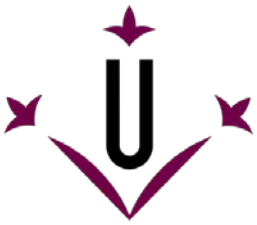

Enhancing the carotenoid content of tomato fruit with pulsed electric field treatments: effects on respiratory activity and quality attributes

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ABSTRACT

Pulsed electric field (PEF) may be used to elicit the accumulation of carotenoids in plant tissues. The stress-adaptive response to PEF is dependent on the treatments conditions and could lead to undesirable effects on the final quality of tomato fruit. This study was aimed at assessing the changes in the respiratory activity and the main quality attributes of tomato fruit when PEF treatments were used to elicit an increased concentration in their carotenoids content. Whole tomatoes (cv. Raf) were subjected to different electric field strengths (40, 120 and 200 kV m^{-1}) and number of pulses (5, 18 and 30 pulses). After being treated, the fruit were immediately stored at 4 ºC for 24 h. Total carotenoids and lycopene concentrations were enhanced by 50 % and 53 %, respectively, after applying 30 pulses at 200 kV m^{-1} (2.31 kJ kg^{-1}). Concurrently, a significant improvement in lipophilic antioxidant capacity was observed. At such treatment conditions, a deceleration in the R_{O_2} and R_{CO_2}, a drop in the ethylene production and the induction of acetaldehyde synthesis were observed, as an evidence of the stress injury caused to tomato tissues. In addition, several quality attributes of tomato were significantly affected. Tomatoes subjected to 200 kV m^{-1} exhibited the greatest values of total soluble solids and pH, as well as a marked reddening and softening of the fruit. Results suggest that selected PEF conditions could be proposed as a pre-processing treatment to produce tomato-based products with enhanced carotenoid contents.

KEYWORDS

Pulsed electric fields, antioxidant capacity, carotenoids, quality attributes, respiratory activity, tomato

ABBREVIATIONS

PEF, pulsed electric fields; TSS, total soluble solids; TCC, total carotenoids content; LC, lycopene content; LAC, lipophilic antioxidant capacity; ROS, reactive oxygen species; BHT, butyl hydroxytoluene; DPPH, 2,2-diphenil-1-picrylhydrazyl; $R_{O_2,}$ oxygen consumption; R_{CO_2}, carbon dioxide production; *L**, lightness; *a**, green-red chromaticity; *b**, blue-yellow chromaticity; TE, Trolox equivalents; ANOVA, analysis of variance; E, electric field strength.

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NOTES

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1. INTRODUCTION

Epidemiological studies have shown that the increased consumption of tomato and tomato-based products may reduce the risk of cardiovascular diseases, certain types of cancer and atherosclerosis (Hedges and Lister, 2005). The reduction of these chronic diseases has been attributed to the presence of high amounts of some valuable bioactive compounds, such as carotenoids, especially to lycopene, which is the most abundant carotenoid in red-ripe tomatoes (Dannehl et al., 2010). The accumulation of carotenoids in tomato normally occurs during ripening. However, carotenoid production has been recently reported to be promoted by enzymatically-mediated softening phenomena triggered by reactive oxygen species (ROS) generated upon exposure to oxidative stress (Fanciullino et al., 2014).

During the last decades, several research works have reported the feasibility of PEF treatments to stimulate the biosynthesis of defensive secondary metabolites in fruit, such as polyphenols and carotenoids (Balasa, Janositz and Knorr, 2011; Soliva-Fortuny et al., 2017; Vallverdú-Queralt et al., 2013b). It has been suggested that the electropermeabilization of cells induced by PEF may trigger the accumulation of ROS (Teissié et al., 1999; Ye et al., 2004). These ROS would induce the bioproduction of secondary metabolites as a way of plants to overcome unfavourable conditions (Sharma et al., 2012). In this regard, Vallverdú-Queralt et al. (2013a, 2013b) reported a significant improvement in carotenoids and phenolic compounds in whole tomatoes after the application of PEF treatments which was attributed to the activation of some metabolic pathways and to the permeabilization of cellular membranes. Besides producing several changes in metabolism of metabolically-active plants, PEF treatments could induce the modification of respiration rate in plants. Some authors have reported

72 that the respiratory activity of plants was increased by the application of abiotic stress,
73 such as wounding, water deficiency and salinity (Fraire-Velazquez and Emmanuel,
74 2013; Galindo et al., 2007; Jacobo-Velázquez et al., 2011; Łukaszuk, E. & Ciereszko,
75 2012). However, literature data concerning the PEF-induced changes in respiration rate
76 in whole fruit and vegetables are not available.

77 In concomitance with the acceleration of tomato metabolism after the application of
78 PEF, several changes in quality attributes may be affected. It is known that PEF can
79 strongly affect the tissue firmness of fruit and vegetables, such as carrots, potatoes and
80 apples, because of its action at the cell membrane level (Lebovka et al., 2004; Shayanfar
81 et al., 2013). Moreover, plant secondary metabolites are known to contribute to colour,
82 flavour and taste of the foods (Balasa and Knorr, 2011). All these parameters determine
83 the final quality of tomato fruit, and hence, their end use or even their acceptance by
84 consumers. However, to the best of our knowledge, there are no previous studies aimed
85 at evaluating the effect of the application of PEF treatments on quality attributes of
86 whole fruit and vegetables.

87 Therefore, the objective of this study was to evaluate the respiratory activity and quality
88 properties of tomato fruit as affected by PEF treatment conditions applied to elicit an
89 enhancement in their carotenoids content.

