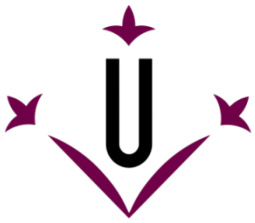

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Enhancing hydroxycinnamic acids and flavan-3-ol contents by pulsed electric fields without affecting quality attributes of apple

Albert Ribas-Agustí, Olga Martín-Belloso, Robert Soliva-Fortuny, Pedro Elez-Martínez*

Department of Food Technology, Agrotecnio Center, University of Lleida, Av. Alcalde Rovira Roure 191, Lleida E-25198, Spain.

E-mail addresses: Albert Ribas-Agustí, albert.ribas@tecal.udl.cat; Olga Martín-Belloso, omartin@tecal.udl.cat; Robert Soliva-Fortuny, rsoliva@tecal.udl.cat; Pedro Elez-Martínez*, pelez@tecal.udl.cat (corresponding author).

*Corresponding author

Tel.: +34 973 702 601; fax: +34 973 702 596.

Abstract

Pulsed electric fields (PEF) have arisen as a promising tool for enhancing plant-based food bioactive compounds, although side effects on quality attributes might compromise consumer acceptance. This work was aimed at filling the gap in the understanding of whole effects of PEF on apple phenolic compounds profile and quality parameters. Treatment specific energy was a critical factor affecting phenolic profile and quality attributes, which in turn varied from 0 to 24 h after treatment. Treatments at 1.8 and 7.3 kJ kg^{-1} (140 and 260 μs total treatment times, respectively) induced important quality changes, mainly discoloration and firmness loss, while overall phenolic contents decreased, except those of flavonols. However, 24 h after treatment at 0.01 kJ kg^{-1} (20 μs total treatment time), the main apple phenolic compounds as determined by HPLC-DAD-MS had enhanced contents (28% 5-caffeoylquinic acid; 35% procyanidin B2). Accordingly, total phenolics and total flavan-3-ols contents increased (26% and 35%, respectively), while physicochemical quality attributes were not affected. Therefore, 0.01 kJ kg^{-1} PEF treatment is proposed for obtaining apples with optimal quality and enhanced functional value.

Keywords

Apple, Pulsed Electric Fields, Phenolic compounds, 5-Caffeoylquinic acid, Procyanidin B2, Antioxidant capacity, Quality attributes, HPLC-DAD-MS.

1. Introduction

Food industry needs to address the growing demand for foods with high functional value, *i.e.*, containing high amounts of bioactive compounds with beneficial effects on human health. Fruits and vegetables are essential in a healthy diet, in large part due to their content in phenolic compounds. These phytochemicals possess antioxidant and other biological properties that have been related to important health benefits (Shahidi & Ambigaipalan, 2015). Apple (*Malus domestica* Borkh) is a highly consumed fruit that represents one of the main sources of dietary phenolic compounds (Tresserra-Rimbau et al., 2013; Wolfe et al., 2008); therefore, it is a strategic commodity in terms of influence on human health. Eating apples provides with significant amounts of flavan-3-ols (epicatechin and procyanidins) and hydroxycinnamic acids (mainly 5-caffeoylquinic acid), amongst others (Jakobek, García-Villalba, & Tomás-Barberán, 2013). In fact, epidemiological studies have associated flavan-3-ols and hydroxycinnamic acids intake with a decreased risk of cardiovascular diseases and cancer (Clifford, 2000; Schroeter et al., 2010).

Apple phenolic compounds may be affected by several factors, from genetic characteristics of the variety, to growing, climatic, ripening and post-harvest conditions (Carbone, Giannini, Picchi, Lo Scalzo, & Cecchini, 2011). During post-harvest management, food processing can be used in an innovative way to protect or enhance the apple phenolic content, in the pursuit of apple products with higher functional value (Ribas-Agustí, Martín-Belloso, Soliva-Fortuny, & Elez-Martínez, 2017). In this sense, pulsed electric fields (PEF) have gained interest, as they achieve microbial inactivation with less decline in bioactive compounds and quality attributes, with respect to conventional thermal treatment (Elez-Martínez, Odriozola-Serrano, Oms-Oliu, Soliva-Fortuny, & Martín-Belloso, 2017).

PEF processing consists in the application of electrical discharges in very short pulses (microseconds), which has reversible or irreversible consequences on cell integrity depending on treatment intensity (Martín-Belloso & Soliva-Fortuny, 2010). PEF have been used in liquid foods for extending shelf life and nutritional quality. In solid foods, it has been used as a pretreatment to improve fruit drying and for extraction of some phytochemicals (Donsì, Ferrari, & Pataro, 2010). Low intensity PEF (<1.5 kV cm^{-1}) have been proposed to induce abiotic stress in intact fruit, resulting in increased contents of phenolic compounds and other secondary metabolites (Toepfl, Heinz, & Knorr, 2005; Soliva-Fortuny et al.,2009). However, the deep study of this PEF application has started recently. In this sense, increased total phenolic content has been described in tomato treated at 1.2 kV cm^{-1} (Vallverdú-Queralt et al., 2012) and apple treated at 1.85 kV cm^{-1} (Wiktor et al., 2015). Soliva-Fortuny et al. (2017) explored the effects of different PEF intensities, as well as time and temperature after treatment, on the phenolic content of apple fruits. These authors reported 13%, 92% and 67% increases in the total phenolic, total flavan-3-ol contents and antioxidant capacity (respectively) in apples treated at 0.01 kJ kg^{-1} and stored for 24 h at 22 °C, although possible effects on individual phenolic compounds and apple quality attributes were not evaluated.

PEF-induced changes in phenolic compounds profile would add valuable information about which metabolic pathways have been stimulated (Treutter, 2001). In this sense, there is very limited knowledge about the effects of PEF on individual phenolic contents, even if it is known that chemical structure and matrix interactions may have an influence on their stability under treatment (Nayak, Liu, & Tang, 2015). Very few works have shown the effects of PEF treatment on the individual phenolic contents of a fruit. Vallverdú-Queralt et al. (2013) reported increased contents in caffeic

acid, caffeoylquinic acid, caffeoyl glucoside, ferulic acid, coumaric acid and flavanones in tomato after low and moderate PEF treatments, while feruloyl glucoside, coumaroyl glucoside and flavonols (quercetin derivatives) contents did not increase. Effects on tomato quality attributes were not determined by those authors, although colour and textural changes may appear after PEF treatment (Gonzalez & Barrett, 2010; Wiktor et al., 2015). More recently, Sotelo et al. (2018) have examined for the first time the effect of PEF on cherry physicochemical properties and phenolic compounds contents. These authors have found significant effects on the contents of selected phenolic compounds, as well as on titratable acidity and total soluble solids of cherries stored for 24 h after PEF treatment. These results have highlighted the need for an individual assessment of phenolic compounds to fully address the effects of PEF on fruit phenolic content.

