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► To cite this version:

Simona Birtic-Pindat, Sylvaine Regis, Carine Le Bourvellec, Catherine M.G.C. Renard. Impact of air-drying on polyphenol extractability from apple pomace. *Food Chemistry*, 2019, 296, pp.142-149. 10.1016/j.foodchem.2019.05.131 . hal-02620761

HAL Id: hal-02620761

<https://hal.inrae.fr/hal-02620761>

Submitted on 25 Oct 2021

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1 Impact of air-drying on polyphenol 2 extractability from apple pomace 3

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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, flavonol, dihydrochalcone, chlorogenic acid

42 **1- Introduction**

43

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

56 Recently, there has been a renewed interest in apple pomaces as a rich-source of functional
57 components and extracts (fibres, pectins, antioxidant polyphenols), as summarized in a recent review
58 by Perussello, Zhang, Marzocchella, & Tiwari (2017). Still, few articles report actual polyphenolic
59 compositions of these extracts, while most use antioxidant assays or “total polyphenols” using the
60 Folin-Ciocalteu assay to quantify yields. Solvents, water and water-ethanol mixtures have been
61 demonstrated to extract polyphenols efficiently from apple pomace (Plaza, Abrahamsson, & Turner,
62 2013; Wijngaard & Brunton, 2009, 2010) in pressurized liquid extraction (PLE). However, there a
63 range of extractions conditions have been used; Reis, Rai, & Abu-Ghannam (2012) reported that
64 water at room temperature extracted 67% of total polyphenols from an apple pomace and Plaza et
65 al.(2013) that PLE at 120°C for 3 min gave the highest yield of flavonols. Wijngaard & Brunton (2010)
66 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

92 stored for polyphenol production, they need to be dried to avoid microbial spoilage, otherwise they
93 require a lot of storage space at sub-zero temperatures. Air-drying down to 100 g water per kg
94 increases considerably pomace stability while decreasing the volume rapidly at a reasonable cost
95 (Lavelli & Corti, 2011). However, drying can impact strongly the properties of pomace, such as
96 polyphenol extractability and integrity, and is costly in terms of energy. Few articles, however, have
97 dealt with the impact of pomace drying pre-treatments on polyphenol extraction. Lavelli & Corti
98 (2011) reported that air-drying at 60°C was better than vacuum drying at 40°C for retention of
99 anthocyanins and flavanols in apple pomace during nine months storage, with the maximum stability
100 at the lowest water activity. Heras-Ramirez et al., (2012) indicated that drying led to significant
101 reductions in antioxidant activity, while dry unblanched pomace reduced the concentrations of
102 epicatechin and caffeic acid compared with dry blanched pomace. However, the pomaces were first
103 dipped in an antioxidant solution. Ferrentino, Morozova, Mosibo, Ramezani, & Scampicchio (2018),
104 comparing extraction of polyphenols from freeze-dried, oven-dried (50°C, 4 days) and frozen apple
105 pomace, found higher yields from freeze-dried than oven-dried pomaces (comparison with “frozen”
106 cannot be done as their results were not normalized for dry matter content). Yan & Kerr (2013),
107 comparing vacuum-belt dried to freeze-dried pomace, reported higher total polyphenol contents
108 (extracted with 80% acetone) after vacuum-belt drying. To our knowledge, no article has examined
109 systematically the impact of drying on polyphenolic composition and extractability from apple
110 pomace.

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

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

120

121 **2- Materials and methods**

122 **2.1 Pomace material**

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

130 For pressing without oxidation, the press was flushed with water first. Inerting was carried out by
131 flushing the water out of the press with CO₂ and connecting the press to the exit of the grinder
132 (Stossier, Malters, Switzerland) via plastic tubing. Crushing started when a burning match was
133 inserted into the entrance of the grinder and combustion could not maintained. A standing time of
134 20 min was observed between crushing and pressing of the apples. For pressing with oxidation,
135 apples were pressed as above, except crushed apples were dropped into a 30 L-drum that was closed
136 and agitated "head-over-tail" for 20 min at room temperature prior to pressing.