90 ## 2. MATERIALS AND METHODS

91 ### 2.1. REAGENTS

92 Butyl hydroxytoluene (BHT) was acquired from Scharlau Chemie S.A. (Barcelona,
93 Spain). DPPH (2,2-diphenyl-1-picrylhydrazyl) and Trolox (6-hydroxy-2,5,7,8-

tetramethylchroman-2-carboxylic acid) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. TOMATOES

Tomato fruit *(Lycopersicum esculentum* cv. Raf*)* grown in Almería (Spain) were purchased at turning stage, characterized by more than 10 % but not more than 30 % of the surface showing a definite change in color from green to red (USDA, 1991). The fruit were stored at 12 ± 1 °C until they reached a light red-ripe stage, hence exhibiting red color in more than 60 % but not more than 90 % of the surface (USDA, 1991). Prior to PEF processing, tomatoes were rinsed with tap water. The excess of water was carefully removed from the surface with a paper cloth.

2.3. PULSED ELECTRIC FIELD TREATMENTS

PEF treatments were conducted in a batch mode PEF system (Physics International, San Leandro, CA, USA). The equipment delivers monopolar exponential-wave pulses from a capacitor of 0.1 μF at a frequency of 0.1 Hz. The treatment chamber consists of a parallelepiped methacrylate container (0.2 x 0.08 m) with two parallel stainless steel electrodes separated by a gap of 10 cm. Tomatoes were placed into the treatment chamber filled with tap water (conductivity of 0.03 S m^{-1}). Different electric field strengths (40, 120 and 200 kV m^{-1}) and number of pulses (5, 18 and 30 pulses) were applied. The specific energy input corresponding to each treatment was calculated according to Luengo, Condón-Abanto, Álvarez, & Raso (2014b) and is displayed in Table 1. Untreated and PEF-treated tomatoes were immediately stored at 4ºC for 24 h, as previously described by Vallverdú-Queralt et al. (2013). Respiratory activity and physicochemical properties of tomatoes were then measured. Afterwards, tomatoes

were ground for 20 seconds in a blender (Solac Professional Mixter BV5722, Spain), immediately freeze-dried and stored at −40 °C prior to carotenoids analysis.

2.4. EXTRACTION AND ANALYSIS OF CAROTENOID COMPOUNDS

2.4.1. Extraction

Carotenoids were extracted following the methodology proposed by Odriozola-Serrano et al., (2007) with slight modifications. Freeze-dried tomato samples (0.2 g) were weighed and mixed with 20 mL of 1 % (w/v) of butylated hydroxytoluene (BHT) in ethanol:hexane (4:3 v/v). The mixture was homogenized at 6 x*g* for 15 min at 4 ºC in a Beckman Coulter centrifuge (Avanti J-26 XP, California, United States). Then, 3 mL of distilled water were added and the mixture was shaken and kept at room temperature to allow phase separation. The organic phase was collected and used to determine total carotenoids and lycopene contents as well as lipophilic antioxidant capacity. All the extractions were repeated twice. All procedures were performed in dim lighting in order to prevent carotenoids photodegradation.

2.4.2. Determination of total carotenoids

Total carotenoids content (TCC) was determined spectrophotometrically (CECIL CE 2021; Cecil Instruments Ltd., Cambridge, UK) following the methodology proposed by Talcott & Howard (1999). The absorbance of the organic phase was measured in triplicate at 470 nm versus a blank of hexane. TCC was calculated using the following equation (1):

$$Total\ carotenoids\ content\ (mg\ kg^{-1}) = \frac{A_{470}\ x\ V\ x\ 10^4}{A^{1\%}_{1cm}\ x\ G} \quad (1)$$

where A_{470} is the absorbance at 470 nm, V is the total volume of extract (mL), $A_{1cm}^{1\%}$ is the extinction coefficient of a mixture of carotenoids established in 2500 by Gross (1991) and G is the sample weight (g). Total carotenoids were expressed as mg kg^{-1}.

2.4.3. Determination of lycopene

Lycopene content (LC) was determined spectrophotometrically following the methodology proposed by Fish, Perkins-Veazie, & Collins (2002). The absorbance of the extracts was measured at 503 nm using hexane as a blank. LC was calculated according to equation 2.

$$\text{Lycopene content } (mg\ kg^{-1}) = \frac{A_{503}\ x\ MW\ x\ DF\ x\ 10^6}{\varepsilon\ x\ L} \quad (2)$$

where A_{503} is the absorbance at 503 nm, MW is the molecular weight of lycopene (536.9 g mol^{-1}), DF is the dilution factor, ε is the molar extinction coefficient for lycopene (17.2 x 10^4 L mol^{-1} cm^{-1}) and L is the pathlength (1 cm). Lycopene content was expressed as mg kg^{-1}.

2.4.4. Lipophilic antioxidant capacity

LAC was evaluated on the same extract used for TCC and LC determination using the colorimetric method reported by Vallverdú-Queralt et al. (2012) which is based on the free radical scavenging effect of 2,2-diphenyl-1-picrylhydrazyl (DPPH). Ten microliters of tomato extract were mixed with 90 µL of distilled water and 3.9 mL of $DPPH^{\cdot}$ solution. The mixture was shaken vigorously in a vortex and kept in the dark for 30 min. The absorbance was measured at 515 nm. Results were expressed as Trolox equivalents (µmol kg^{-1}).

