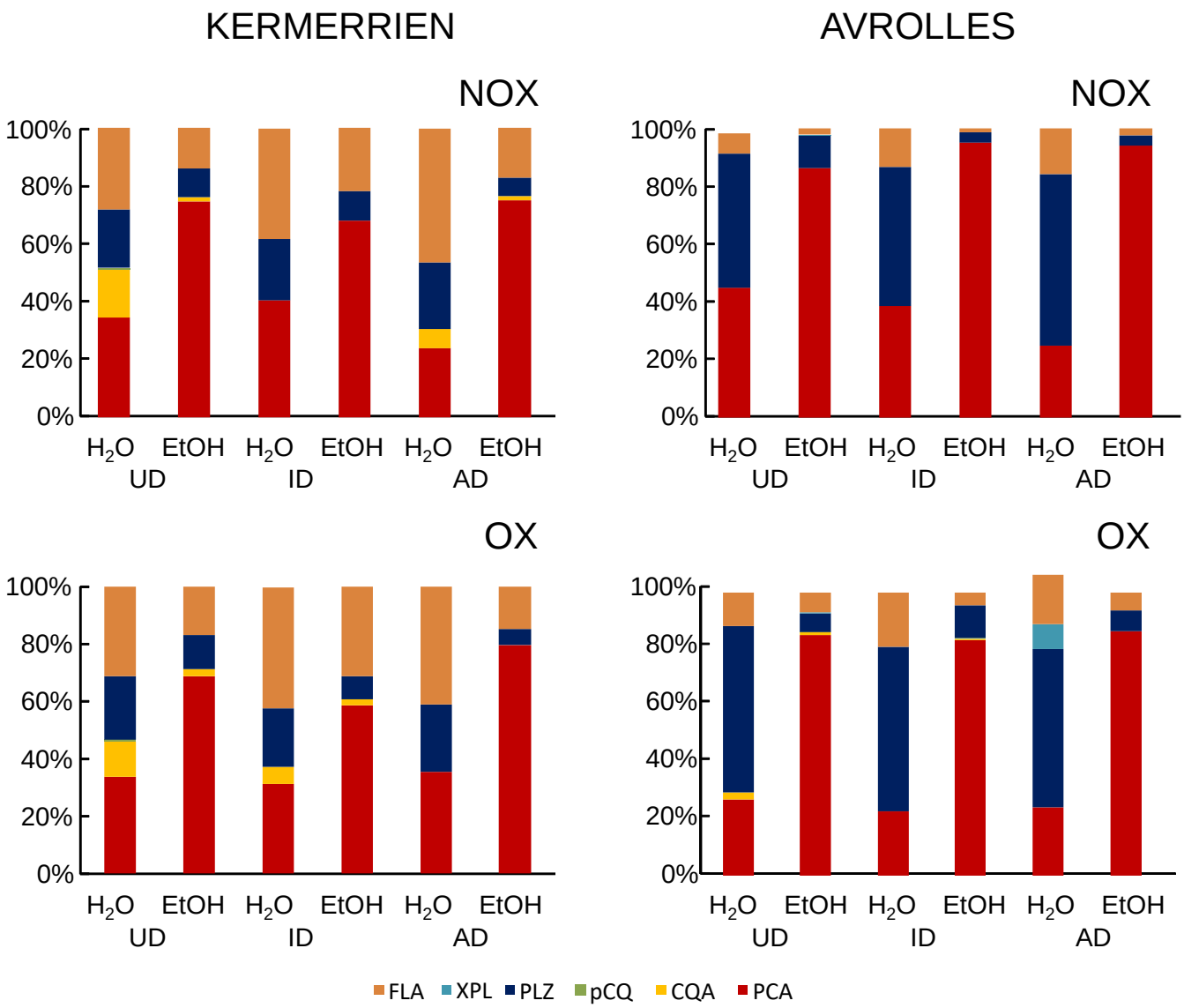
Birtic et al.,
apple pomace,

Fig 1



Birtic et al.,
apple pomace,

Fig 2



Tables

Table 1: Yields and sugar compositions of the cell walls isolated as alcohol-insoluble solids from the pomaces of 'Kermerrien' and 'Avrolles' apples, produced with (OX) or without (NOX) oxidation during pressing, and air-dried at 70°C (AD) or not (UD).