Literature data describes two main effects of PEF on raw fruit. In one hand, there are quality changes, such as loss of turgor and enzymatic browning, due to the loss of intracellular water, electrolytes, enzymes and other compounds after cell membranes permeabilization (Jemai & Vorobiev, 2002; Lebovka, Praporscic, & Vorobiev, 2004). On the other hand, PEF induces plant response to abiotic stress, *e.g.*, enhancing the secondary metabolism with the accumulation of phenolic compounds in plant tissue (Elez-Martínez, Odriozola-Serrano, Oms-Oliu, Soliva-Fortuny, & Martín-Belloso, 2017). It is very likely that the underlying mechanisms of the occurrence of both effects are independent from each other. In that case, a given PEF intensity with effects on texture or color does not necessarily entail effects on the phenolic profile, and vice versa.

The use of PEF as an innovative approach to enhance apple functional quality needs reliable information regarding both effects on phenolic compounds and quality attributes in order to meet consumers' acceptance. The identification of a food process

enhancing apple nutritional quality without affecting its physicochemical properties would be of economic significance given the importance of apple in the global fruit market. To our knowledge, a work encompassing the effects of PEF on individual phenolic compounds and quality attributes of intact apple is still lacking. In this context, this work investigated the effects of PEF intensity at 0 and 24 h after treatment at three specific energies (0.01, 1.8 and 7.3 kJ kg^{-1}), on apple individual phenolic contents as well as antioxidant capacity and quality attributes, including flesh firmness, skin and flesh color, titratable acidity (TA), pH and soluble solids content (SSC).

2. Materials and Methods

2.1. Reagents

Ultrapure water was obtained with a Milli-Q system (Millipore Ibérica, Madrid, Spain). Methanol (HPLC grade), Folin-Ciocalteu reagent and sodium hydroxide were purchased from Scharlab (Sentmenat, Spain). Sodium carbonate and sulfuric acid were obtained from Poch (Gliwice, Poland). Sodium nitrite, aluminum chloride and vanillin were obtained from Thermo Fisher Scientific (Geel, Belgium). 5-Caffeoylquinic acid (chlorogenic acid), procyanidin B2, coumaric acid, catechin, epicatechin, quercetin-3-*O*-rutinoside (rutin), phloretin-2'-β-D-glucoside (phloridzin), 2,2-Diphenyl-1-picrylhydrazyl (DPPH), formic acid and meta-phosphoric acid were purchased from Sigma-Aldrich (Darmstadt, Germany).

2.2. PEF processing of apples

Commercially mature apples (cv. 'Golden Delicious') were purchased in a local store (Lleida, Spain) and kept at 6 °C until processing. Apples had uniform weight (203 ± 6 g), firmness (10.69 ± 0.49), soluble solids content (12.83 ± 0.14 °Brix), titratable

acidity (0.35 ± 0.00 % malic acid), pH (4.06 ± 0.06), flesh color (L* 82.32 ± 0.35, a* -3.66 ± 0.35, b* 23.10 ± 2.00) and skin color (L* 73.96 ± 1.51, a* -14.51 ± 0.94, b* 45.24 ± 1.24). Whole unpeeled apples were processed in a batch equipment (Physics International, San Leandro, CA, USA) equipped with a 0.1 μF capacitor which delivered pulses with exponentially decaying waveform, a TG-70 gas control unit and a PT55 pulse generator (Pacific Atlantic Electronics Inc., El Cerrito, CA, USA) The pulse generator had a charge voltage of +5 to +7 kV DC, a trigger pulse of +250 V and an output voltage of +50 kV. The system supplied 4 μs monopolar pulses at a fixed frequency of 0.1 Hz. The treatment chamber consisted of a parallelepiped methacrylate container with two parallel stainless steel electrodes (20 × 10 cm) separated by a gap of 10 cm. The conductive medium was tap water at 20 °C, which had a conductivity of 370 μS cm^{-1}. The characteristics of the electric field and the energy of the treatments, such as frequency, shape, polarity, width and difference of potential of the electric pulses, as well as the electric current generated across the electrodes, were monitored by using a digital oscilloscope (model THS720, Tektronix Inc., Beaverton, OR, USA). PEF treatments were selected in basis of the results obtained by Soliva-Fortuny, Vendrell-Pacheco, Martín-Belloso, & Elez-Martínez (2017), who found increased total phenolic contents in apple.

Thus, apples (two per batch) were exposed to the following PEF treatments: *i*) 0.4 kV cm^{-1}, 5 pulses (0.01 kJ kg^{-1}, 20 μs total treatment time); *ii*) 2.0 kV cm^{-1}, 35 pulses (1.8 kJ kg^{-1}, 140 μs total treatment time) and *iii*) 3.0 kV cm^{-1}, 65 pulses (7.3 kJ kg^{-1}, 260 μs total treatment time). Apples temperature did not rise significantly after the treatments. Untreated and PEF-treated apples were characterized in terms of phenolic contents and quality attributes just after processing (0 h) or after 24 h at 22 °C. For the analysis of phenolic compounds, a representative part of the apple samples with peel

was cut in small pieces (about 5 mm cubes), quickly plunged in liquid nitrogen and kept at -30 °C until extraction within one month. Each treatment, including control, was replicated twice using batches of 2 intact apples per replica, and every replica was analyzed in duplicate.

2.3. Phenolic contents

2.3.1. Extraction of phenolic compounds

Phenolic compounds were extracted as described by Ribas-Agustí et al. (2012) with some modifications. Five g of frozen apple pieces were blended, mixed with 15 g of methanol and centrifuged at 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 at 50-60 kHz 200 W (J.P. Selecta, Abrera, Spain) for 5 min, centrifuged at 21,612 ×*g* for 20 min at 4 °C and the resulting supernatant was mixed with the previous one and kept at -30 °C until analysis.