2.5. RESPIRATORY ACTIVITY

The respiratory activity of both untreated and PEF-treated tomatoes was determined using a static system. Just after PEF treatments, three tomatoes from each treatment (ca. 130 g) were individually placed in hermetic containers (0.5 L of capacity) for 24 h at 4 ºC. Changes in the composition of the headspace were measured twice using a gas analyser (490 Micro GC, Agilent Technologies, Santa Clara, USA). A 1.7 mL sample was withdrawn from the headspace atmosphere through an adhesive rubber septum with a syringe. Portions of 0.25 and 0.33 mL were injected for O_2 and CO_2 determination, respectively. The O_2 content was analysed with a CP-Molsieve 5Å column (10 m x 0.32 mm, df = 30 µm) at 60 ºC and 100 kPa. For quantification of CO_2, ethylene (C_2H_4) and acetaldehyde (C_2H_4O), a Pora-PLOT Q column (10 m x 0.32 mm, df = 10 µm) at 70 ºC and 200 kPa, was used. Both columns were equipped with a thermal conductivity detector. Respiration as oxygen consumption (R_{O_2}) and carbon dioxide production (R_{CO_2}) was expressed as mg kg^{-1} h^{-1} according to Fonseca, Oliveira, & Brecht (2002). In addition, the production of ethylene (µg h^{-1} kg^{-1}) and acetaldehyde (ng h^{-1} kg^{-1}) was determined.

2.6. PHYSICOCHEMICAL PROPERTIES

Colour. The CIELab parameters (lightness, L^*; green-red chromaticity, a^*; and blue-yellow chromaticity, b^*) were utilized to characterise the external colour of three tomato fruit from each PEF-treatment using a Minolta colorimeter (Minolta CR-400, Konica Minolta Sensing, Inc., Osaka, Japan). The apparatus was set up for a D65 illuminant and 10º observer angle. A white standard plate (Y = 94.00, x = 0.3158, y = 0.3322) was used for calibration. The colour was assessed by measuring the lightness (L^*) and the a^*/b^* ratio.

183 ***Firmness.*** Whole tomato firmness was determined in three fruit with a TA-XT2 texture
184 analyser (Stable Micro Systems Ltd., Surrey, England), with a 4-mm-diameter steel
185 probe at a penetration speed of 5 mm s^{-1}. Results were expressed in Newtons (N).

186 ***pH.*** pH was determined using a Crison 2001 pH-meter (Crison Instruments S.A., Alella,
187 Barcelona, Spain) at 25 ºC.

188 ***Soluble solids.*** Total soluble solids content (TSS) was determined by measuring the
189 refraction index with an Atago RX-1000 refractometer (Atago Company Ltd., Tokyo,
190 Japan) at 25 ºC. The results were expressed as % of soluble solids.

191 **2.7. STATISTICAL ANALYSIS**

192 Statistical analyses were carried out using the JMP Pro v.12.0.1 software (SAS Institute,
193 Cary, NC, USA). Each PEF treatment replicate was obtained from two fruits. Three
194 different replicates for each assayed condition were used. For the reproducibility, each
195 analysis was conducted twice (n = 6). Results were reported as the mean ± standard
196 deviation. Results were subjected to a factorial analysis of variance (ANOVA) followed
197 by Tukey–Kramer *post hoc* test in order to establish statistical differences among mean
198 values. The relationship between variables was determined using the Pearson
199 correlation coefficient. The statistical significance level was set up at $p < 0.05$.

200 **3. RESULTS AND DISCUSSION**

201 **3.1. EFFECTS OF PEF ON CAROTENOIDS AND LIPOPHILIC**
202 **ANTIOXIDANT CAPACITY OF TOMATO FRUIT**

203 The application of PEF treatments significantly enhanced ($p < 0.05$) total carotenoids
204 (TCC) and lycopene (LC) concentrations in tomato fruit (Figures 1 and 2). TCC and LC

were significantly ($p < 0.001$) influenced by the specific energy input applied. The electric field strength was the main treatment parameter affecting the TCC and LC of tomato, regardless the pulse number applied. Thus, TCC and LC were remarkably higher in tomatoes subjected to 200 kV m^{-1}. The maximum enhancement in TCC was attained in tomatoes subjected to treatments delivering an energy input of 2.31 kJ kg^{-1} (200 kV m^{-1} - 30 pulses), leading to a 50 % increase in comparison to the content in untreated fruit. However, tomatoes treated with specific energy inputs of 0.02 kJ kg^{-1} (40 kV m^{-1} - 5 P), 0.09 kJ kg^{-1} (40 kV m^{-1} - 30 P) and 0.83 kJ kg^{-1} (120 kV m^{-1} - 30 P), did not exhibit any significant ($p > 0.05$) change in TCC with respect to untreated fruit. Lycopene concentration increased by 53 % when tomatoes were treated at 2.31 kJ kg^{-1} (200 kV m^{-1} - 30 P). A similar trend was already observed by Vallverdú-Queralt et al. (2013) in tomato fruits *cv.* Daniella. Authors found that PEF treatments conducted at 120 kV m^{-1} and 5 pulses led to maximum increases in α-carotene, 9- and 13-cis-lycopene, as well as in total carotenoid concentrations, which was attributed to both the activation of carotenoids metabolic pathway and the increase in the extractability from the food matrix due to the permeabilization of the cell membranes. However, they reported that treatments conducted at 200 kV m^{-1} resulted in a decrease in trans-lycopene and 9-, 13- and 15-cis-lycopene, as well as in total carotenoid concentrations, probably as a result of the irreversible electroporation of cell membranes. These observations differ from those obtained in this work and may be linked to the different varietal response of tomato fruits to PEF treatments.