Variety	Pressing	Drying	Yield (g/g d.w.)	Composition (mg/g alcohol insoluble solids)								
				Rha	Fuc	Ara	Xyl	Man	Gal	Glc	AUA	MeOH
Kermerrien	NOX	UD	0.71	5	3	54	24	6	35	327	105	24
Kermerrien	NOX	AD	0.66	5	2	51	31	4	36	339	120	21
Kermerrien	OX	UD	0.69	5	2	51	26	6	34	297	139	21
Kermerrien	OX	AD	0.69	5	2	50	30	6	33	312	130	20
Avrolles	NOX	UD	0.56	6	4	74	33	15	48	223	144	22
Avrolles	NOX	AD	0.60	3	2	59	29	8	55	224	152	20
Avrolles	OX	UD	0.66	6	4	66	29	17	53	220	166	21
Avrolles	OX	AD	0.65	6	6	90	26	9	76	100	124	20
Pooled standard deviation				*0.3*	*0.2*	*2.4*	*5.5*	*0.4*	*1.3*	*8.1*	*19*	*1.2*

Pooled standard deviation was calculated from all the replicates.

AUA: anhydrouronic acids

Table 2: Initial polyphenol composition (mg/g dry weight) of the pomaces of 'Kermerrien' and 'Avrolles' apples, produced with (OX) or without (NOX) oxidation during pressing, and air-dried at 70°C (AD) or not (UD).

Variety	Pressing	Drying	Flavan-3-ols				OH-cinnamic acids		Dihydro-chalcones		Sum of flavonols
			CAT	EPI	PCA	DP	CQA	pCQ	XPL	PLZ	
Kermerrien	NOX	UD	12	72	1373	7	113	4	3	113	85
Kermerrien	NOX	AD	nd	nd	282	7	3	nd	nd	10	31
Kermerrien	OX	UD	10	55	1188	6	99	4	4	158	157
Kermerrien	OX	AD	nd	nd	342	7	5	nd	nd	25	67
Avrolles	NOX	UD	nd	nd	3162	23	62	18	15	213	43
Avrolles	NOX	AD	nd	nd	1335	31	11	5	4	90	30
Avrolles	OX	UD	nd	nd	2240	22	44	17	14	207	43
Avrolles	OX	AD	nd	nd	1195	30	10	6	3	77	30
Pooled standard deviation			*0.6*	*4*	*148*	*0.9*	*5*	*0.8*	*0.5*	*14*	*3*

Pooled standard deviation was calculated from all the replicates containing the compound (n from 6 for CAT and EPI to 24); nd: not detected.

CAT: (+)-catechin; EPI: (-)-epicatechin; PCA: procyanidins; DP: number average degree of polymerisation of procyanidins; CQA: 5-O-caffeoylquinic acid; p-CQ: 4-*p*-coumaroylquinic acid; XPL: phloretin xyloglucoside; PLZ: phloridzin.

Table 3: Yields of freeze-dried extract (mg/g initial apple pomace dry weight) and proportion (%) of total polyphenols (TP, Folin-Ciocalteu reactive substances) in the pomaces of 'Kermerrien' and 'Avrolles' apples, produced with (OX) or without (NOX) oxidation during pressing. 48 % EtOH: ethanol:water 48/52 mL:mL. Data represent mean ± SD of 18 samples.

Presence of O_2 during pomace production	OX				NOX			
Extraction solvent	H_2O		48% EtOH		H_2O		48% EtOH	
	Yield	% TP	Yield	% TP	Yield	% TP	Yield	% TP
Kermerrien	325 ± 21	2 ± 0.4	21 ± 3	12 ± 3.5	337 ± 28	2 ± 0.4	14 ± 5	24 ± 3.4
Avrolles	400 ± 38	2 ± 0.6	33 ± 7	14 ± 4.6	374 ± 20	2 ± 0.4	32 ± 12	12 ± 3.9