137

138 **2.2 Standards and chemicals**

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

141 (Genay, France). Phloridzin was obtained from Fluka (Buchs, Switzerland). Sugar standards were from
142 Fluka (Buchs, Switzerland). D₃-methanol was from Acros organics (Geel, Belgium). Sodium
143 borohydride, N-methyl imidazole, acetic anhydride, toluene- α -thiol, Folin-Ciocalteu's phenol reagent
144 were from Sigma-Aldrich (Deisenhofen, Germany). Acetonitrile, ethanol and methanol were
145 analytical grade and from Fisher Scientific (Fair Lawn, New Jersey, USA).

146

147 **2.3 Drying treatment**

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

154 Initially, to evaluate pomace water content (WC), samples of 5 g (n=6) were dried until they achieved
155 a constant weight (117°C, 17h). Water content is expressed on a fresh weight basis. Pomace samples
156 from 'Kermerrien' (OX and NOX) and 'Avrolles' (OX and NOX) were prepared in triplicate by drying
157 down to 72%, 62%, 34%, 12%, 4%, 2% and 0% (no detectable weight loss) (using drying durations
158 calculated from the drying kinetics) and used, subsequently, for polyphenol extractions and analyzes.
159 Actual water contents are summarized in Supplementary Table 1.

160

161 **2.4 Preparation of alcohol insoluble solids**

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

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

167

168 **2.5 Extraction of polyphenols**

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

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

186

187 **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 with 100 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 Na₂CO₃ (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 acid equivalents 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 **2.7 Individual 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

212 45 min; followed by washing and reconditioning the column. Catechins and their thioesters were
213 quantified at 280 nm against an epicatechin standard. Chlorogenic acid (5-O-caffeoylquinic acid), 4-*p*-
214 coumaroylquinic acid and their methyl derivatives were quantified at 320 nm using as standards 5-O-
215 caffeoylquinic acid and *p*-coumaric acid, respectively. All flavonols were quantified at 350 nm against
216 a quercetin standard. Phloridzin and phloretin xyloglucoside were quantified at 280 nm against a
217 phloridzin standard.

218

219 **2.8 Polysaccharide analysis**

220 All AIS were analyzed in duplicate for neutral sugars, uronic acids and methanol content. Neutral
221 sugars were analyzed using gas chromatography (GC) with flame ionization detector (FID) as alditol
222 acetates after acid hydrolysis: samples (c.a. 10 mg of AIS) were subjected to pre-hydrolysis with 250
223 μL sulphuric acid (12 mol/L) for 1 hour at room temperature (Saeman, Moore, Mitchell, & Millett,
224 1954), which was diluted to 1 mol/L sulphuric acid with the addition of water and the internal
225 standard (inositol). All samples were placed in oven at 100 °C for 3 hours for hydrolysis. Afterwards,
226 they were derivatized to volatile alditol acetates by reduction with sodium borohydride and, then,
227 acetylation in acetyl anhydride catalysed by N-methyl imidazole, and extracted in dichloromethane
228 (Englyst, Wiggins, & Cummings, 1982). Extracts were injected onto a GC-FID HP 5890 Serie II (Agilent,
229 Inc, Palo Alto, USA) with a capillary column of 30 m x 0.25 mm i.d. coated with DB225 MS, 0.25 μm
230 film thickness (J&W Scientific, Agilent, Inc, Palo Alto, USA). The conditions were: temperature of
231 injection 250°C in split mode (ratio 1:25); hydrogen as carrier gas at 45 cm/s (at 215 °C), column flow
232 was 1.3 ml/min; the oven temperature was isothermal at 215 °C.