The increased concentration of carotenoids by PEF was also accompanied by an enhancement of the LAC of tomato fruit compared to the baseline values found in the untreated fruits. This increase in LAC values correlated well with the accumulation of TCC ($r = 0.60$, $p < 0.001$) and LC ($r = 0.62$, $p < 0.001$) (Supplementary table 1). Thus,

PEF treatments produced a significant ($p < 0.05$) increase in LAC values of tomato (Figure 3), ranging from 17 % (0.02 kJ kg^{-1}) to 60 % (0.38 kJ kg^{-1}). The electric field strength was the main treatment parameter affecting the LAC of tomato fruit. Thus, treatments carried out at 200 kV m^{-1} led to the highest increase in LAC values, regardless the number of pulses applied. The maximum enhancement in LAC was attained after applying 5 pulses at 200 kV m^{-1} (0.38 kJ kg^{-1}) thus reaching values of 2.78 ± 0.08 mmol kg^{-1}, TE. This is in line with the results reported by Vallverdú et al. (2012) who also found an increase in the antioxidant capacity, ranging from 10.4 to 37.4 % in PEF-processed tomato fruits.

3.2. EFFECTS OF PEF ON THE RESPIRATORY ACTIVITY OF TOMATO FRUIT

The effect of PEF on the respiratory activity of tomato fruit is displayed in Table 2. The application of PEF treatments to tomato fruits had a determinant impact on the modification of the respiration rate, leading to increased oxygen consumption (R_{O_2}) and carbon dioxide production (R_{CO_2}). Statistical analysis revealed that R_{O_2} or R_{CO_2} of tomato fruits were strongly affected by the electric field strength. A peak value in oxygen consumption in tomatoes subjected to 200 kV m^{-1} and 5 pulses (0.38 kJ kg^{-1}) (Table 2) was found, corresponding to a 156 % increase with respect to that found in those untreated fruits. Similarly, CO_2 production markedly rose after the application of 0.38 kJ kg^{-1} (200 kV m^{-1} - 5 P), thus reaching a maximal Rco_2 value of 7.5 ± 0.5 mg h^{-1} kg^{-1} of CO_2. Further increase in the amount of energy delivered resulted into a progressive reduction of the respiratory rates compared to the reported peak values (Table 2). In line with our results, Dellarosa et al., (2016) reported that PEF treatments with electric field strengths of 10 kV m^{-1} triggered the increase in R_{O2} and R_{CO2} of fresh-

254 cut apples, whereas more intense treatments led to a sharp decrease of both R_{O2} and
255 R_{CO2} as a consequence of a severe loss of cell viability. The increased respiratory
256 activity in plants under abiotic stress has been observed by many authors, proving that
257 respiration plays a special role in the metabolic adaptation of plants to adverse
258 conditions (Fraire-Velazquez and Emmanuel, 2013; Łukaszuk, E. & Ciereszko, 2012;
259 Rakhmankulova et al., 2003; Sabbagh et al., 2014; Yuan et al., 2016).

260 The significant correlations ($p < 0.01$) found between TCC and both R_{O2} ($r = 0.41$) and
261 R_{CO2} ($r = 0.36$) (Supplementary table 1) indicate that the acceleration of respiratory
262 activity of tomatoes after PEF treatments may be connected to the activation of the
263 carotenoids biosynthetic pathway as a way to overcome oxidative stress. However, the
264 lack of a strong correlation found could be explained by the complexity of chemical
265 reactions occurring in natural systems as well as by the severe structural injuries caused
266 beyond a certain energy input value, which would lead to cell death and the subsequent
267 reduction of respiratory rates.

268 Ethylene production was significantly influenced by the application of PEF treatments
269 (Table 2). Ethylene concentration was remarkably higher in tomatoes treated with the
270 lowest electric field strength. A maximum 53 % increase was reached after the
271 application of treatments with an energy input of 0.09 kJ kg^{-1} (40 kV m^{-1} - 30 P).
272 Further increase in the intensity of PEF treatments led to a depletion in ethylene
273 concentration. This fact could be associated to the sharp rise in CO_2 (Table 2), which
274 has been suggested to act as a competitive inhibitor of ethylene (Soliva-Fortuny et al.,
275 2004). Ethylene biosynthesis has already been reported to be involved in several
276 processes such as ripening as well as pathogen and wounding responses, leaf senescence
277 and biotic or abiotic stress responses (Alexander and Grierson, 2002). This allows

confirming the hypothesis proposed by Vallverdú-Queralt et al., (2013a) who suggested that PEF could evoke ethylene production and in turn, the activation of carotenoids biosynthesis. Moreover, the drop in ethylene concentration and the deceleration of the R_{O2} and R_{CO2} (Table 2) when tomatoes were treated with the highest energy inputs suggests that these processing conditions trigger a severe loss of cell viability. It has been reported that increasing the treatment intensity would promote formation of large pores and reversible permeabilisation would turn into irreversible breakdown, leading to the loss of cell viability (Soliva-Fortuny et al., 2009)

It is worth highlighting the induction of acetaldehyde synthesis when tomatoes were subjected to specific energy inputs above 0.38 kJ kg^{-1}, reaching the maximum values (1.41 ± 0.15 ng h^{-1} kg^{-1}) in tomatoes treated with 0.83 kJ kg^{-1} (120 kV m^{-1} – 30 P). The presence of acetaldehyde confirms the triggering of anaerobic processes, which was possibly associated to the flooding of intracellular spaces as a result of the leaking of cellular contents. This is in line with the results obtained by Dellarosa et al., (2016) who confirmed that anaerobic fermentative metabolism took place in fresh-cut apples treated with electric field strengths ranging from 10 to 40 kV m^{-1}.

