Influence of pulsed electric fields processing on the bioaccessible and non-bioaccessible fractions of apple phenolic compounds

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Abstract

Pulsed electric fields (PEF) are known to influence the chemical and microstructural factors governing apple phenolic compounds fate upon digestion. However, the effect of PEF on fruit phenolic compounds bioaccessibility has yet to be determined. This work assessed the effects of PEF treatment (0 and 24 h after 0.01, 1.8 and 7.3 kJ kg^{-1}) on the bioaccessible and non-bioaccessible fractions of apple phenolic compounds. Bioaccessible and non-bioaccessible 5-caffeoylquinic acid increased at 24 h after delivering 0.01 kJ kg^{-1} (61 and 35%, respectively). At 1.8 and 7.3 kJ kg^{-1}, the overall bioaccessible content decreased, although the percentage of compounds released (bioaccessibility) increased in some cases. Bioaccessibility of overall phenolic compounds increased from 14% (untreated) to 27% (24 h after 7.3 kJ kg^{-1}). Therefore, PEF processing could modulate the apple functional value, by either increasing phenolic contents in the bioaccessible and non-bioaccessible fractions or the phenolic bioaccessibility.

Keywords

Apple, Bioaccessible, Bioaccessibility, Non-bioaccessible, Pulsed Electric Fields, Phenolic compounds.

1. Introduction

Apple fruit (*Malus pumila* Mill.) is one of the most consumed fruits in the world, either raw or as processed food. In 2013, the world average production of apple and apple products was 28 g capita^{-1} day^{-1}, and it reached 50 g capita^{-1} day^{-1} in the US and EU (Food and Agriculture Organization of the United Nations, 2019). Its consumption has been linked to important health benefits, mainly attributed to their high content in phenolic compounds, most notably hydroxycinnamic acids and flavan-3-ols (Boyer & Liu, 2004). In particular, apple is a very important dietary source of 5-caffeoylquinic acid, epicatechin and procyanidins (Bars-Cortina, Macià, Iglesias, Romero, & Motilva, 2017), whose intake has been correlated with decreased risk of cardiovascular disease and cancer (Clifford, 2000; Schroeter et al., 2010).

Therefore, the apple functional value is strongly determined by its phenolic content. However, only a percentage of the apple phenolic content can be biologically active in the body, as it has to be absorbed through the gastrointestinal tract and reach the bloodstream, which is known as *bioavailability.* On the other hand, *bioaccessibility* is the term used to describe the percentage of food compounds released from the food matrix during digestion, which is a required step to their absorption and bioavailability (Rein et al., 2013). Hence, the phenolic content in the duodenal lumen after ingestion of apple can be divided into two fractions: *i) Bioaccessible content*, which is the amount of phenolic compounds readily available for small intestinal absorption; and *ii) Non-bioaccessible content*, which is the amount of phenolic compounds that will continue their journey to the colon. The bioaccessible and non-bioaccessible phenolic compounds fractions of food can be assessed by using an *in vitro* simulated digestion with dialysis of the digested food. Bioaccessible compounds are dialyzable, while the non-bioaccessible compounds will be retained within the non-dialyzed content (Minekus et al., 2014). In recent years, non-bioaccessible phenolic compounds have gained interest due to their two-sided interaction with the colon microbiota, leading to important health benefits. On one hand, they assist the good preservation of the colonic mucosa and a balanced bacterial population, which has direct implications on digestion regulation and host health (Mills et al., 2015). On the other hand, the metabolism of the colon microbiota transforms the non-bioaccessible phenolic compounds to absorbable forms, thus contributing to their bioavailability (Selma, Espín, & Tomás-Barberán, 2009).

Phenolic compounds are known to be poorly absorbed, which limits their biological efficacy (Rein et al., 2013). Chemical structure, concentration and matrix interactions are three basic pillars that govern bioaccessibility of phenolic compounds from fruits. It has been shown that food processing can interact with all these three factors, hence its use has been proposed to modulate phenolic compounds bioaccessibility (Ribas-Agustí, Martín-Belloso, Soliva-Fortuny, & Elez-Martínez, 2018a). In particular, low and moderate-intensity pulsed electric fields (PEF) have been shown to increase phenolic compounds contents in apple fruit (Soliva-Fortuny, Vendrell-Pacheco, Martín-Belloso, & Elez-Martínez, 2017; Wiktor et al., 2015). It has been stated that PEF at non-lethal conditions induce phenolic compounds accumulation in plant tissues in response to abiotic stress (Elez-Martínez, Odriozola-Serrano, Oms-Oliu, Soliva-Fortuny, & Martín-Belloso, 2017).

Furthermore, PEF has known effects on the apple matrix and its capacity to retain phenolic compounds (Jemai & Vorobiev, 2002; Lohani & Muthukumarappan, 2016). The effects on fruit tissue structure are derived from changes in the integrity and permeability of cell membranes, as described by the theory of electroporation (Martín-Belloso & Soliva-Fortuny, 2010). These changes may be reversible or irreversible, depending on the capacity of cells to rearrange the cell membranes (Gonzalez & Barrett, 2010). According to Angersbach, Heinz, & Knorr (2000), the field strength of electric pulses must be higher than 0.4-0.8 kV cm^{-1} for significant membrane breakdown of apple cells, although the critical value depends on membrane thickness and electrical conductivity, among other cell factors. It can be suggested that phenolic compounds would have a facilitated release from a PEF-treated apple tissue where the permeability of the cell membranes has been fostered. In this line, higher release of phenolic compounds bound in apple pomace matrix has been described (Lohani & Muthukumarappan, 2016). Also, increased bioaccessibility of phenolic compounds has been found in fruit juices following the application of PEF (Buniowska, M., Carbonell-Capella, J. M., Frigola, A., & Esteve, M. J., 2017; Rodríguez-Roque et al., 2015). A work of Jemai & Vorobiev (2002) indicated that PEF treatment had greater effect than thermal treatment on the structure and permeability of apple tissue. As occurring under thermal treatment, the PEF-induced modification of the fruit matrix entail changes in the fruit textural properties (Lebovka,

Praporscic, & Vorobiev, 2004). Thus, the assessment of textural properties is important when evaluating the effects of PEF on fruit.

Therefore, PEF arises as a very promising technology to influence phenolic compounds bioaccessibility from apple. In this line, the use of food processing technologies for enhancing fruit phenolic compounds bioaccessibility is very relevant to the food industry, which is in the need for providing food products with high functional value. Though it is known that PEF can affect the foremost factors controlling phenolic compounds fate upon digestion (*i.e.* chemical structure, concentration and matrix interactions) (Barba et al., 2017; Cilla, Bosch, Barberá, & Alegría, 2018; Ribas-Agustí, Martín-Belloso, Soliva-Fortuny, & Elez-Martínez, 2018a), to the best of our knowledge, no works have been performed to determine the effect of PEF on phenolic compounds bioaccessibility of a whole fruit.