2.3.2. HPLC-DAD-MS determination of phenolic compounds

Phenolic compounds were analyzed by HPLC using the method described by Ribas-Agustí et al. (2012) with minor modifications. Identification of compounds was performed in an Acquity UPLC system equipped with diode array (DAD) and triple quadrupole mass spectrometer (MS) detectors using electrospray ionization interface (Waters, Milford, MA, USA). The chromatographic separation was performed in a reversed-phase HSS T3 column (2.1 × 150 mm, 1.8 μm particle size, Waters) using a mobile phase composed of A (ultra-pure water-methanol-formic acid 97.9: 2.0: 0.1 v/v/v) and B (methanol-formic acid 99.9: 0.1 v/v) in a linear gradient from 100% A to 80% A (6 min), 60% A (15-18 min) and 10% A (19-23 min). MS scan mode was used

to obtain parent molecular ions and MS^2 daughters mode was used to obtain their fragmentation patterns, with argon as collision gas and 15-25 V as collision energies. Peaks were identified in basis of their retention times, DAD, MS and MS^2 data, by comparison with data obtained from pure standards. When pure standards were not available, peaks were tentatively identified in basis of data obtained from literature (Sánchez-Rabaneda et al., 2004).

Quantitative analysis was performed in a HPLC-DAD system (Waters), by transferring the peaks identification from UPLC chromatograms, by comparing their retention times, relative intensities and DAD spectra. A reversed-phase SunFire column (3 × 150 mm, 3.5 µm particle size, Waters) was used for chromatographic separation, with gradient elution of a mobile phase composed of A (ultra-pure water-methanol-phosphoric acid 94.966: 5.00: 0.034 v/v/v) and B (methanol-phosphoric acid 99.966: 0.034 v/v). The mobile phase ranged from 95% A to 70% A (5 min), 60% A (25-45 min) and 10% A (50-55 min). Quantification was made by creating external calibration curves with pure standards. When a commercial standard was not available, quantification was made with calibration of a similar compound. Coumaric acid was used for quantification of coumaroyl derivatives, 5-caffeoylquinic acid for caffeoyl derivatives, procyanidin B2 for procyanidin dimer and trimer, quercetin-3-*O*-rutinoside for quercetin derivatives and phloretin-2'-β-D-glucoside for phloretin derivatives.

2.3.3. Spectrophotometric determinations

2.3.3.1. Total phenolic content

The total phenolic content was evaluated according to the method described by Singleton and Rossi (1965), with some modifications. An aliquot of the methanolic extract (0.4 mL) was mixed with 4 mL deionized water and 0.4 mL Folin Ciocalteu

reagent. After 5 min, 4 mL 5% (w/v) sodium carbonate and 1.2 mL deionized water were added and the mixture was kept in the dark for 90 min at room temperature. Afterwards, the absorbance was recorded at 750 nm (2021 spectrophotometer, Cecil Instruments, Cambridge, UK). Quantification was carried out by calibration with standard curve of 5-caffeoylquinic acid in 80% methanol at 0-40 mg 100 mL^{-1} range. The results were expressed as mg of 5-caffeoylquinic acid equivalents per 100 g (fresh weight) of apple.

2.3.3.2. Total flavonoid content

The total flavonoid content was determined as described by Zhishen et al. (1999), using a method adapted to microplate spectrophotometer. An aliquot of the methanolic extract (50 µL) was added to a microplate well pre-filled with 120 µL deionized water, then 10 µL 5% (w/v) $NaNO_2$ were added and the mixture was let stand for 5 min. After that, 10 µL 10% (w/v) aluminum chloride were added, the mixture was left 1 min and then 60 µL 1 N NaOH were added, followed by 50 µL distilled water. The absorbance was measured at 510 nm (Multiskan GO, ThermoFisher, Waltham, MA USA), and total flavonoids were quantified using a catechin calibration curve at 0-12 mg 100 mL^{-1}. The results were expressed as mg of catechin equivalents per 100 g (fresh weight) sample.

2.3.3.3. Total flavan-3-ol content

The total flavan-3-ol content was assessed using a method adapted from Sun et al. (1998). An aliquot of the methanolic extract (50 µL) was added to a microplate well with 125 µL 1% (w/v) vanillin. After 5 min, 125 µL 25% (v/v) sulfuric acid were added and left to stand for 25 min. The absorbance was measured at 500 nm (Multiskan GO,

ThermoFisher), and total flavan-3-ols were quantified using a catechin calibration curve at 0-10 mg 100 mL^{-1}.

2.4. Antioxidant capacity

The antioxidant activity of apple extracts was evaluated by their DPPH radical scavenging capacity using a method based on Rodriguez-Roque et al. (2013) and adapted to microplate spectrophotometer. An aliquot of methanolic extract (20 µL) was mixed with 280 µL of freshly prepared 0.025 g L^{-1} DPPH in methanol, and the absorbance at λ = 515 nm was read after 25 min in the dark at 25 °C (Multiskan Go, ThermoFisher). The rate of inhibition of DPPH radical at 25 min was calculated as regards the absorbance shown by a blank consisting in 80% methanol instead of sample extract (equation 1).

(1) $$\%DPPH\ inhibition = \left(\frac{Abs\ control - Abs\ sample}{Abs\ control}\right) \times 100$$

2.5. Quality attributes

Firmness of untreated and PEF-treated apples was measured using a texture analyzer (TA-XT2, Stable Micro Systems, Godalming, UK) using a cylinder probe of 4 mm diameter, which penetrated 10 mm the apple flesh at a constant rate of 5 mm s^{-1}. Firmness (N s) was determined as area under the curve between the graph of y (force) and x (time).

The soluble solids content (SSC) was estimated by refractometry (RX-1000, Atago, Tokyo, Japan). Titratable acidity (TA) was measured by titration to pH 8.1 endpoint, and expressed as equivalents of malic acid; pH was measured using a pH meter (2001, Crison L'Hospitalet de Llobregat, Spain).