3.3. EFFECTS OF PEF ON PHYSICOCHEMICAL PROPERTIES OF TOMATO FRUIT

PEF processing had a significant effect ($p < 0.05$) on the physicochemical properties of tomato fruit (Table 3). With regard to colour, both *L** and *a*/b** ratio significantly changed 24 h after the application of PEF. Statistical analysis indicated that the electric field strength was the main PEF processing parameter affecting tomato colour ($p < 0.001$). However, a correlation between colour parameters and pulse number or specific energy input delivered could not be drawn. On the one hand, the application of PEF led

to a decrease in lightness values, especially after delivering energy inputs beyond 0.14 kJ kg^{-1} ($E \geq 120$ kV m^{-1}). Changes in tomato lightness could be triggered by a decompartimentalization process which allows enzymes to come into contact with their substrates as a consequence of electroporation-driven migration of cell contents (Asavasanti et al., 2010). On the other hand, high energy inputs, especially those corresponding to 200 kV m^{-1} treatments, promoted an increase in *a*/b** values. This change was related to an increase in *a** values, which ranged from 8.3 ± 1.8 (untreated tomatoes) to 15.3 ± 0.9 (2.31 kJ kg^{-1}) (data not shown). A significant ($p < 0.001$) correlation between *a*/b** ratio and both TCC ($r = 0.67$) and LC ($r = 0.73$) (Supplementary table 1) was found, which is consistent with the well-established relationship between the reddening of tomato and the accumulation of carotenoids (Arias et al., 2000).

The structural integrity of tomato tissues was strongly related to the specific energy input of the treatment (Table 3). Hence, the higher the treatment intensity the greater the softening effect. Thus, the most intense PEF treatment assessed (2.31 kJ kg^{-1}: 200 kV m^{-1} – 30 P) cause an 80 % reduction in firmness values. Nevertheless, the firmness of tomato fruit was dramatically affected even for low energy treatments. This is in agreement with previous works which found that the application of electric fields of 0.1 to 500 kV m^{-1} can induce severe tissue damage through membrane breakdown (Asavasanti et al., 2010). Additionally, the inverse correlation found between the firmness of tomato and both TCC ($r = -0.60$, $p < 0.001$) and LC ($r = -0.63$, $p < 0.001$) (Supplementary table 1) suggests that those conditions leading to the highest carotenoid content were also those resulting into the highest firmness loss. This could in turn favour the extraction of carotenoids from the food matrix, as reported for other

326 vegetable tissues after the application of PEF treatments (Luengo et al., 2014a; Zderic
327 et al., 2013).

328 PEF treatments also induced changes in total soluble solids (TSS) content of tomato.
329 The initial TSS of untreated fruit was 4.6 ± 0.4 % and was significantly ($p < 0.05$)
330 influenced by both the electric field strength and the number of pulses applied (Table 3).
331 Thus, TSS values rose by 24 % and reached highest values in those fruit subjected to the
332 most intense treatments (2.31 kJ kg^{-1}: 200 kV m^{-1} – 30 P). It is known that soluble
333 sugars act as metabolic and structural components of cells, however, they also take part
334 in some processes linked to growth, development and metabolic responses of plants
335 (Rosa et al., 2009). As soluble sugars are very sensitive to stress factors, it has already
336 been reported an active accumulation of solutes in response to osmotic stress (Atkinson
337 et al., 2011; Fraire-Velazquez and Emmanuel, 2013). According to Toepfl et al., (2005)
338 the membrane rupture triggered by PEF produce osmotic imbalances in cells. Therefore,
339 the accumulation of sugars in PEF-treated tomatoes may play a role in osmoregulation
340 as a strategy of tomato to restore the cell activity (Galindo et al., 2009, 2007). In
341 addition, the increased concentration of soluble solids could be linked with the
342 acceleration of tomato ripening associated to the increased metabolic activity induced
343 by PEF. Moreover, the application of these treatments may produce the disorganization
344 of cell wall polysaccharides and molecular bonds (Cholet et al., 2014) which could lead
345 to the release of soluble solids into the aqueous phase at membrane interfaces,
346 modifying the TSS content.

347 PEF treatments also modified the natural pH of tomato. The pH of untreated tomatoes
348 was 4.06 ± 0.01 and significantly ($p < 0.05$) increased when tomatoes were subjected to
349 PEF treatments delivering energy inputs beyond 0.09 kJ kg^{-1} (40 kV m^{-1} - 30 P). The

maximum pH values were found in tomatoes treated at 200 kV m^{-1} and 5 pulses (0.38 kJ kg^{-1}). After such treatments, tomato fruit also exhibited their maximum peak on both R_{O2} and R_{CO2}. Therefore, the increased pH values could be related to higher respiration rate after PEF treatments where organic acids were used as substrate. To the best of our knowledge there are no previous studies explaining the changes in pH when PEF treatments were applied to whole fruit, even though Kader and Lindberg, (2010) reported that changes in intracellular pH acts as secondary messenger in response of plants to different stress conditions. In addition, the modification of pH in PEF-treated tomatoes may be attributed to the electrical breakdown of cell membranes, which could become more permeable to molecules and ions that are sufficiently small to traverse membrane pores (Garner et al., 2007). However, the complexity of pH signalling against stress factors makes necessary to carry out additional studies in order to clarify the specific role of pH in plant defence mechanism to PEF-induced stress.