This work aimed at filling the gap between PEF processing and the fate of apple phenolic compounds after digestion. To this end, phenolic compounds bioaccessibility and contents in the bioaccessible and non-bioaccessible fractions were evaluated upon PEF processing at three specific energies (0.01, 1.8 and 7.3 kJ kg^{-1}). The effects were assessed at 0 and 24 h after treatments, in order to evaluate post-treatment changes. The results will provide the food industry with relevant information enabling the use PEF technology for enhancing the nutritional quality of apple products.

2. Materials and Methods

2.1. Reagents

Ultrapure water was obtained with a Milli-Q system (Millipore Ibérica, Madrid, Spain). Sodium chloride, ammonium carbonate, magnesium chloride hexahydrate and methanol (HPLC grade) were obtained from Scharlab (Sentmenat, Spain). Potassium chloride was obtained from Panreac (Castellar del Vallès, Spain). Calcium chloride dihydrate was purchased from Merck (Darmstadt, Germany). Potassium dihydrogen phosphate and sodium hydrogen carbonate were obtained from VWR (Llinars del Vallès, Spain). 5-Caffeoylquinic acid (chlorogenic acid), coumaric acid, (+)-catechin, (–)-epicatechin, procyanidin B2, quercetin-3-*O*-rutinoside (rutin), phloretin-2'-β-D-glucoside (phloridzin), sodium sulfide, formic acid, meta-phosphoric acid,

119 porcine α-amylase, porcine pepsin, porcine bile extract and porcine pancreatin were purchased
120 from Sigma-Aldrich (Darmstadt, Germany).
121
122 *2.2. PEF processing of apples*
123 Locally produced apples, commercially mature cv. 'Golden Delicious', were obtained
124 shortly (one month) after season from a local shop (Lleida, Spain). Before purchasing, they
125 were stored in cold store (0-4 °C) except for a short period (<48 h) at retail at ambient
126 temperature (22 °C). After purchasing, they were stored at 6 °C until processing within one
127 week. Apple samples had uniform weight (203 ± 6 g) and ripeness, as determined by toughness
128 (10.69 ± 0.49 N s), soluble solids content (12.83 ± 0.14 °Brix), titratable acidity (0.35 ± 0.00 %
129 malic acid), pH (4.06 ± 0.06) and skin color (L* 73.96 ± 1.51, a* -14.51 ± 0.94, b* 45.24 ± 1.24).
130 Apples were processed in a PEF batch equipment (Physics International, San
131 Leandro, CA, USA), equipped with a 0.1 μF capacitor, a TG-70 gas control unit and a PT55
132 pulse generator (Pacific Atlantic Electronics Inc., El Cerrito, CA, USA) (Fig. 1a). The treatment
133 chamber, which was isolated by a methacrylate case, had two parallel stainless steel electrodes
134 (20 × 10 cm) separated by 10 cm, and contained tap water (20 °C, 370 μS cm^{-1}) as a
135 conductive medium (Fig. 1b). The device delivered monopolar pulses of 4 μs width with
136 exponentially decaying waveform. The specific energy input (Q, kJ kg^{-1}) was calculated using
137 the following equation:
138 $$Q = \frac{V^2\ C\ n}{2\ m}$$
139 where V is the voltage (V), C is the capacitance (F), n is the number of pulses and m is the
140 mass of sample (g).
141 Treatments were applied at three specific energy inputs with the aim of influencing
142 apple metabolism and/or microstructure, since the effects on either or both may lead to effects
143 on phenolic compounds bioaccessible contents and bioaccessibility. In this regard, it has been
144 suggested that 0.01 kJ kg^{-1} stimulated apple secondary metabolism, as shown by increased
145 total phenolic content (as determined by spectrophotometric method) at 24 h after application
146 (Soliva-Fortuny, Vendrell-Pacheco, Martín-Belloso, & Elez-Martínez, 2017). Therefore, apples
147 were treated at 0.01 kJ kg^{-1} (0.4 kV cm^{-1}, 5 pulses). Furthermore, apples were treated at higher

148 specific energy inputs in order to induce more important microstructural changes: 1.8 kJ kg^{-1}
149 (2.0 kV cm^{-1}, 35 pulses) and 7.3 kJ kg^{-1} (3.0 kV cm^{-1}, 65 pulses), as suggested by the literature
150 (Barba et al., 2015).

151 Sampling consisted of four representative cylinders each apple (2 cm diameter × 2 cm
152 length, containing peel), from opposite sides in order to overcome the possible heterogeneity of
153 the response within fruit (Fig. 1c). Each treatment, including control, was replicated twice using
154 batches of two apples per replica, and every replica was digested and analyzed in duplicate.
155 Apple cylinders from PEF-treated and untreated apples, just treated and after 24 h at 22 °C,
156 were used for the determination of flesh toughness and phenolic compounds contents, or they
157 were digested to assess bioaccessible and non-bioaccessible compounds (Fig. 2). The samples
158 for the determination of phenolic compounds contents in undigested apple were cut in small
159 pieces of approximately 5 mm^3, quickly frozen in liquid nitrogen and kept at −30 °C for one
160 month until extraction.

161

162 *2.3. Toughness*

163 Flesh toughness was determined by penetration into 2 × 2 cm apple flesh cylinders,
164 using a texture analyzer (TA-XT2, Stable Micro Systems, Godalming, UK) with a cylinder probe
165 of 4 mm diameter. Tests were performed at a constant rate of 5 mm s^{-1} to a depth of 10 mm
166 (Rojas-Graü et al., 2007). Toughness (N s) was determined as area under the force-time curve,
167 on eight samples (cylinders) obtained from four different apples each treatment.

168

169 *2.4. Simulated gastrointestinal digestion*

170 Phenolic compounds in the bioaccessible and non-bioaccessible fractions were
171 evaluated using an *in vitro* static digestion according to Minekus et al. (2014), who described a
172 complete and internationally-agreed protocol using electrolyte and enzymatic solutions to
173 simulate the oral, gastric and duodenal phases of human digestion. The oral phase was initiated
174 by blending 10 g of sample and 10 mL of simulated salivary fluid (Minekus et al., 2014) with α-
175 amylase (pH 7) for 2 min in a paddle blender (Masticator, IUL Instruments, Barcelona, Spain). A
176 gastric phase followed by putting the simulated oral bolus in a glass bottle with 20 mL of
177 simulated gastric fluid (Minekus et al., 2014) and pepsin (pH 3). After 2 h of incubation at 37 °C

with agitation, the duodenal phase was initiated by inserting a cellulose-membrane dialysis bag (molecular weight cut-off 12,000 Da, Sigma-Aldrich) containing simulated intestinal fluid (Minekus et al., 2014). At this stage, the dialysis bag is used to mimic the role of the intestinal epithelium and separate the compounds that have been released from the undigested product (bioaccessible fraction) (Minekus et al., 2014). After a transition period of 30 min to reach pH 7, a solution containing simulated intestinal fluid, bile extract and pancreatin was added to the chyme and the mixture was left to incubate for further 2 h. At the end of digestion, the dialysis bags were rinsed with water (10-20 mL) until clean, using rinsing bottle. Their content was weighed and stored at −40°C until analysis of the bioaccessible fraction within 5 months. The remaining substance, which contained undialyzed compounds, was centrifuged at 21612 ×*g* for 20 min at 4 °C to remove debris and was stored at −40°C until analysis of the non-bioaccessible fraction within 5 months. Gastric and intestinal phases were performed in the dark, in absence of oxygen (bottles were flushed with nitrogen gas), in an orbital incubator (Ovan, Badalona, Spain) at 37 °C and 120 rpm. Electrolyte concentrations and enzyme activities followed the indications provided by Minekus et al. (2014). Blank samples (bioaccessible and non-bioaccessible), consisting in water instead of apple, were made in identical conditions.