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

236 Methanol was determined by stable isotope dilution assay against D₃-methanol by headspace-GC-MS
237 (mass spectrometry) after saponification, as described by Renard & Ginies (2009). The GC apparatus
238 was a GC-MS QP2010 Shimadzu with capillary column (Cp_wax_52cb 30m x 0.32mm x 0.5 μm;
239 Varian, Inc, Palo Alto, USA) equipped with auto sampler AOC5000. Sealed vials were placed at 50 °C
240 for 15 min and then 0.5 mL of head-space was injected in split injector (ratio 1:10). GC conditions
241 were: helium as carrier gas at 45 cm/s, oven temperature isothermal at 40°C. Mass detector
242 conditions were: electronic impact ionization mode (70eV), temperature of source 200°C with data
243 collected using selected ions (m/z 31; 32; 35) at 5 scans/s. The degree of methylation (DM) was
244 calculated as molar ratio of methanol to galacturonic acid.

245

246 **2.9 Statistical analysis**

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

253

254 **3 Results and discussion**

255 **3.1 Reduction of apple pomace water contents by air-drying**

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

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

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

268

269 **3.2 Dried pomaces composition**

270 **3.2.1 Polysaccharide composition**

271 Polysaccharides, arising from the cell walls of the fruits, constituted most of the pomace dry matter
272 (> 500 g/kg), compared with circa 150 g/kg of the fresh fruit dry matter. Similar polysaccharides or
273 dietary fibre contents in apple pomace have been reported previously (Perussello, et al., 2017):
274 recent examples include Yan & Kerr (2013) who reported 442 - 495 g/kg total dietary fibre (TDF) in
275 dried apple pomaces versus 124 mg/g in freeze-dried apple, while Kolodziejczyk et al. (2009)
276 reported an average TDF content of 524 g/kg for pomace from clear juice production. There were no
277 significant differences in polysaccharide yield and composition as a function of drying or oxidation.

278 Table 1 shows the yields and composition recorded for the two extreme water contents for non-
279 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 'Kermerrien') and flavonols. Small amounts of 4-*p*-
293 coumoylquinic 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 'Kermerrien' 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

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

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

347

348 **3.3 Impact of drying on polyphenol extractability**

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

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

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

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

368

369 **3.4 Composition of the extracts**

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

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

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

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

401

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

408

409 **4 Conclusion**

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

423

424 **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

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544 apple pomace powders produced by vacuum-belt drying. *Journal of the Science of Food and*
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546

547

548 **Captions to figures**

549

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

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

554

555 Fig 2: Proportions (% in weight of identified polyphenols) of the different polyphenols in the extracts
556 from pomaces of Kerrmerrien and Avrolle apples by pressurized liquid extraction using water (H₂O) or
557 48% ethanol (EtOH) at 70°C, after different levels of drying. Pomaces had been generated either in
558 the presence (OX) or absence (NOX) of oxygen.

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

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

562

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

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

566

567 Supplementary Figure 2: Drying curves of 'Kermerrien' apple pomaces in monolayer (< 0.5 cm) at
568 70°C in a ventilated oven. Pomaces were generated in the presence (solid circles) or in the absence
569 (empty circles) of oxygen. Each point is the average of three replicates, the lines indicate the
570 standard deviations.