CONCLUSIONS

Pulsed electric field (PEF) treatments enhanced the amount of carotenoids in tomato fruit. PEF treatments conducted at 200 kV m^{-1} and 30 pulses (2.31 kJ kg^{-1}) led to the maximum increase in total carotenoids (50 %) and lycopene (53 %) concentration. The stress-induced accumulation of carotenoids was accompanied by changes in the respiratory activity as well as in the main physicochemical properties of tomato fruit. Increased values of pH and TSS, as well as changes in the surface colour were found after applying PEF treatments. However, irreversible damage in tomato tissue promoted by PEF led to a dramatic loss of firmness, which in turn affected the appearance and overall quality of tomato fruit. Therefore, PEF could be proposed as a pre-processing treatment to produce tomato-based products with high antioxidant potential. However,

374 the precise control of processing conditions is fundamental for the feasible application
375 of this promising technology.

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TABLE CAPTIONS

- ***Table 1.*** *PEF-processing treatment conditions.*
- ***Table 2:*** *Effect of PEF treatment conditions on the respiratory activity of tomato.*
- ***Table 3:*** *Physicochemical properties of untreated and PEF-treated tomato.*

Table 1. *PEF-treatment processing conditions*

Electric field strength (kV m^{-1})	Number of pulses	Specific energy input (kJ kg^{-1})
0	0	Untreated
40	5	0.02
40	18	0.06
40	30	0.09
120	5	0.14
120	18	0.50
120	30	0.83
200	5	0.38
200	18	1.38
200	30	2.31

554 ***Table 2:*** *Effect of PEF treatment conditions on the respiratory activity of tomato*

Specific energy input (kJ kg^{-1})	Electric field strength (kV m^{-1})	Number of pulses	Oxygen consumption (mg h^{-1} kg^{-1})	Carbon dioxide production (mg h^{-1} kg^{-1})	Ethylene production (µg h^{-1} kg^{-1})	Acetaldehyde production (ng h^{-1} kg^{-1})
Untreated	-	-	2.09 ± 0.51^{c}	2.80 ± 0.16^{c}	1.70 ± 0.87^{bc}	ND^{c}
0.02	40	5	2.29 ± 0.24^{c}	3.97 ± 0.26^{abc}	2.19 ± 0.89^{ab}	ND^{c}
0.06	40	18	2.75 ± 0.58^{bc}	3.80 ± 0.13^{bc}	1.90 ± 0.90^{abc}	ND^{c}
0.09	40	30	2.26 ± 0.25^{c}	4.09 ± 0.22^{abc}	2.59 ± 0.94^{a}	ND^{c}
0.14	120	5	3.15 ± 0.64^{abc}	5.40 ± 0.34^{abc}	1.29 ± 0.54^{bcd}	ND^{c}
0.38	200	5	5.37 ± 0.40^{a}	7.48 ± 0.48^{a}	1.31 ± 0.60^{bcd}	1.09 ± 0.12^{abc}
0.5	120	18	3.24 ± 0.79^{abc}	6.33 ± 0.34^{ab}	1.88 ± 0.73^{bc}	0.67 ± 0.01^{abc}
0.83	120	30	3.29 ± 0.47^{abc}	4.78 ± 0.17^{abc}	1.73 ± 0.26^{bc}	1.41 ± 0.15^{a}
1.38	200	18	3.10 ± 0.30^{ab}	4.33 ± 0.14^{abc}	1.21 ± 0.15^{cd}	0.32 ± 0.03^{bc}
2.31	200	30	2.11 ± 0.67^{c}	3.83 ± 0.26^{abc}	0.72 ± 0.45^{d}	1.10 ± 0.29^{ab}

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556 Values are expressed as mean ± standard deviation (n = 6). Different letters within the same column mean statistically significant differences ($p < 0.05$)

557 between treatments. ND: no detected.

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560 ***Table 3:*** *Physicochemical properties of untreated and PEF-treated tomato*

Specific energy input (kJ kg^{-1})	Electric field strength (kV m^{-1})	Number of pulses	Fruit colour		Firmness (N)	Soluble solids (%)	pH
			*L**	*a*/b**			
Untreated	-	-	43.9 ± 2.4 ^{a}	0.40 ± 0.11 ^{d}	17.4 ± 2.1 ^{a}	4.6 ± 0.3 ^{d}	4.06 ± 0.01 ^{ef}
0.02	40	5	43.6 ± 2.1 ^{a}	0.48 ± 0.18 ^{cd}	14.2 ± 2.7 ^{bc}	4.9 ± 0.1 ^{cd}	4.10 ± 0.07 ^{de}
0.06	40	18	42.5 ± 1.9 ^{ab}	0.54 ± 0.22 ^{bcd}	14.9 ± 1.8 ^{ab}	5.2 ± 0.3 ^{abc}	4.05 ± 0.01 ^{f}
0.09	40	30	41.9 ± 1.3 ^{ab}	0.56 ± 0.15 ^{bcd}	10.7 ± 2.0 ^{c}	4.8 ± 0.1 ^{cd}	4.13 ± 0.02 ^{cd}
0.14	120	5	41.1 ± 1.8 ^{abc}	0.92 ± 0.15 ^{a}	8.7 ± 1.7 ^{cd}	4.7 ± 0.3 ^{cd}	4.11 ± 0.03 ^{cd}
0.38	200	5	39.1 ± 4.7 ^{bc}	0.90 ± 0.09 ^{a}	6.3 ± 0.4 ^{de}	4.7 ± 0.1 ^{cd}	4.70 ± 0.10 ^{a}
0.5	120	18	36.9 ± 0.9 ^{c}	0.78 ± 0.08 ^{ab}	6.1 ± 1.2 ^{de}	5.0 ± 0.7 ^{bcd}	4.18 ± 0.05 ^{b}
0.83	120	30	39.2 ± 1.4 ^{bc}	0.71 ± 0.11 ^{abc}	5.9 ± 0.7 ^{de}	5.3 ± 0.2 ^{abc}	4.20 ± 0.10 ^{bc}
1.38	200	18	38.7 ± 0.6 ^{bc}	0.92 ± 0.17 ^{a}	6.8 ± 1.6 ^{de}	5.6 ± 0.3 ^{ab}	4.10 ± 0.10 ^{cd}
2.31	200	30	40.5 ± 1.9 ^{abc}	0.88 ± 0.08 ^{a}	3.1 ± 0.7 ^{e}	5.7 ± 0.9 ^{a}	4.15 ± 0.06 ^{bc}