2.5 Phenolic contents

2.5.1. Extraction of phenolic compounds

The phenolic compounds contents in undigested apples were estimated from methanolic extracts (Ribas-Agustí, Cáceres, Gratacós-Cubarsí, Sárraga, & Castellari, 2012). Frozen apple samples were blended (5 g) and mixed with methanol (1:4) and centrifuged (21,612 ×*g*) for 20 min at 4 °C. The clear supernatant was kept and the residue was further homogenized with 5 g of methanol, treated with ultrasounds (50-60 kHz) for 5 min, centrifuged again and the resulting supernatant was mixed with the previous one and kept at -30 °C until analysis within four months.

The non-bioaccessible fraction of digested apple was a mixture of apple components dissolved in simulated digestion fluid and tissue debris. Preliminary tests showed that there were no significant differences ($p<0.05$) between the phenolic content of the methanolic extracts (including digestion fluid and tissue debris) and the direct analysis of the digestion fluid (data not

208 shown). Similarly, the phenolic content of the bioaccessible fraction showed no significant
209 difference if evaluated by either direct analysis or after methanolic extraction. Therefore, the
210 analysis of phenolic compounds from the bioaccessible and non-bioaccessible fractions did not
211 require extraction.
212
213 *2.5.2. HPLC-DAD-MS^2 analysis of individual phenolic compounds*
214 Phenolic compounds concentrations in undigested and digested samples were
215 analyzed according to Ribas-Agustí, Cáceres, Gratacós-Cubarsí, Sárraga, & Castellari (2012)
216 with some modifications. An UPLC-DAD-MS^2 system (Waters, Milford, MA, USA) was utilized
217 for identification purposes, using a reversed-phase HSS T3 column (2.1 × 150 mm, 1.8 µm
218 particle size, Waters). The volume of injection was 10 µL and the column was maintained at 35
219 °C. The mobile phase, at a flow rate of 0.3 mL min^{-1}, was composed of A (ultrapure water-
220 methanol-formic acid 97.9: 2.0: 0.1 v/v/v) and B (methanol-formic acid 99.9: 0.1 v/v). A linear
221 gradient of mobile phase was performed: 0–6 min 0–20% B, 6–15 min 20–40% B, 15–18 min
222 40% B (isocratic) and 18–19 min 40–90% B. Electrospray ionization tandem mass spectrometry
223 experiments were performed in a triple quadrupole system, operating in the negative mode.
224 Parent molecular ions were obtained in scan mode and daughters mode was used to acquire
225 fragmentation patterns, with collision energies at 15-25 V. Peaks retention times, DAD spectra
226 and mass/charge ratios from parent and daughter ions were contrasted for identification with
227 those obtained from pure standards or, when no available, for tentative identification with
228 literature data (Sánchez-Rabaneda et al., 2004).
229 An HPLC-DAD system (Waters) was used for quantification purposes. Peaks
230 identification from UPLC chromatograms were transferred in basis of their retention times,
231 relative intensities and DAD spectra. Separation was carried out in a reversed-phase SunFire
232 column (3 × 150 mm, 3.5 µm particle size, Waters) under gradient elution of a mobile phase
233 composed of A (ultrapure water-methanol-phosphoric acid 94.966: 5.00: 0.034 v/v/v) and B
234 (methanol-phosphoric acid 99.966: 0.034 v/v). The volume of injection was 40 µL and the
235 column chamber was set at 35 °C. The mobile phase, at a flow rate of 0.4 mL min^{-1}, varied
236 using the following gradient: 0–5 min 5–30% B, 5–25 min 30–40%, 25–45 min 40% (isocratic),
237 45–50 min 90% B. Phenolic compounds were detected at their maximum absorption

238 wavelength, and quantification was made by using external calibration curve of their pure
239 standard, or when no available, a standard of a chemically similar compound. Coumaric acid
240 was used for quantification of coumaroyl derivatives, 5-caffeoylquinic acid for caffeoyl
241 derivatives, quercetin-3-*O*-rutinoside for quercetin derivatives and phloretin-2'-β-D-glucoside for
242 phloretin derivatives. The limits of quantification were determined at the signal-to-noise ratio of
243 10.
244
245 *2.6. Bioaccessibility, bioaccessible and non-bioaccessible contents.*
246 Concentration of phenolic compounds in the bioaccessible and non-bioaccessible
247 fractions was assessed from the dialyzed and non-dialyzed fractions (respectively) of digested
248 samples. Results were expressed as amount of bioaccessible/non-bioaccessible compound per
249 amount of sample (fresh weight). Bioaccessibility, *i.e.*, the percentage of dietary phenolic
250 compounds that are bioaccessible, was calculated as the ratio of bioaccessible compounds to
251 the compounds from undigested samples (methanolic extracts).
252
253 *2.7. Statistical analysis*
254 Results showed no homogeneity in their variance according to the Levene's test, due
255 to the higher variance of results from PEF-treated apples compared to untreated apples.
256 Therefore, differences between means of untreated and PEF-treated apples, at 0 h or 24 h after
257 treatment, were assessed by Welch's *t*-test, which does not assume homogeneity of the
258 variances. Correlation between toughness and phenolic compounds bioaccessibility was
259 determined by Pearson correlation coefficient. Level of significance was set at $\alpha = 0.05$. (JMP,
260 SAS Institute, Cary, NC, USA).
261
262 **3. Results and discussion**
263
264 *3.1. Apple phenolic contents in the bioaccessible fraction as affected by PEF*
265 PEF had significant influence on the amount of phenolic compounds that was released
266 from the apple matrix and became bioaccessible during *in vitro* digestion. Different responses
267 were found depending on the compound and the treatment specific energy (Table 1).