571

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

577

578

Fig 1

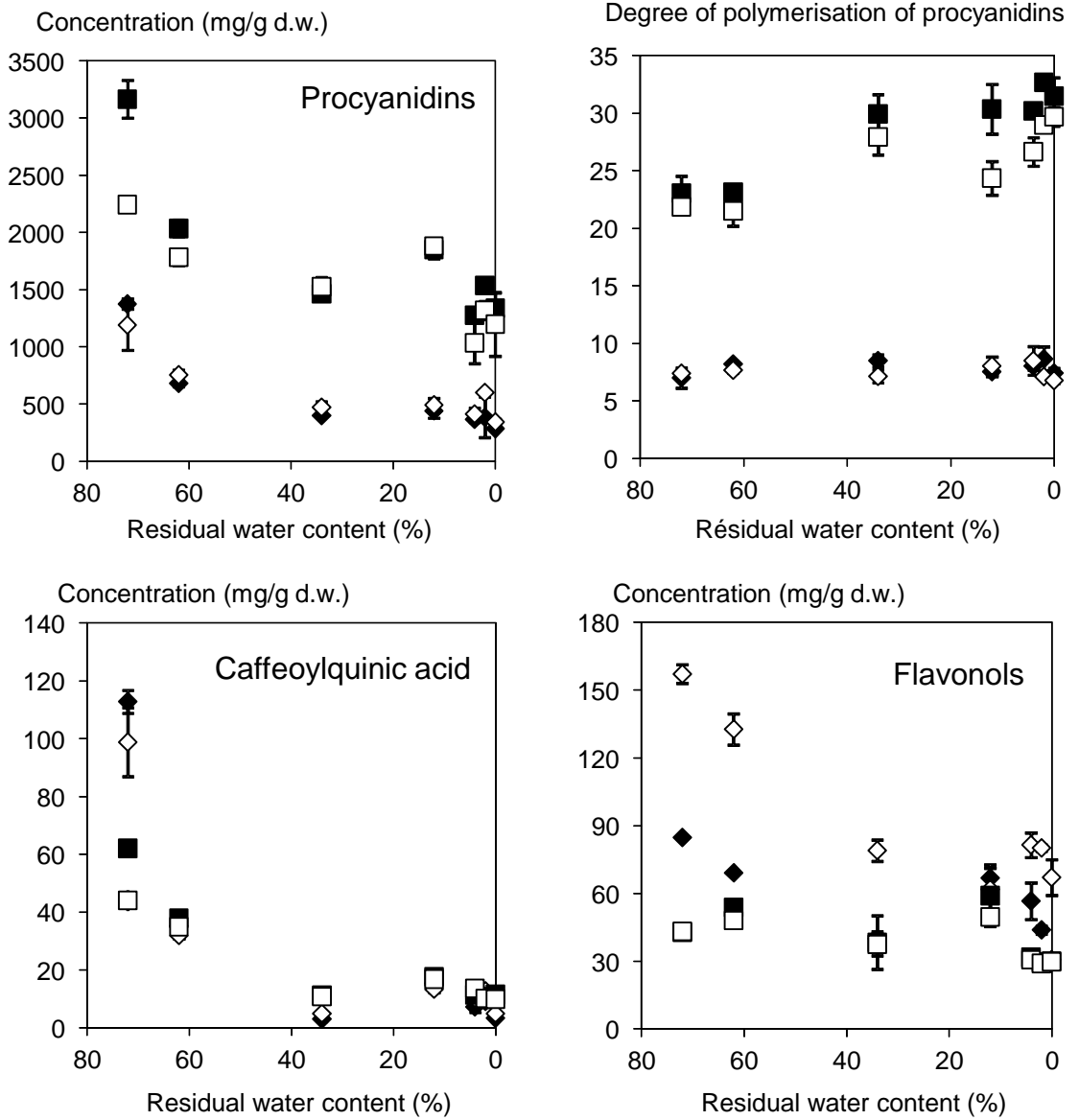


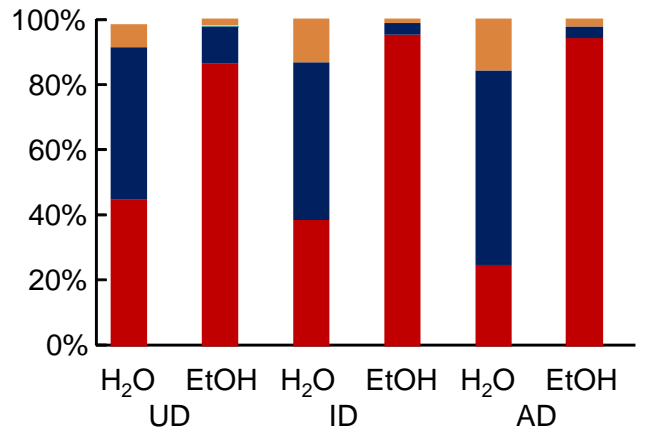
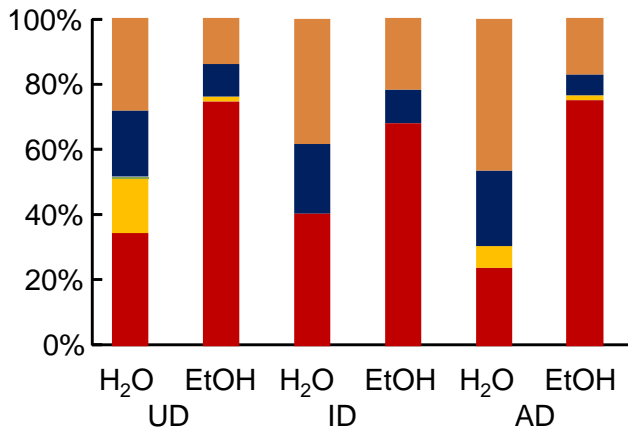
Fig 2