561 Values are expressed as mean ± standard deviation (n=6). Different letters within the same column represent statistically significant differences ($p < 0.05$)

562 between treatments.

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FIGURE CAPTIONS

- ***Figure 1:*** *Total carotenoid content (mg kg^{-1}) of untreated and PEF-treated tomatoes.*
- ***Figure 2****: Lycopene content (mg kg^{-1}) of untreated and PEF-treated tomatoes.*
- ***Figure 3:*** *Lipophilic antioxidant capacity (µmol kg^{-1}) of untreated and PEF-treated tomatoes measured by DPPH assay.*

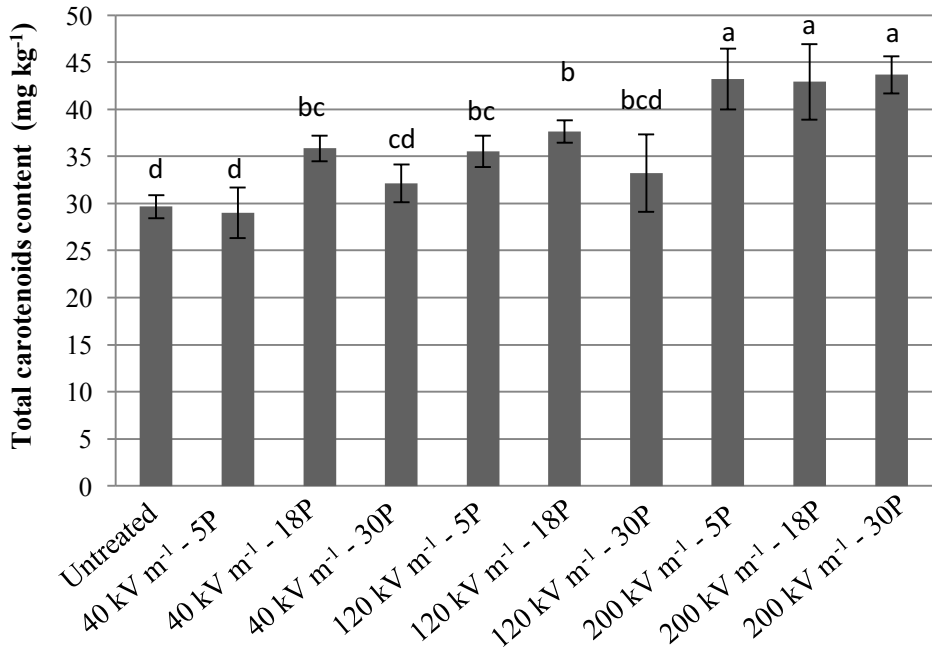
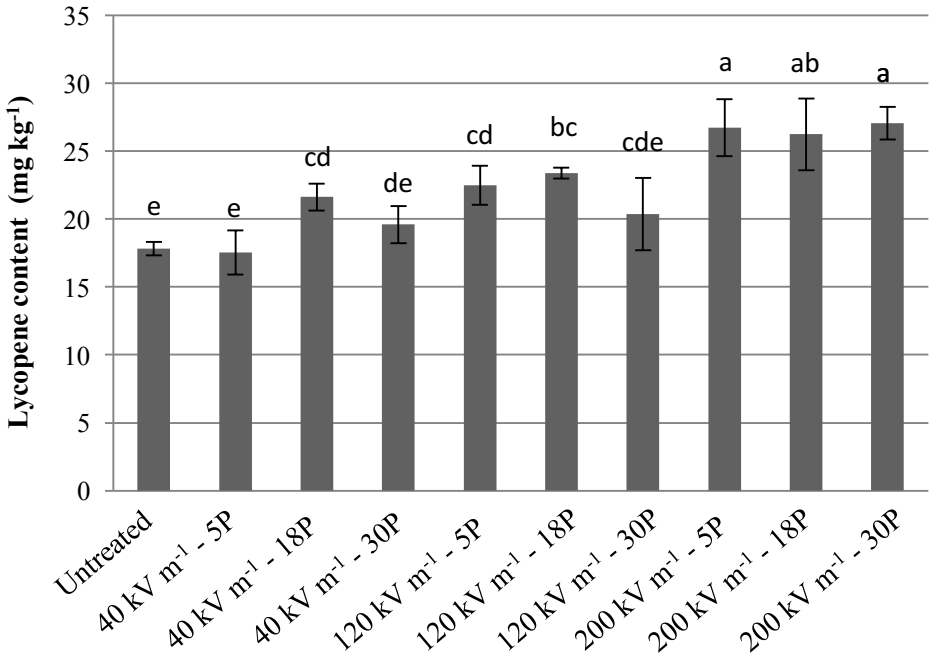


Figure 1: *Total carotenoid content (mg kg^{-1}) of untreated and PEF-treated tomatoes.* Fruits were subjected to different electric field strength (kV m^{-1}) and number of pulses (P). Values are expressed as mean ± standard deviation (n = 6). Different letters mean significant differences ($p < 0.05$) on TCC between treatments.