Just after treatment, apple fruits subjected to 0.01 kJ kg^{-1} had 29% lower 5-caffeoylquinic bioaccessible content than untreated apple. This decrease could be related to temporary microstructural changes hampering their release from the matrix during digestion, as their contents were not affected by the 0.01 kJ kg^{-1} treatment in undigested apples (Ribas-Agustí, Martín-Belloso, Soliva-Fortuny, & Elez-Martínez, 2018b). In this sense, Cholet et al. (2014) described much greater thickness of grape skin immediately after non-lethal PEF treatment, as consequence of cell wall reorganization. Such changes in fruit tissue might be compatible with decreased release of phenolic compounds during digestion from their intracellular compartments. A possible correlation between thickness of apple cell walls and bioaccessibility of phenolic compounds needs to be further addressed in future studies.

However, 24 h after 0.01 kJ kg^{-1} treatment, apples had 61% higher bioaccessible 5-caffeoylquinic acid and 26% higher sum of bioaccessible phenolic compounds than untreated apple. The increase in these bioaccessible phenolic contents could be due to increased contents in the undigested apple. In this sense, it has been previously found that 0.01 kJ kg^{-1} treatment enhanced the contents of 5-caffeoylquinic acid and procyanidin B2 in undigested apple at 24 h after treatment (Ribas-Agustí, Martín-Belloso, Soliva-Fortuny, & Elez-Martínez, 2018b). These results are in line with previous works that suggested the use of PEF to stress plant material and stimulate the biosynthesis of phenolic compounds (Elez-Martínez, Odriozola-Serrano, Oms-Oliu, Soliva-Fortuny, & Martín-Belloso, 2017). It is unlikely that the higher amount of phenolic compounds in the bioaccessible fraction at 24 h after 0.01 kJ/kg treatment, compared to untreated apple, was due to higher extractability of compounds, due to the following reasons:

i) No increase in the bioaccessible phenolic contents were found at 0 h after 0.01 kJ kg^{-1} treatment (Table 1). Any modification of the extractability due to electroporation or membranes breakage would have been detected immediately after treatment.

ii) On the contrary, the 0.01 kJ kg^{-1} treatment leaded to increased bioaccessible 5-caffeoylquinic and sum of bioaccessible phenolic compounds at 24 h after treatment (Table 1). This is compatible with an activation of the stress metabolism and an accumulation of phenolic compounds within 24 h following the application of PEF.

297 *iii)* The lack of texture changes (toughness) at 0 and 24 h after 0.01 kJ kg^{-1} treatment
298 (Fig. 3) indicated very limited effect on the cell capacity to retain water (*i.e.*, no turgor loss), and
299 most probably, on the extractability of vacuolar hydrophilic compounds such as phenolic
300 compounds.
301 The present work gives support to the use of PEF technology for enhancing apple
302 functional value, given the important health benefits attributed to phenolic compounds once they
303 have been absorbed into the organism (Crozier, Jaganath, & Clifford, 2009).
304 A different behavior was found following 1.8 and 7.3 kJ kg^{-1} treatments, with
305 bioaccessible contents tending to decrease at higher energy density and time after treatment.
306 Different effects were found across the families of compounds, which indicates different
307 susceptibility according to the chemical structure. The highest decrease was found in the family
308 of hydroxycinnamic acids. The bioaccessible flavan-3-ol and dihydrochalcone contents were
309 also affected (Table 1). On the contrary, flavonols (quercetin derivatives) were not affected by
310 any of the PEF treatments, at 0 h or 24 h. The sum of bioaccessible phenolic compounds
311 decreased by 34% and 44% at 24 h after treatments at 1.8 and 7.3 kJ kg^{-1} (respectively),
312 compared to untreated apples. The overall decrease in bioaccessible compounds at 1.8 and 7.3
313 kJ kg^{-1} can be linked to decreased contents in undigested apple, which was probably
314 consequence of their degradation due to process-induced oxidative reactions. In this sense,
315 lower content in total phenolics has been reported after PEF treatment, due to the leakage of
316 cell contents facilitating the oxidative reactions mediated by polyphenol oxidase (PPO) (Wiktor
317 et al., 2015).
318
319 *3.2. Apple phenolic contents in the non-bioaccessible fraction as affected by PEF*
320 The non-bioaccessible fraction of the *in vitro* digestion represents the dietary phenolic
321 compounds that are accessible to the colon microbiota metabolism after their passage through
322 the small intestine. 4-Caffeoylquinic acid, epicatechin and phloretin xyloglucoside contents were
323 below the limit of quantification in control and PEF-treated samples (1.2, 1.0 and 1.0 mg kg^{-1} in
324 the non-bioaccessible fraction, respectively).
325 The effects of PEF treatments on non-bioaccessible contents are shown in Table 2.
326 Treatment at 0.01 kJ kg^{-1} induced a 19% decrease at 0 h and 35% increase after 24 h in the

non-bioaccessible content of 5-caffeoylquinic acid, with respect to untreated apple. It has been described that non-bioaccessible caffeoylquinic acid modulates the colon microbiota population and metabolism, which could be beneficial to host health (Mills et al., 2015). No significant effects on all other individual compounds were found at 0.01 kJ kg^{-1}, except for a 17% decrease in phloretin glucoside at 0 h after treatment.

PEF at 1.8 and 7.3 kJ kg^{-1} induced a decrease in the non-bioaccessible contents of 5-caffeoylquinic acid, *p*-coumaroylquinic acid and phloretin glucoside (Table 2). Flavonols, on the other hand, showed high stability against PEF, given that their bioaccessible and non-bioaccessible contents were not affected by any treatment. Overall non-bioaccessible phenolic compounds content was not significantly affected at 24 h after treatments (Table 2).

3.3. Bioaccessibility of apple phenolics as affected by PEF and its relationship with apple toughness

Bioaccessibility, *i.e.* the release of compounds from the apple matrix during digestion, was modified upon PEF treatments, showing two different behaviors depending on their specific energy. One scenario appeared in apples treated at 0.01 kJ kg^{-1}. Immediately after treatment (0 h), 0.01 kJ kg^{-1} induced a decrease in the bioaccessibilities of 5-caffeoylquinic acid (from 14% to 7%) and the sum of phenolic compounds (from 17% to 13%), compared to untreated apples. However, no significant effects on any compound were observed at 24 h after treatment (Fig. 4). Effects on bioaccessibility require changes in the food matrix structure or in the molecular interactions that have an influence on the capacity of a given compound to be extracted during digestion (Ribas-Agustí, Martín-Belloso, Soliva-Fortuny, & Elez-Martínez, 2018a). The decrease in the bioaccessibilities of 5-caffeoylquinic acid and the sum of phenolic compounds immediately after 0.01 kJ kg^{-1} treatment might be due to a temporary effect on the apple matrix, although it disappeared at 24 h after treatment. In fact, the absence of relevant effects on compounds bioaccessibility indicate limited effects on apple matrix at the specific energy input of 0.01 kJ kg^{-1} at 24 h after treatment.