The color of flesh and skin was determined by a chroma meter using the CIELAB color space (CR-400, Konica Minolta, Osaka, Japan) and data were expressed as lightness (L*), browning index (BI, equation 2) and hue (h°, equation 3) (McLellan, Lind, & Kime, 1995; Oms-Oliu, Aguilo-Aguayo, Martín-Belloso, & Soliva-Fortuny, 2010).

(2) $$BI = \frac{100(x-0.31)}{0.172}, \ where\ x = \frac{a^*+1.75L^*}{5.645L^*+a^*-3.012b^*}$$

(3) $$h° = arctan\left(\frac{b^*}{a^*}\right) \text{ , if a* > 0}$$

$$h° = arctan\left(\frac{b^*}{a^*}\right) + 180, \text{ if a* < 0}$$

2.6. Statistical analysis

One-way ANOVA was used to determine the effects of PEF treatments at 0 and 24 h after treatment, using the JMP v.12 software (SAS Institute, Cary, NC, USA). Differences between means were assessed by the Student's t test, with a level of significance $\alpha = 0.05$.

3. Results

3.1. Phenolic compounds profile

The apple phenolic profile was dominated by flavan-3-ols, hydroxycinnamic acids, dihydrochalcones and flavonols, as determined by HPLC-DAD-MS. PEF had different effects on phenolic compounds contents according to treatment specific energy and time after treatment (Table 1). Furthermore, the different classes of phenolic compounds showed different behaviour upon PEF. Hence, hydroxycinnamic acids and flavan-3-ol relative contents in apples treated at 0.01 kJ kg^{-1} were higher than in

untreated apples, while they diminished at 1.8 and 7.3 kJ kg^{-1} in favor of dihydrochalcones and flavonols, especially at 24 h after treatment.

The main hydroxycinnamic acids found in the apples were 5-caffeoylquinic acid (chlorogenic acid), *p*-coumaroylquinic acid and 4-caffeoylquinic acid (cryptochlorogenic acid). 5-Caffeoylquinic and *p*-coumaroylquinic acids had the highest sensitivity to PEF treatment. Their contents decreased at 1.8 and 7.3 kJ kg^{-1} and continued decreasing 24 h after treatment with respect to untreated apple, by 88-95% (*p*-coumaroylquinic acid) and 86-91% (5-caffeoylquinic acid) (Table 1). 4-Caffeoylquinic acid also showed high sensitivity, with 38-40% reduction in its content at 24 h after treatment at 1.8 or 7.3 kJ kg^{-1}. On the contrary, 0.01 kJ kg^{-1} had no detrimental effect on hydroxycinnamic acids; quite the opposite, 5-caffeoylquinic acid, a main apple phenolic compound, exhibited 28% higher content at 24 h after treatment.

Regarding flavan-3-ols, this work assessed the contents in epicatechin, procyanidin B2 and two co-eluting procyanidins (trimer+dimer). Similarly to hydroxycinnamic acids, their contents had a significant reduction after 1.8 and 7.3 kJ kg^{-1} treatments (Table 1). Namely, epicatechin content decreased by 82-89%, procyanidin B2 by 72-82% and dimer+trimer procyanidins by 64-76% at 24 h after these treatments. On the contrary, contents clearly increased just after treatment: 32% (epicatechin), 46% (procyanidin B2) and 38% (trimer+dimer) and 24 h after treatment: 32% (procyanidin B2) and 27% (trimer+dimer).

Phloretin glucoside, phloretin xyloglucoside and hydroxyphloretin xyloglucoside were identified as the main apple dihydrochalcones. They showed less sensitivity to 1.8 kJ kg^{-1} treatment than hydroxycinnamic acids and flavan-3-ols (Table 1). Only phloretin xyloglucoside, which was found to be the most PEF-unstable dihydrochalcone, declined significantly (44-58%). However, all dihydrochalcones contents decreased under 7.3 kJ

kg^{-1}, just after treatment (26% phloretin glucoside and 49% phloretin xyloglucoside) and after 24 h (71% phloretin xyloglucoside, 56% phloretin glucoside and 30% hydroxyphloretin xyloglucoside). On the contrary, treatment at 0.01 kJ kg^{-1} did not induce changes in the dihydrochalcones contents.

Five quercetin derivatives (galactoside, glucoside, arabinoside, xyloside and rhamnoside) were found as main apple flavonols. On the contrary to what was shown for the rest of phenolic classes, no significant differences existed between flavonol contents in untreated and PEF-treated apples (Table 1).

3.2. Total phenolic, total flavonoid, total flavan-3-ol contents and antioxidant capacity

As occurred with individual compounds, PEF had significant effects on total contents (as determined by spectrophotometric methods) and antioxidant capacity (DPPH radical inhibition) depending on the specific energies of the treatments and time after treatment. Apples treated at 0.01 kJ kg^{-1} showed 24% higher antioxidant capacity than untreated apples, although the increase was not significant 24 h after treatment (Table 2). At higher intensities, the antioxidant capacity decreased significantly just after treatment at 7.3 kJ kg^{-1} (39% decrease) and after 24 h at 1.8 kJ kg^{-1} (46%) and 7.3 kJ kg^{-1} (62%).

Similar effects of PEF were found on total phenolic and flavan-3-ol contents. The treatment at 0.01 kJ kg^{-1} induced a 25-26% increase in total phenolics and 43-35% in total flavan-3-ols just after and 24 h after treatment (respectively). On the other hand, total flavonoids were not affected by PEF treatment at 0.01 kJ kg^{-1} (Table 2). At higher treatment intensities, contents showed an important reduction: total phenolic content decreased by 32% (1.8 kJ kg^{-1}) and 43% (7.3 kJ kg^{-1}) just after treatment and 50% (1.8

kJ kg^{-1}) and 66% (7.3 kJ kg^{-1}) after 24 h. Total flavan-3-ols decreased by 19% (1.8 kJ kg^{-1}) and 51% (7.3 kJ kg^{-1}) just after treatment and 52% (1.8 kJ kg^{-1}) and 59% (7.3 kJ kg^{-1}) after 24 h. Total flavonoids decreased by 28% (1.8 kJ kg^{-1}) and 50% (7.3 kJ kg^{-1}) just after treatment and 60% (1.8 kJ kg^{-1}) and 68% (7.3 kJ kg^{-1}) 24 h after treatment. Effects of PEF on total phenolic, flavonoid and flavan-3-ol contents, as measured by the spectrophotometric methods, were in concordance with those showed by the sum of single compounds as measured by HPLC, particularly with those belonging to the main classes in apple: hydroxycinnamic acids and flavan-3-ols.