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571 ***Figure 2:*** *Lycopene content (mg kg^{-1}) of untreated and PEF-treated tomatoes.* Fruits were
572 subjected to different electric field strength (kV m^{-1}) and number of pulses (P). Values are
573 expressed as mean ± standard deviation (n = 6). Different letters mean significant differences (p
574 < 0.05) on LC between treatments.

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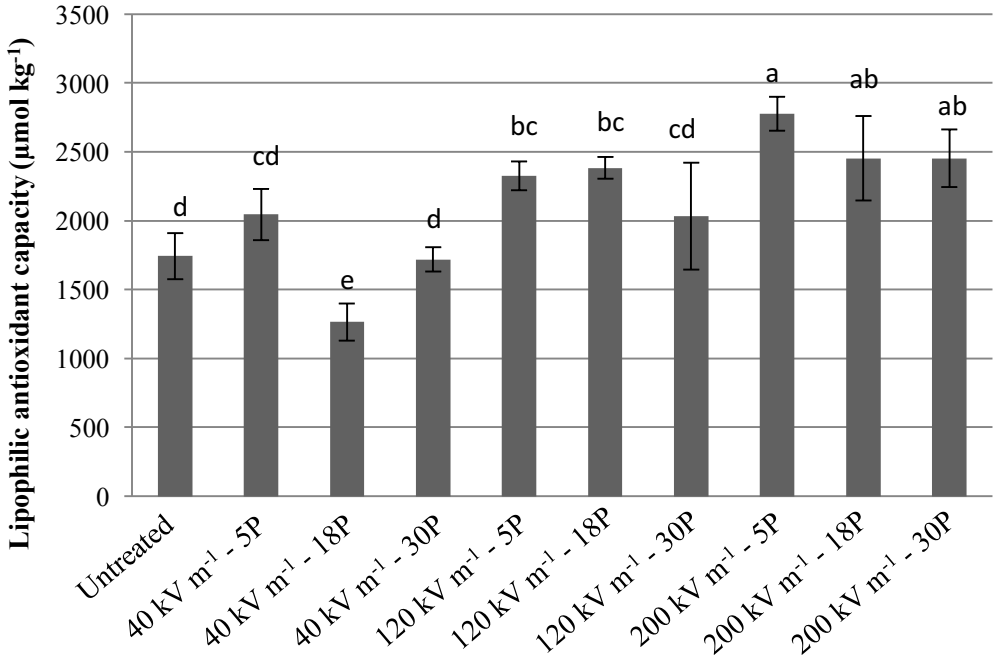

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585 ***Figure 3:*** *Lipophilic antioxidant capacity (LAC) (µmol kg-1,TE) of untreated and PEF-treated*
586 *tomatoes measured by DPPH assay.* Values are expressed as mean ± standard deviation (n = 6).
587 Different letters mean significant differences ($p < 0.05$) on LAC between treatments.

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SUPPLEMENTARY MATERIAL

- ***Supplementary table 1.*** *Pearson correlation coefficients between carotenoids content, lipophilic antioxidant activity, respiratory activity and the quality attributes of tomato fruit*

Supplementary table 1. *Pearson correlation coefficients between carotenoids content, lipophilic antioxidant activity, respiratory activity and the quality attributes of tomato fruit*

	TCC	LC	LAC	Ro2	Rco2	Ethylene	Acetaldehyde	*L**	*a*/b**	Firmness	TSS
TCC											
LC	0,9858***										
LAC	0,6***	0,6195***									
Ro2	0,4084**	0,4084**	0,3799**								
Rco2	0,3552**	0,3539**	0,3823**	0,6755***							
Ethylene	-0,3841**	-0,3848**	-0,2597*	0,0971	0,0527						
Acetaldehyde	0,2162	0,2406	0,2451	0,0858	-0,0196	-0,1983					
*L**	-0,4637***	-0,4599***	-0,4089	-0,179	-0,3994*	0,2417	-0,1031				
a/b**	0,6983***	0,7315***	0,532***	0,2604*	0,3597**	-0,3923**	0,1724	-0,6352***			
Firmness	-0,6041***	-0,6303***	-0,633***	-0,2779*	-0,327*	0,3211*	-0,3778**	0,55***	-0,6492***		
TSS	0,4377***	0,4183***	0,2322	0,0401	-0,1311	-0,1801	0,355**	-0,2457	0,2836*	-0,3735**	
pH	0,4592***	0,461***	0,561***	0,3679*	0,4712***	-0,156	0,1327	-0,304*	0,3747**	-0,3828**	-0,234

Significant correlation at $p < 0.05$ (*), $p < 0.01$ and (**) and $p < 0.001$(***). TCC, total carotenoids concentration; LC, lycopene concentration; LAC, lipophilic antioxidant capacity; R_{O_2}, oxygen consumption; R_{CO_2}, carbon dioxide production; *L**, lightness; TSS, total soluble solids.