The maintenance of the tissue toughness at 0 and 24 h after 0.01 kJ kg^{-1} treatment (Fig. 3) was also consistent with limited effects on apple matrix, as it denotes that the capacity of the apple tissue to retain intracellular water was not altered (Lebovka, Praporscic, & Vorobiev,

2004). As mentioned earlier, the increase in the sum of phenolic compounds in the apple bioaccessible fraction at 0.01 kJ kg^{-1} was due to an increase in phenolic compounds in undigested apple, as the bioaccessibility rate was not affected. In this sense, Toepfl, Heinz, & Knorr (2006) stated that PEF inducing reversible (non-lethal) pore formation in plant cells could be used to increase desirable fruit phenolic compounds, due to the induction of stress reactions, secondary metabolites biosynthesis, and the maintenance of cells viability. Therefore, it is very likely that 0.01 kJ kg^{-1} PEF leaded to non-lethal effects on apple tissue, as it induced phenolic compounds biosynthesis at 24 h and an apparent preservation of the apple tissue integrity, as shown by unaltered toughness and bioaccessibility.

A second scenario of effects was found in fruits subjected to 1.8 and 7.3 kJ kg^{-1} (Fig. 4). At 24 h after these treatments, apples showed an important increase in the sum of phenolic compounds bioaccessibility: from 14% (untreated) to 23% (1.8 kJ kg^{-1}) and 27% (7.3 kJ kg^{-1}). Main apple phenolic compounds, 5-caffeoylquinic acid and epicatechin, showed significant changes at 24 h after 1.8 kJ kg^{-1} treatment. In the case of epicatechin bioaccessibility, it was found a very substantial increase, from 12% in untreated apple to 49% in PEF-treated apple. This could be partially due to higher formation of epicatechin resulting from procyanidins degradation during digestion of treated apples (Kahle et al., 2011), in the likely event of higher exposition to the effects of gastric digestion after PEF-induced matrix changes. Apple is a main dietary source of 5-caffeoylquinic acid and epicatechin. Thus, the bioaccessibility enhancement of these phenolic compounds by PEF treatment acquires special relevance.

PEF treatment at 1.8 kJ kg^{-1} also induced significant increase in phloretin glycosides bioaccessibility and decrease in quercetin glycosides bioaccessibility, with respect to untreated apples (Fig. 4). 4-Caffeolquinic acid also showed decreased bioaccessibility at 0 h after 7.3 kJ kg^{-1}. Bouayed, Deußer, Hoffmann, & Bohn (2012) described the isomerization of 5-caffeoylquinic acid to 4-caffeoylquinic acid during *in vitro* digestion. The results of the present work suggest that bioaccessibility of 4-caffeoylquinic acid was dominated by 5-caffeoylquinic isomerization during digestion, instead of the release of native 4-caffeoylquinic acid from the apple matrix. This was shown by, on one hand, the higher bioaccessibility of 4-caffeoylquinic compared to its isomer 5-caffeoylquinic acid, and on the other hand, the decrease in bioaccessibility at 7.3 kJ kg^{-1}. The latter, could be explained by the fact that higher specific

energy leaded to higher degradation of 5-caffeoylquinic, which was at the expense of the bioaccessible 4-caffeoylquinic acid formed from 5-caffeoylquinic acid isomerization, even if matrix changes may prompt higher release of this compound.

Changes in apple tissue integrity at 1.8 and 7.3 kJ kg^{-1} were put in evidence by the toughness evaluation. Compared to untreated apple, the treatment at 1.8 kJ kg^{-1} resulted in a 72% decrease of the apple toughness, which further decreased by 79% at 24 h after treatment (Fig. 3). More severely, treatment at 7.3 kJ kg^{-1} caused a decrease of 83%, which was sustained at 24 h after application. As it has been documented in the literature, significant membrane breakdown in plant cells has been described under field strengths above 0.4-0.8 kV cm^{-1} (Angersbach, Heinz, & Knorr, 2000) and 1-2 kV cm^{-1} (Martín-Belloso & Soliva-Fortuny, 2010), which results in a loss of intracellular water, components, tissue turgor and firmness (Gonzalez & Barrett, 2010; Lebovka, Praporscic, & Vorobiev, 2004). Treatment specific energy input (kJ kg^{-1}) and toughness were robustly correlated (Pearson correlation coefficient=-0.79, n=32, $p<0.0001$), showing a clear interdependence between these two variables. Toughness was also negatively correlated with the bioaccessibility of phloretin xyloglucoside ($p=0.0001$), 5-caffeoylquinic acid ($p=0.0009$) and epicatechin ($p=0.0058$). These results suggest microstructural effects consistent with the above mentioned scenario, where the release of 5-caffeoylquinic acid, epicatechin and phloretin xyloglucoside from the apple matrix during *in vitro* digestion was facilitated. On the contrary, the release of *p*-coumaroylquinic acid, phloretin glucoside and quercetin derivatives during digestion appeared to be independent from the matrix integrity.

Very low bioaccessibilities were found for procyanidin B2, procyanidin trimer and hydroxyphloretin xyloglucoside in untreated and PEF-treated apples, as their bioaccessible contents were always below the limit of quantification (1.0 mg kg^{-1}).

Previous works have shown increased bioaccessibility of phenolic compounds in thermally-treated food (Ribas-Agustí, Martín-Belloso, Soliva-Fortuny, & Elez-Martínez, 2018a). However, to the best of our knowledge, this is the first time that effects of PEF on the bioaccessibility of phenolic compounds from a whole fruit are presented.

4. Conclusion

The results of the present work contribute to the understanding of PEF effects on fruit bioactive compounds, showing for the first time, increased contents in bioaccessible phenolic compounds from PEF-treated fruit. PEF processing leaded to important changes in apple bioaccessible and non-bioaccessible phenolic compounds, especially on the sum of bioaccessible compounds. Very different effects were found according to the treatment intensity, depicting two different scenarios: *i)* increase in the bioaccessible and non-bioaccessible contents but no effects on toughness and compounds bioaccessibility (0.01 kJ kg^{-1}); and *ii)* decrease in the bioaccessible and non-bioaccessible contents but effects on toughness and increased bioaccessibility (1.8 and 7.3 kJ kg^{-1}). Results clearly showed that the extent of the effects was dependent on the chemical class of phenolic compound. Furthermore, effects showed to be dynamic over 24 h, hence the importance of assessing PEF effects at a certain time after processing and on a representative array of chemical compounds. A dual use of PEF can be proposed for apples processing: on one hand, as a promoter of apple fruit functional properties by increasing the sum of bioaccessible phenolic compounds and non-bioaccessible 5-caffeoylquinic acid contents (0.01 kJ kg^{-1}). On the other hand, as a promoter of apple phenolic compounds bioaccessibility in food products where apple texture is not to be retained (1.8 and 7.3 kJ kg^{-1}). In the latter case, further studies comparing performances are encouraged.

Acknowledgement

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Figure captions

Figure 1

Scheme of the pulsed electric fields (PEF) experimental set-up and sampling. (a) Circuit diagram of the PEF device. (b) Treatment chamber. (c) Fruit sampling.

[1]High-voltage source.

[2]Capacitor (0.1 µF).

[3]Trigger (pulses generator).