3.3. Quality attributes

PEF had significant effects on quality attributes depending on treatment specific energy and time after treatment. Apples' TA was only modified when treated at 0.01 kJ kg^{-1}, increasing by 23% just after treatment and 27% after 24 h. On the contrary, pH of apples treated at 0.01 kJ kg^{-1} did not change, while it increased about 0.2 units at 24 h after treatments at 1.8 and 7.3 kJ kg^{-1} (Table 3). The SSC only changed after 24 h following treatment at 0.01 kJ kg^{-1}, thus increasing by 14% with respect to untreated apples.

The effects on firmness and color also depended upon the specific energy of the treatments. The mildest PEF treatment (0.01 kJ kg^{-1}) did not affect firmness (Table 3) or color of skin or flesh (Table 4), except for a slight hue shift (+1.4 h°) in flesh just after treatment. However, stronger PEF intensities led to important firmness and color changes. The tissue was 72% and 83% softer just after treatment at 1.8 and 7.3 kJ kg^{-1} (respectively) and, after 24 h, it softened further to 79% of the initial firmness in the case of 1.8 kJ kg^{-1} (table 3). Regarding color changes, as a general rule, apples treated at 1.8 and 7.3 kJ kg^{-1} were browner (as shown by higher BI), darker (as shown by lower

L*) and more yellowish (as shown by smaller h°) than untreated apples or treated at 0.01 kJ kg^{-1} (Table 4). On apple skin, these two PEF treatments resulted in 6-9% higher BI (24 h after treatment), a decrease in L* of 12-13% (just after treatment) and 22-24% (after 24 h) and a hue shift of −4 ° (just after treatment) and −17 ° (after 24 h) at 1.8 kJ kg^{-1}, and −12 ° (just after treatment) and −19 ° (after 24 h) at 7.3 kJ kg^{-1}. The color change was more pronounced in the flesh than on the skin. In flesh, PEF treatments at 1.8 and 7.3 kJ kg^{-1} caused respectively 79% and 55% higher BI just after treatment and 117% and 105% after 24 h. The 1.8 kJ kg^{-1} treatment appeared to induce higher flesh browning than the 7.3 kJ kg^{-1} treatment. A significant effect of these two PEF treatments on flesh lightness was found, with 19% (1.8 kJ kg^{-1}) and 16% (7.3 kJ kg^{-1}) reductions in L* values just after treatment and 23% (1.8 kJ kg^{-1}) and 22% (7.3 kJ kg^{-1}) reductions 24 h after treatment. They also induced more yellowish flesh hue, with −19 ° (0-24 h) at 1.8 kJ kg^{-1} and −17 ° (just after treatment) and −19 ° (after 24 h) at 7.3 kJ kg^{-1} (Table 4).

4. Discussion

Important modifications of raw apple phenolic profile and quality attributes were induced by PEF, depending on the specific energy and time after treatment. Apples treated by PEF at 0.01 kJ kg^{-1} had higher contents in the main phenolic compounds 5-caffeoylquinic acid and oligomeric procyanidins, which was reflected in higher contents in total phenolic and flavan-3-ols contents. This effect was probably due to a response of the apple tissue to oxidative stress (Elez-Martínez, Odriozola-Serrano, Oms-Oliu, Soliva-Fortuny, & Martín-Belloso, 2017), which resulted in an accumulation of compounds at 0 h (procyanidins) and 24 h (5-caffeoylquinic acid) after treatment. Flavonols (quercetin derivatives) and dihydrochalcones (phloretin derivatives) contents

were not affected. The initial increase in phenolic content after treatment was accompanied by an increase in antioxidant capacity, as an evidence of the biological role that these compounds exert in apple tissue. It has been stated that plants accumulate phenolic compounds after abiotic stress as a mechanism for neutralization of reactive oxygen species (ROS) and other harmful oxidative molecules that have been generated in the fruit tissue (Gill & Tuteja, 2010). Otherwise, it could be argued that the increase in phenolic compounds contents and antioxidant capacity after treatment was due to matrix changes and an increased extractability of these compounds during their determination. However, the lack of firmness and color changes after treatment at 0.01 kJ kg^{-1} indicates that matrix modifications were irrelevant, thus their involvement in compounds extractability would have been very limited in any case. Also, the fact that the firmness decrease at 1.8 and 7.3 kJ kg^{-1} was not tied to increased phenolic compounds contents agrees with the hypothesis that secondary metabolism was enhanced upon PEF treatment.

To the best of our knowledge, this is the first time that apple individual phenolic compounds have been evaluated by HPLC-DAD-MS, together with quality changes, after PEF treatment. Changes in phenolic classes are in agreement with Soliva-Fortuny et al. (2017), who found increased contents in total phenolics, flavonols and flavan-3-ols (as determined by spectrophotometric methods) in apples stored for 24 h after treatment at 0.01 kJ kg^{-1}, although quality changes and individual contents remained unknown. Wiktor et al. (2015) reported increased total phenolic content and antioxidant capacity in apple tissue treated at 1.13 kJ kg^{-1}, while color was not affected. The finding of 5-caffeoylquinic acid and flavan-3-ols as stress-response compounds in PEF-treated apples is supported by the work of Mayr, Batzdorfer, Treutter, and Feucht (1993), which showed that apple fruit and leaves accumulated 5-caffeoylquinic acid and flavan-

3-ols as a response to surfactant treatment. Therefore, it can be signaled that apple tissue under mild PEF treatment reacts as if it was against other wounding agents, activating the same regulatory metabolism consisting in the activation of, at least, phenylalanine ammonia-lyase (PAL) and dihydroflavonolreductase (DFR), two enzymes that prompt the accumulation of 5-caffeoylquinic acid and flavan-3-ols (Treutter, 2001). Interestingly, the results are similar to those described for PEF-treated tomato (Vallverdú-Queralt et al., 2013), which accumulated 5-caffeoylquinic and other hydroxycinnamic acids rather than flavonols. Therefore, the apple stress-induced metabolism after mild PEF treatment showed close similarities to tomato, which makes reasonable to expect further similarities to other fruits and vegetables.