KERMERRIEN

AVROLLES

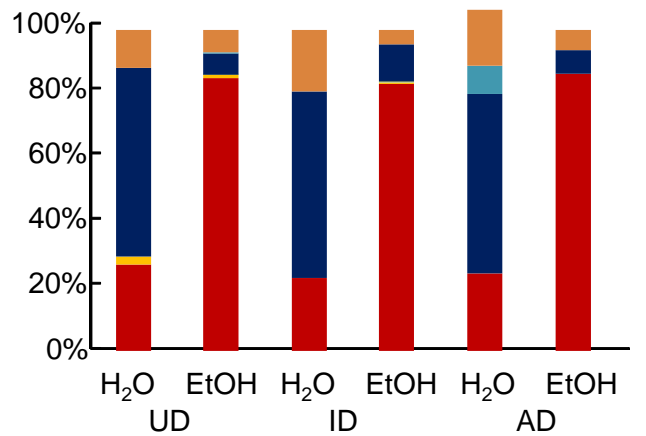
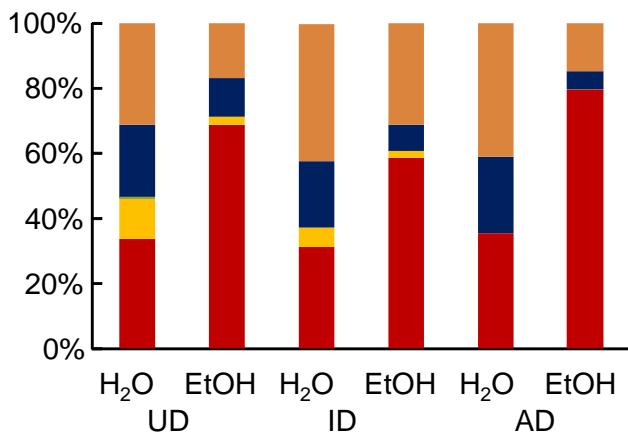
NOX

NOX



OX

OX



FLA XPL PLZ pCQ CQA PCA

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
<i>Pooled standard deviation</i>				<i>0.3</i>	<i>0.2</i>	<i>2.4</i>	<i>5.5</i>	<i>0.4</i>	<i>1.3</i>	<i>8.1</i>	<i>19</i>	<i>1.2</i>

Pooled standard deviation was calculated from all the replicates.

AUA: anhydrouronic acids

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

3

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
<i>Pooled standard deviation</i>			<i>0.6</i>	<i>4</i>	<i>148</i>	<i>0.9</i>	<i>5</i>	<i>0.8</i>	<i>0.5</i>	<i>14</i>	<i>3</i>

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

5 CAT: (+)-catechin; EPI: (-)-epicatechin; PCA: procyanidins; DP: number average degree of polymerisation of procyanidins; CQA: 5-O-caffeoylquinic acid; p-CQ:
 6 4-*p*-coumaroylquinic acid; XPL: phloretin xyloglucoside; PLZ: phloridzin.

7 Table 3: Yields of freeze-dried extract (mg/g initial apple pomace dry weight) and proportion (%) of total polyphenols (TP, Folin-Ciocalteu reactive
 8 substances) in the pomaces of 'Kermerrien' and 'Avrolles' apples, produced with (OX) or without (NOX) oxidation during pressing. 48 % EtOH: ethanol:water
 9 48/52 mL:mL. Data represent mean \pm SD of 18 samples.

10

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

11