[4]Treatment chamber.

[5]Stainless steel electrodes.

[6]Sample.

[7]Conductive medium (tap water).

[8]Sample cylinders (peel and flesh).

Figure 2

Scheme of the experimental design.

Figure 3

Effect of pulsed electric fields on apple toughness (relative to untreated apple, 100%) at 0 h (dashed bars) and 24 h (solid bars) after treatment. Error bars indicate standard deviation. Different letters indicate significant difference ($p<0.05$) among treatments and time after treatment.

Figure 4

Bioaccesibility of phenolic compounds in untreated apples and apples treated by pulsed electric fields. Dashed bars, 0 h after processing; solid bars, 24 h after processing. (a) Hydroxycinnamic acids. (b) Flavan-3-ols. (c) Dihydrochalcones. (d) Flavonols. (e) Sum of phenolic compounds. Error bars indicate standard deviation. [a] Asterisks indicate significant difference with respect to untreated apple. *P <0.05. **P <0.01. ***P <0.001.

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586

587 **Table 1**

588 Effect of pulsed electric fields (PEF) on phenolic contents (mean ± SD)[a] in the bioaccessible

589 fraction of apples digested at 0 h and 24 h after treatment.

	PEF treatment	Time after treatment 0 h		24 h	
Hydroxycinnamic acids					
5-Caffeoylquinic acid	Untreated	5.09 ± 0.51		4.06 ± 0.22	
(mg kg^{-1})	0.01 kJ kg^{-1}	3.60 ± 0.96	*	6.54 ± 0.57	*
y	1.8 kJ kg^{-1}	3.54 ± 0.65	*	1.22 ± 0.30	***
	7.3 kJ kg^{-1}	1.63 ± 0.52	***	0.91 ± 0.11	***
4-Caffeoylquinic acid	Untreated	1.95 ± 0.17		1.78 ± 0.11	
(mg kg^{-1})	0.01 kJ kg^{-1}	1.67 ± 0.32		1.72 ± 0.38	
	1.8 kJ kg^{-1}	1.36 ± 0.25	*	0.89 ± 0.11	***
	7.3 kJ kg^{-1}	1.06 ± 0.09	***	0.76 ± 0.07	***
***p*-Coumaroylquinic acid**	Untreated	0.56 ± 0.06		0.46 ± 0.03	
(mg kg^{-1})	0.01 kJ kg^{-1}	0.39 ±0.12		0.52 ± 0.21	
	1.8 kJ kg^{-1}	0.25 ±0.07	***	0.09 ± 0.02	***
	7.3 kJ kg^{-1}	0.16 ± 0.04	***	0.04 ± 0.01	***
Flavan-3-ols					
Epicatechin	Untreated	2.96 ± 0.25		2.73 ± 0.23	
(mg kg^{-1})	0.01 kJ kg^{-1}	2.10 ± 0.61		3.13 ± 0.48	
	1.8 kJ kg^{-1}	2.51 ± 0.45		2.12 ± 0.24	*
	7.3 kJ kg^{-1}	2.02 ± 0.43	*	1.74 ± 0.57	
Dihydrochalcones					
Phloretin glucoside	Untreated	1.24 ± 0.13		1.15 ± 0.07	
(mg kg^{-1})	0.01 kJ kg^{-1}	1.28 ± 0.32		1.30 ± 0.46	
	1.8 kJ kg^{-1}	1.00 ± 0.18		1.16 ± 0.18	
	7.3 kJ kg^{-1}	0.73 ± 0.18	**	0.66 ± 0.13	**
Phloretin xyloglucoside	Untreated	1.22 ± 0.11		1.15 ± 0.05	
(mg kg^{-1})	0.01 kJ kg^{-1}	1.19 ± 0.20		1.38 ± 0.38	
	1.8 kJ kg^{-1}	0.83 ± 0.09	**	0.76 ± 0.07	***
	7.3 kJ kg^{-1}	0.77 ± 0.09	***	0.62 ± 0.09	**
Flavonols					
Quercetin-3-*O*-rhamnoside	Untreated	0.95 ± 0.02		1.00 ± 0.06	
(mg kg^{-1})	0.01 kJ kg^{-1}	0.95 ± 0.13		1.15 ± 0.31	
	1.8 kJ kg^{-1}	1.00 ± 0.30		0.90 ± 0.09	
	7.3 kJ kg^{-1}	1.00 ± 0.17		0.88 ± 0.17	
Quercetin-3-*O*-xyloside	Untreated	0.72 ± 0.03		0.76 ± 0.03	
(mg kg^{-1})	0.01 kJ kg^{-1}	0.74 ± 0.06		0.87 ± 0.24	

	1.8 kJ kg^{-1}	0.65 ± 0.13		0.74 ± 0.06	
	7.3 kJ kg^{-1}	0.82 ± 0.12		0.75 ± 0.13	
Quercetin-3-*O*-galactoside	Untreated	0.71 ± 0.04		0.80 ± 0.07	
(mg kg^{-1})	0.01 kJ kg^{-1}	0.79 ± 0.13		1.05 ± 0.46	
	1.8 kJ kg^{-1}	0.65 ± 0.13		0.78 ± 0.05	
	7.3 kJ kg^{-1}	0.92 ± 0.24		0.85 ± 0.23	
Quercetin-3-*O*-arabinoside	Untreated	0.65 ± 0.01		0.67 ± 0.02	
(mg kg^{-1})	0.01 kJ kg^{-1}	0.67 ± 0.04		0.76 ± 0.12	
	1.8 kJ kg^{-1}	0.69 ± 0.07		0.65 ± 0.04	
	7.3 kJ kg^{-1}	0.70 ± 0.06		0.62 ± 0.07	
Quercetin-3-*O*-glucoside	Untreated	0.68 ± 0.00		0.69 ± 0.01	
(mg kg^{-1})	0.01 kJ kg^{-1}	0.70 ± 0.03		0.82± 0.14	
	1.8 kJ kg^{-1}	0.67 ± 0.11		0.67 ± 0.04	
	7.3 kJ kg^{-1}	0.71 ± 0.05		0.65 ± 0.08	
Sum of phenolic compounds	Untreated	16.74 ± 0.65		15.19 ± 0.62	
(mg kg^{-1})	0.01 kJ kg^{-1}	14.08 ± 2.06		19.23± 2.34	*****
	1.8 kJ kg^{-1}	13.17 ± 1.61	*****	9.99 ± 0.95	*******
	7.3 kJ kg^{-1}	10.52 ± 1.35	*******	8.48 ± 1.41	*******

[a] Asterisks indicate significant difference with respect to untreated apple. *P <0.05. **P <0.01. ***P <0.001.

593 **Table 2**

594 Effect of pulsed electric fields (PEF) on phenolic contents (mean ± SD)[a] in the non-
595 bioaccessible fraction of apples digested at 0 h and 24 h after treatment.