The contents in 5-caffeoylquinic acid and flavan-3-ols showed an important decrease at higher PEF specific energies (1.8 and 7.3 kJ kg^{-1}), due to degradation of these compounds even though there had been a stress-induced biosynthesis. Similarly, Sotelo et al. (2018) reported the loss of 4-hydroxybenzoic acid and isorhamnetin rutinoside in cherries after treatments above 30 kJ kg^{-1}, although the cyanidin glucoside content was enhanced. The important firmness decrease and colour changes in apples occurring at 1.8 and 7.3 kJ kg^{-1} suggest irreversible permeabilization of the cell membranes and permanent cell damage, as it has been described in literature for PEF treatments above 1.5 kV cm^{-1} (Elez-Martínez, Odriozola-Serrano, Oms-Oliu, Soliva-Fortuny, & Martín-Belloso, 2017). The loss in 5-caffeoylquinic acid and flavan-3-ols, together with other hydroxycinnamic acids and dihydrochalcones, contrasted with the higher stability of flavonols. This leads to suggest that they are actively involved in the redox reactions that take place in PEF-damaged apple tissue due to the generation of reactive oxygen species (Galindo, Wadsö, Vicente, & Dejmek, 2008). On the contrary, the absence of firmness loss and colour changes at 0.01 kJ kg^{-1} may indicate that the

damaged cell membranes could have resealed, which would have facilitated the preservation of higher phenolic contents after PEF treatment.

Regarding physicochemical quality attributes, apples treated at 0.01 kJ kg^{-1} had higher TA but similar pH than the untreated ones, showing that even if concentration in dissociated protons did not change, there was an accumulation of undissociated acids (Lobit, Soing, Génard, & Habib, 2002). This fact, together with increased SSC at 24 h after treatment, suggested that very low specific energy PEF treatment stimulated an accumulation of organic acids and sugars in apple (Bureau, Ścibisz, Le Bourvellec, & Renard, 2012), which was more important at 24 h after treatment. In this sense, it is known that some organic acids and sugars, amongst other molecules, are involved in abiotic stress response in plants (Shulaev, Cortes, Miller, & Mittler, 2008). On the contrary, apples treated at 1.8 and 7.3 kJ kg^{-1} had same TA, same SSC and slightly higher pH than untreated apples, suggesting no accumulation of acids or sugars. Firmness and color were also affected depending on the specific energy of the treatments. The 0.01 kJ kg^{-1} treatment had no relevant consequences. However, the most intense treatments produced an important decrease in firmness and substantial color changes. The skin and flesh were browner, darker and more yellowish, especially at 24 h after PEF treatment, and more intensely in the flesh than in the skin. Process-induced browning in apple is associated to enzymatic and non-enzymatic reactions (Nicolas, Richard-Forget, Goupy, Amiot, & Aubert, 1994). Enzymatic browning occurs by oxidation of phenolic compounds to quinones and derived pigments, which is initially catalyzed by polyphenol oxidases. Non-enzymatic browning, such as Maillard reaction, takes place when reducing sugars react with amino acids, which is common in processed foods at high temperature (Jaeger, Janositz, & Knorr, 2010). Given the PEF capacity of breaking cell membranes, it is more likely that the color changes showed by

the 1.8 and 7.3 kJ kg^{-1} treatments had been consequence of enzymatic browning, as a result of the release of phenolic compounds and polyphenol oxidases from their respective cell compartments. Some works have assessed inhibitory effects of PEF to polyphenol oxidases (Soliva-Fortuny, Balasa, Knorr, & Martín-Belloso, 2009), although the intensities used in this work probably did not reach an inhibitory threshold. Both firmness decrease and color changes obtained in apple tissues at 1.8 and 7.3 kJ kg^{-1} are consistent with matrix changes related to the rupture of cell walls and/or membranes (Gonzalez & Barrett, 2010; Lebovka, Praporscic, & Vorobiev, 2004). Indeed, these matrix changes did not take place after a treatment of 0.01 kJ kg^{-1}.

In this work, the specific energy of the PEF treatment appeared as a key factor influencing apple phenolic profile and quality attributes. These changes were derived from the ability of PEF to trigger metabolic changes and reversible or irreversible cell membranes permeabilization. A turning point can be deduced, in terms of PEF specific energy, in which higher content of phenolic compounds and little matrix changes turn to lower phenolic content and visible matrix changes, most probably due to the irreversibility of the damage induced in the tissue. In this work, this turning point was clearly between 0.01 and 1.8 kJ kg^{-1}.

5. Conclusions

PEF influenced both phenolic compounds contents and quality attributes of apple fruit, with more important changes at 24 h after treatment. Apple quality was clearly enhanced when applying a treatment of 0.01 kJ kg^{-1}, with apples having higher contents in 5-caffeoylquinic acid, procyanidins (as determined by HPLC-DAD-MS), SSC and TA, together with color and texture attributes of untreated apples. The increase in phenolic compounds contents was most probably related to a stress-induced

biosynthesis of hydroxycinnamic acids and flavan-3-ols, while dihydrochalcones and flavonols contents were not influenced. On the other hand, treatments at 1.8 and 7.3 kJ kg^{-1} resulted in softer and darker apples, due to irreversible tissue damage. These two treatment intensities had a detrimental effect on the phenolic compounds contents, except for flavonols, showing that this class is less prone to be affected by either PEF-induced metabolism or degradation. This work defined, for the first time, PEF operating conditions to be used for enhancing apple functional quality and assuring consumers acceptance. Namely, PEF at 0.01 kJ kg^{-1} provided apples with fresh-like appearance and higher contents in the health beneficial phenolic compounds 5-caffeoylquinic acid and procyanidins.

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Table 1

Effect of pulsed electric fields (PEF) and time after treatment (22 °C) on apple phenolic compounds as determined by HPLC-DAD-MS (mg kg^{-1} fresh weight, mean ± SD)[a].