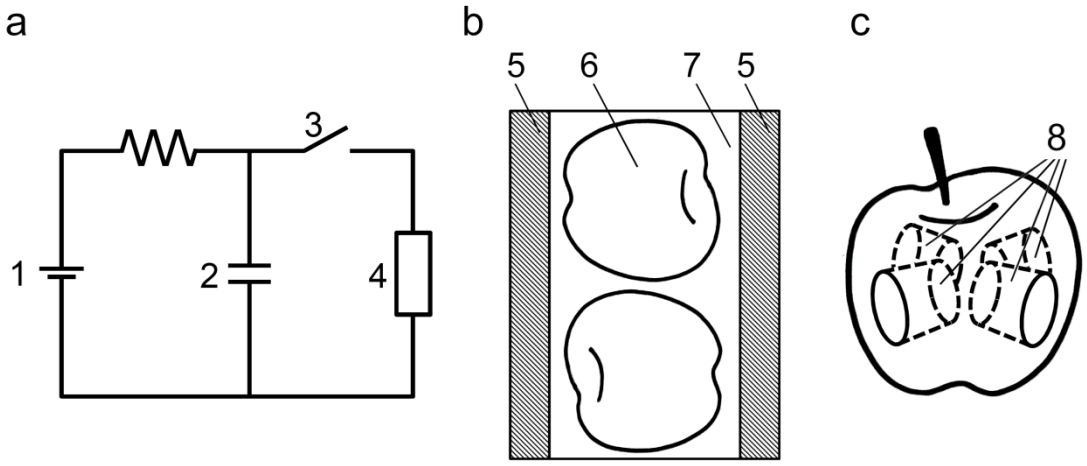
	PEF treatment	Time after treatment 0 h		24 h	
Hydroxycinnamic acids					
5-Caffeoylquinic acid	Untreated	2.70 ± 0.19		2.33 ± 0.23	
(mg kg^{-1})	0.01 kJ kg^{-1}	2.20 ± 0.31	*	3.15 ± 0.59	*
	1.8 kJ kg^{-1}	BLQ[b]		BLQ[b]	
	7.3 kJ kg^{-1}	BLQ[b]		BLQ[b]	
***p*-Coumaroylquinic acid**	Untreated	0.54 ± 0.21		0.48 ± 0.10	
(mg kg^{-1})	0.01 kJ kg^{-1}	0.32 ± 0.09		0.61 ± 0.27	
	1.8 kJ kg^{-1}	BLQ[c]		BLQ[c]	
	7.3 kJ kg^{-1}	BLQ[c]		BLQ[c]	
Dihydrochalcones					
Phloretin glucoside	Untreated	1.83 ± 0.09		1.77 ± 0.18	
(mg kg^{-1})	0.01 kJ kg^{-1}	1.48 ± 0.22	*	1.66 ± 0.28	
	1.8 kJ kg^{-1}	1.47 ± 0.05	***	2.20 ± 0.41	
	7.3 kJ kg^{-1}	1.42 ± 0.25		1.40 ± 0.16	*
Flavonols					
Quercetin-3-*O*-rhamnoside	Untreated	3.40 ± 0.40		3.94 ± 0.64	
(mg kg^{-1})	0.01 kJ kg^{-1}	3.56 ± 0.85		4.88 ± 2.00	
	1.8 kJ kg^{-1}	3.86 ± 1.42		3.60 ± 0.58	
	7.3 kJ kg^{-1}	3.35 ± 1.22		3.64 ± 1.19	
Quercetin-3-*O*-xyloside	Untreated	2.11 ± 0.02		2.51 ± 0.30	
(mg kg^{-1})	0.01 kJ kg^{-1}	2.14 ± 0.29		3.32 ± 1.18	
	1.8 kJ kg^{-1}	1.94 ± 0.11		2.64 ± 0.28	
	7.3 kJ kg^{-1}	2.36 ± 0.77		2.42 ± 0.67	
Quercetin-3-*O*-galactoside	Untreated	1.92 ± 0.07		2.65 ± 0.67	
(mg kg^{-1})	0.01 kJ kg^{-1}	2.03 ± 0.12		2.54 ± 0.52	
	1.8 kJ kg^{-1}	1.82 ± 0.24		2.82 ± 0.36	
	7.3 kJ kg^{-1}	2.00 ± 0.53		2.36 ± 0.63	
Quercetin-3-*O*-arabinoside	Untreated	1.78 ± 0.19		1.89 ± 0.14	
(mg kg^{-1})	0.01 kJ kg^{-1}	1.83 ± 0.25		2.37 ± 0.69	
	1.8 kJ kg^{-1}	1.64 ± 0.08		1.92 ± 0.14	
	7.3 kJ kg^{-1}	1.65 ± 0.20		1.79 ± 0.29	
Quercetin-3-*O*-glucoside	Untreated	1.70 ± 0.20		1.75 ± 0.12	
(mg kg^{-1})	0.01 kJ kg^{-1}	1.72 ± 0.14		2.12 ± 0.63	
	1.8 kJ kg^{-1}	1.78 ± 0.28		1.82 ± 0.12	

	7.3 kJ kg^{-1}	1.75 ± 0.31		1.90 ± 0.42
Sum of phenolic compounds (mg kg^{-1})	Untreated	15.98 ± 0.70		17.33 ± 1.89
	0.01 kJ kg^{-1}	15.29 ± 1.50		20.65 ± 4.59
	1.8 kJ kg^{-1}	12.52 ± 1.78	*	14.99 ± 1.58
	7.3 kJ kg^{-1}	12.52 ± 2.69		13.51 ± 2.62

596 [a] Asterisks indicate significant difference with respect to untreated apple. *P <0.05. **P <0.01.
597 ***P <0.001.[b]BLQ, below 1.2 mg kg^{-1} (limit of quantifaction). [c]BLQ, below 0.1 mg kg^{-1} (limit of
598 quantifaction).
599