	PEF treatment	Time after treatment	
		0 h	24 h
Hydroxycinnamic acids			
p-Coumaroylquinic acid	Untreated	4.92 ± 0.42 b	5.55 ± 1.07 ab
	0.01 kJ kg^{-1}	5.31 ± 0.70 ab	6.36 ± 1.08 a
	1.8 kJ kg^{-1}	2.43 ± 1.12 c	0.26 ± 0.14 d
	7.3 kJ kg^{-1}	2.00 ± 0.66 c	0.67 ± 0.24 d
5-Caffeoylquinic acid	Untreated	36.50 ± 3.64 b	39.27 ± 4.26 b
	0.01 kJ kg^{-1}	40.08 ± 6.16 b	50.41 ± 6.62 a
	1.8 kJ kg^{-1}	22.58 ± 8.92 c	5.39 ± 1.94 e
	7.3 kJ kg^{-1}	13.20 ± 3.38 d	3.49 ± 0.63 e
4-Caffeoylquinic acid	Untreated	3.92 ± 0.30 a	3.92 ± 0.49 a
	0.01 kJ kg^{-1}	3.57 ± 0.28 a	3.75 ± 0.34 a
	1.8 kJ kg^{-1}	3.72 ± 0.69 a	2.43 ± 0.29 b
	7.3 kJ kg^{-1}	3.42 ± 0.37 a	2.35 ± 0.29 b
Flavan-3-ols			

Procyanidin B2	Untreated	24.21 ± 6.72	b	25.68 ± 6.88	b
	0.01 kJ kg^{-1}	36.67 ± 2.93	a	34.59 ± 5.07	a
	1.8 kJ kg^{-1}	10.51 ± 2.51	c	5.33 ± 1.42	c
	7.3 kJ kg^{-1}	5.17 ± 2.13	c	2.80 ± 0.72	c
Procyanidin trimer + dimer	Untreated	25.46 ± 7.21	b	27.19 ± 7.32	b
	0.01 kJ kg^{-1}	36.04 ± 3.82	a	35.13 ± 5.75	a
	1.8 kJ kg^{-1}	21.31 ± 7.88	b	8.08 ± 2.89	c
	7.3 kJ kg^{-1}	10.77 ± 4.17	c	4.66 ± 1.54	c
Epicatechin	Untreated	18.95 ± 2.31	b	22.97 ± 3.36	a
	0.01 kJ kg^{-1}	24.96 ± 2.46	a	24.94 ± 3.42	a
	1.8 kJ kg^{-1}	12.12 ± 3.13	c	4.06 ± 0.56	d
	7.3 kJ kg^{-1}	4.81 ± 0.98	d	2.42 ± 0.19	d
Dihydrochalcones					
Hydroxyphloretin xyloglucoside	Untreated	2.28 ± 0.20	ab	2.56 ± 0.23	a
	0.01 kJ kg^{-1}	2.06 ± 0.12	bc	2.31 ± 0.16	ab
	1.8 kJ kg^{-1}	2.51 ± 0.40	ab	2.45 ± 0.10	a
	7.3 kJ kg^{-1}	2.09 ± 0.19	bc	1.80 ± 0.08	c

Phloretin xyloglucoside	Untreated	6.95 ± 0.71	c	8.31 ± 0.44	ab
	0.01 kJ kg^{-1}	7.90 ± 0.50	bc	9.20 ± 1.52	a
	1.8 kJ kg^{-1}	3.90 ± 0.49	d	3.46 ± 0.26	d
	7.3 kJ kg^{-1}	3.57 ± 0.58	d	2.39 ± 0.45	e
Phloretin glucoside	Untreated	7.88 ± 0.56	ab	9.22 ± 0.81	a
	0.01 kJ kg^{-1}	8.19 ± 1.22	ab	9.31 ± 1.28	a
	1.8 kJ kg^{-1}	7.06 ± 1.52	bc	8.08 ± 1.07	ab
	7.3 kJ kg^{-1}	5.86 ± 1.11	c	4.06 ± 0.97	d
Flavonols					
Quercetin-3-*O*-galactoside	Untreated	3.82 ± 0.15	a	3.16 ± 0.75	a
	0.01 kJ kg^{-1}	3.05 ± 0.51	a	3.93 ± 0.87	a
	1.8 kJ kg^{-1}	3.20 ± 1.24	a	4.74 ± 0.49	a
	7.3 kJ kg^{-1}	5.03 ± 2.24	a	4.28 ± 1.77	a
Quercetin-3-*O*-glucoside	Untreated	2.29 ± 0.23	ab	2.44 ± 0.50	ab
	0.01 kJ kg^{-1}	2.30 ± 0.21	ab	2.61 ± 0.42	a
	1.8 kJ kg^{-1}	2.29 ± 0.24	ab	2.32 ± 0.11	ab
	7.3 kJ kg^{-1}	2.48 ± 0.50	ab	1.99 ± 0.27	b

Quercetin-3-*O*-arabinoside	Untreated	2.69 ± 0.23	ab	2.39 ± 0.09	ab
	0.01 kJ kg^{-1}	2.50 ± 0.27	ab	2.86 ± 0.45	a
	1.8 kJ kg^{-1}	2.44 ± 0.36	ab	2.79 ± 0.10	ab
	7.3 kJ kg^{-1}	2.66 ± 0.38	ab	2.34 ± 0.50	b
Quercetin-3-*O*-xyloside	Untreated	3.83 ± 0.59	ab	3.58 ± 0.19	ab
	0.01 kJ kg^{-1}	3.69 ± 0.50	ab	3.81 ± 0.52	ab
	1.8 kJ kg^{-1}	3.24 ± 0.62	b	4.34 ± 0.27	a
	7.3 kJ kg^{-1}	3.83 ± 0.58	ab	3.38 ± 1.06	b
Quercetin-3-*O*-rhamnoside	Untreated	5.26 ± 0.62	ab	5.47 ± 0.28	ab
	0.01 kJ kg^{-1}	5.29 ± 0.61	ab	5.96 ± 0.91	a
	1.8 kJ kg^{-1}	5.61 ± 1.07	ab	5.54 ± 0.38	ab
	7.3 kJ kg^{-1}	5.62 ± 0.80	ab	4.73 ± 1.15	b

[a] Different letters represent significant difference ($p<0.05$) among treatments and time after treatment.

Table 2

Effect of pulsed electric fields (PEF) and time after treatment (22 °C) on apple total phenolic, flavonoid and flavan-3-ol contents (as determined by spectrophotometric methods), and antioxidant capacity (mean ± SD)[a].