600 **Figure 1**

601



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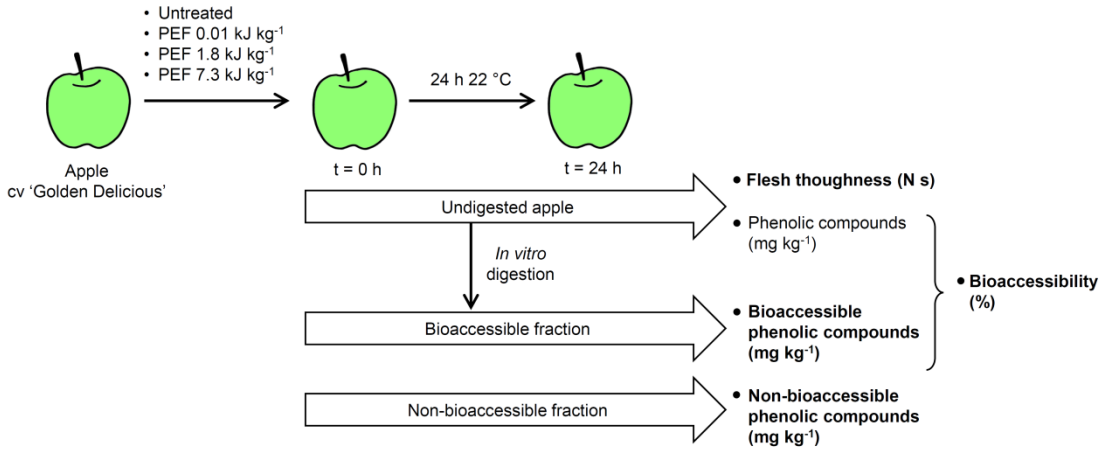
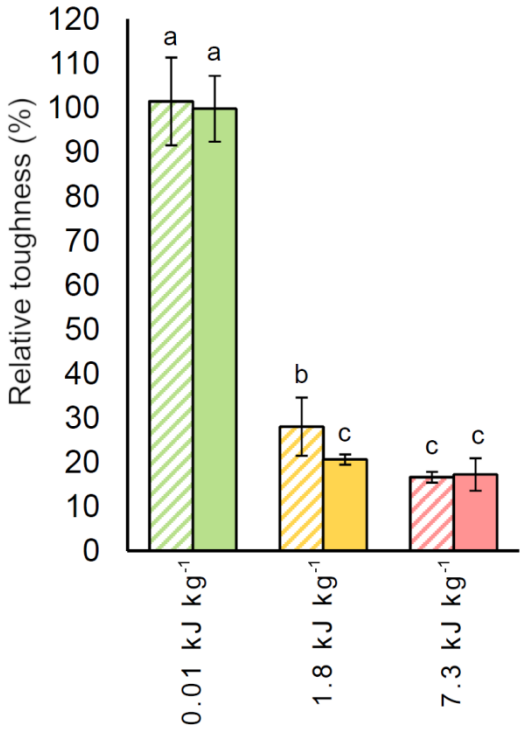
603

Figure 2


• Untreated
• PEF 0.01 kJ kg-1
• PEF 1.8 kJ kg-1
• PEF 7.3 kJ kg-1
24 h 22 °C
Apple
cv 'Golden Delicious'
t = 0 h
t = 24 h
Undigested apple
• Flesh thoughness (N s)
• Phenolic compounds (mg kg-1)
In vitro digestion
Bioaccessible fraction
• Bioaccessible phenolic compounds (mg kg-1)
• Bioaccessibility (%)
Non-bioaccessible fraction
• Non-bioaccessible phenolic compounds (mg kg-1)

608 **Figure 3**



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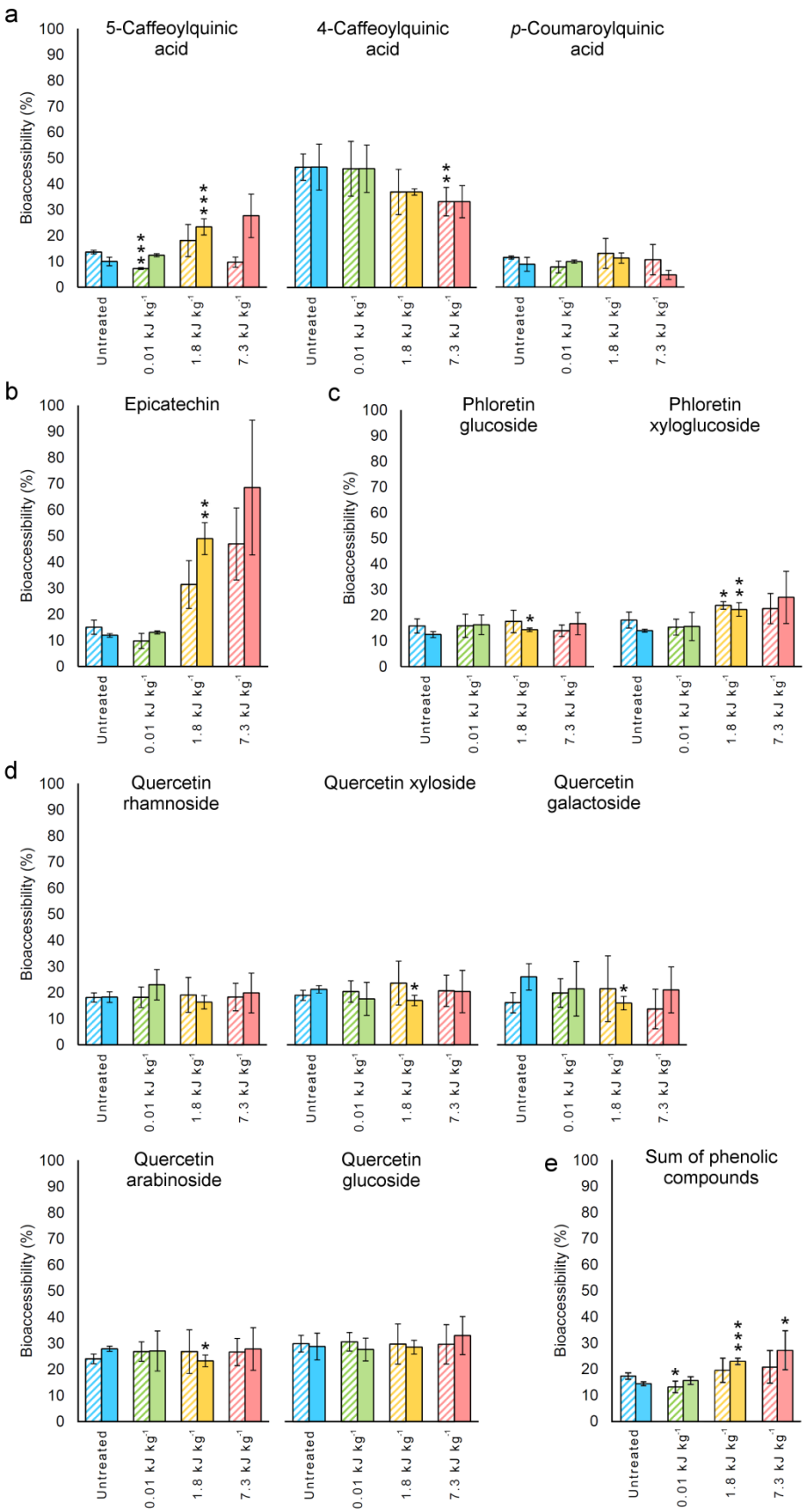
610

Figure 4


a
5-Caffeoylquinic acid
4-Caffeoylquinic acid
p-Coumaroylquinic acid
b
Epicatechin
c
Phloretin glucoside
Phloretin xyloglucoside
d
Quercetin rhamnoside
Quercetin xyloside
Quercetin galactoside
Quercetin arabinoside
Quercetin glucoside
e
Sum of phenolic compounds
Bioaccessibility (%)
Untreated
0.01 kJ kg-1
1.8 kJ kg-1
7.3 kJ kg-1