	PEF treatment	Time after treatment	
		0 h	24 h
Total phenolic content (mg 5-Caffeoylquinic acid 100 g^{-1})	Untreated	60.81 ± 6.37 b	60.98 ± 7.40 b
	0.01 kJ kg^{-1}	75.94 ± 7.74 a	76.74 ± 4.53 a
	1.8 kJ kg^{-1}	41.47 ± 14.08 c	30.32 ± 4.17 de
	7.3 kJ kg^{-1}	34.88 ± 5.37 cd	20.53 ± 2.34 e
Total flavonoid content (mg Catechin 100 g^{-1})	Untreated	23.45 ± 5.37 a	26.51 ± 2.61 a
	0.01 kJ kg^{-1}	27.03 ± 2.81 a	25.71 ± 3.91 a
	1.8 kJ kg^{-1}	16.96 ± 7.72 b	10.65 ± 1.95 c
	7.3 kJ kg^{-1}	11.66 ± 3.15 bc	8.55 ± 1.98 c
Total flavan-3-ol content (mg Catechin 100 g^{-1})	Untreated	23.48 ± 4.50 b	23.69 ± 3.62 b
	0.01 kJ kg^{-1}	33.48 ± 3.18 a	32.08 ± 4.88 a

	1.8 kJ kg^{-1}	19.11 ± 4.54	b	11.26 ± 1.34	c
	7.3 kJ kg^{-1}	11.40 ± 1.41	c	9.66 ± 1.73	c
Antioxidant capacity					
(% DPPH inhibition)	Untreated	30.85 ± 2.64	bc	36.10 ± 5.23	ab
	0.01 kJ kg^{-1}	38.31 ± 2.94	a	41.05 ± 1.78	a
	1.8 kJ kg^{-1}	25.71 ± 6.19	c	19.36 ± 3.09	d
	7.3 kJ kg^{-1}	18.90 ± 2.23	d	13.82 ± 1.35	e

[a] Different letters represent significant difference ($p<0.05$) among treatments and time after treatment.

Table 3

Effect of pulsed electric fields (PEF) and time after treatment (22 °C) on apple quality attributes (mean ± SD)[a].

	PEF treatment	Time after treatment	
		0 h	24 h
Firmness (N s)	Untreated	10.69 ± 0.49 a	10.37 ± 0.19 a
	0.01 kJ kg^{-1}	10.85 ± 1.06 a	10.35 ± 0.77 a
	1.8 kJ kg^{-1}	3.00 ± 0.70 b	2.14 ± 0.12 c
	7.3 kJ kg^{-1}	1.78 ± 0.13 c	1.79 ± 0.38 c
Soluble solids content (°Brix)	Untreated	12.83 ± 0.14 bcd	12.42 ± 0.07 bcd
	0.01 kJ kg^{-1}	12.95 ± 0.09 bc	14.15 ± 1.27 a
	1.8 kJ kg^{-1}	13.28 ± 0.88 ab	12.48 ± 0.98 bcd
	7.3 kJ kg^{-1}	11.87 ± 0.68 d	12.23 ± 0.19 cd
Titratable acidity (% malic acid)	Untreated	0.35 ± 0.00 cd	0.36 ± 0.06 cd
	0.01 kJ kg^{-1}	0.43 ± 0.04 ab	0.45 ± 0.01 a
	1.8 kJ kg^{-1}	0.37 ± 0.05 bc	0.30 ± 0.09 d
	7.3 kJ kg^{-1}	0.41 ± 0.04 abc	0.38 ± 0.04 bc
pH	Untreated	4.06 ± 0.06 bc	4.07 ± 0.02 bc
	0.01 kJ	3.99 ± 0.01 bc	3.96 ± 0.02 c

kg^{-1}		
1.8 kJ kg^{-1}	4.09 ± 0.15 b	4.25 ± 0.08 a
7.3 kJ kg^{-1}	4.10 ± 0.05 b	4.24 ± 0.15 a

[a] Different letters represent significant difference ($p<0.05$) among treatments and time after treatment.

Table 4

Effect of pulsed electric fields (PEF) and time after treatment (22 °C) on apple color (mean ± SD) [a].

		Skin		Flesh
	PEF	Time after treatment		Time after tr
	treatment	0 h	24 h	0 h
Browning index	Untreated	70.24 ± 4.58 b	70.98 ± 0.48 b	28.29 ± 2.74 e
	0.01 kJ kg^{-1}	68.69 ± 2.45 b	71.67 ± 5.39 b	26.73 ± 3.33 e
	1.8 kJ kg^{-1}	64.81 ± 5.44 c	75.57 ± 1.56 a	50.76 ± 2.00 c
	7.3 kJ kg^{-1}	75.64 ± 0.96 a	77.28 ± 2.93 a	43.77 ± 6.59 d
L*	Untreated	73.96 ± 1.51 a	73.42 ± 1.39 a	82.32 ± 0.35 a
	0.01 kJ kg^{-1}	73.07 ± 0.80 a	73.06 ± 0.91 a	82.62 ± 0.25 a
	1.8 kJ kg^{-1}	65.33 ± 1.60 b	55.92 ± 1.71 c	66.87 ± 1.49 c
	7.3 kJ kg^{-1}	64.28 ± 2.48 b	57.47 ± 1.69 c	68.76 ± 1.30 b
Hue (°)	Untreated	107.77 ± 0.62 a	108.33 ± 0.39 a	99.00 ± 0.09 b
	0.01 kJ kg^{-1}	108.26 ± 1.48 a	107.29 ± 2.74 a	100.41 ± 0.95 a
	1.8 kJ kg^{-1}	103.51 ± 2.69 b	91.65 ± 1.81 d	79.95 ± 2.33 d
	7.3 kJ kg^{-1}	95.68 ± 0.76 c	89.63 ± 3.40 d	82.04 ± 1.01 c

[a] Different letters represent significant difference ($p<0.05$) among treatments and time after treatment.

0.01 kJ kg^{-1} PEF enhanced 5-caffeoylquinic acid and procyanidin contents after 24 h.

Flavonol contents were stable after 1.8 and 7.3 kJ kg^{-1} treatments.

Apple physicochemical quality attributes were not affected at 0.01 kJ kg^{-1}.

PEF at 1.8 and 7.3 kJ kg^{-1} induced quality changes and phenolic compounds loss.

PEF at 0.01 kJ kg^{-1} is proposed for obtaining apples with enhanced functional value.