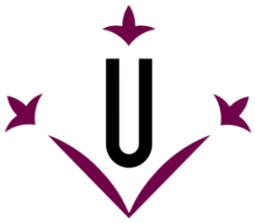

Induced accumulation of individual carotenoids and quality changes in tomato fruits treated with pulsed electric fields and stored at different post-treatments temperatures.

Sandra González-Casado, Olga Martín-Belloso, Pedro Elez-Martínez and Robert Soliva-Fortuny*

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

Corresponding Author

*Tel: +34 973 702678. Fax: +34 973 702596.

E-mail: rsoliva@tecal.udl.cat

ABSTRACT

Pulsed electric fields (PEF) have been proposed to elicit an increase in the content of health-related compounds in plant-based products. It has been previously demonstrated that PEF treatments may be applied to significantly increase the content and bioaccessibility of carotenoids in tomatoes. Nevertheless, the metabolic response of tomato is known to be greatly affected by postharvest storage conditions, which have a determinant impact on the quality characteristics of the product. The effects of PEF processing and post-treatment storage temperature on both carotenoid profile and the main physicochemical properties of tomato fruits were evaluated. Different specific energy inputs (0.02 kJ kg^{-1} and 0.38 kJ kg^{-1}) and storage temperatures (4, 12 and 20 ºC) were studied. The application of PEF treatments significantly improved the accumulation of carotenoids in tomato fruits. Nevertheless, the concentration of total and individual carotenoids during storage was differently influenced by the storage temperature depending on the applied PEF treatment. The increased concentration of carotenoids was noticeably higher in tomatoes stored at 12 ºC than in those fruits stored at 4 or 20 ºC. The mildest PEF treatment (0.02 kJ kg^{-1}) promoted the greatest accumulations of total carotenoids (58 %) and lycopene (150 %) in tomatoes stored during 5 d at 12 ºC without compromising the fresh-like quality of tomato fruits. However, the most intense PEF treatment (0.38 kJ kg^{-1}) triggered a fast accumulation of carotenoids, leading to the greatest increase of β-carotene (77 %), γ-carotene (200 %) and lutein (238 %) concentration in tomatoes stored at 12 ºC for 1 d. Nonetheless, irreversible damage was caused to tomato tissues, thus leading to deleterious quality effects. The results obtained provide valuable information for the future application of PEF in the development of tomato derivative products with increased health-related properties.

KEYWORDS

Individual carotenoids, pulsed electric fields, physicochemical properties, storage temperature, tomato

ABBREVIATIONS

PEF, pulsed electric fields; ROS, reactive oxygen species; BHT, butylated hydroxytoluene; L*, lightness; a*, green to red chromaticity; b*, blue to yellow chromaticity; h^o, hue angle; TSS, total soluble solids; HPLC, high performance liquid chromatography; ANOVA, analysis of variance; LCYB, lycopene β-cyclase; LCYE, lycopene ε-cyclase.

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NOTES

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1. INTRODUCTION

Consumption of raw tomatoes and tomato-based products is nowadays strongly associated with a reduced incidence of certain types of cancer, cardiovascular diseases and atherosclerosis (Hedges & Lister, 2005). These health-promoting properties have been attributed to the presence of high amounts of phytochemicals, including carotenoids, which act as antioxidants in detoxifying free radicals (Ilahy, Hdider, Lenucci, Tlili, & Dalessandro, 2011; Vallverdú-Queralt, Oms-Oliu, Odriozola-Serrano, Lamuela-Raventós, Martín-Belloso, & Elez-Martínez, 2013).

The increased demand of healthy foods provides an opportunity to develop new technologies that allow obtaining products with enhanced functional properties. Pulsed electric fields (PEF) treatments have attracted large interest due to its potential to offer useful applications in the food industry. Inactivation of microorganism and enzymes (Elez-Martínez, Sobrino-López, Soliva-Fortuny, & Martín-Belloso, 2012; Martín-Belloso & Elez-Martínez, 2005), extraction of intracellular compounds (Luengo, Condón-Abanto, Álvarez, & Raso, 2014; Vorobiev & Lebovka, 2006), preservation of certain food components (Odriozola-Serrano, Soliva-Fortuny, & Martín-Belloso, 2009), among others, have been investigated. In addition, some authors have proposed the application of PEF at moderate intensity as an abiotic elicitor capable of inducing an increase in the antioxidant potential of metabolically active fruit tissues (González-Casado, Martín-Belloso, Elez-Martínez, & Soliva-Fortuny, 2018a; Soliva-Fortuny, Vendrell-Pacheco, Martín-Belloso, & Elez-Martínez, 2017; Vallverdú-Queralt et al., 2013). All stresses, either biotic or abiotic, induce oxidative stress in plants, wich is associated with the generation of reactive oxygen species (ROS). The oxidative signalling in turn controls the biosynthesis and accumulation of carotenoids in order to overcome stressful conditions (Balasa, Janositz, & Knorr, 2011;

75 Fanciullino, Bidel, & Urban, 2014). Preliminary studies have demonstrated the feasibility
76 of applying PEF treatments to trigger the accumulation of some phytochemicals. González-
77 Casado et al. (2018a) observed a maximum 1.5-fold increase in the carotenoids content of
78 tomato fruits stored at 4 ºC for 24 h after the application of 30 pulses at 200 kV m^{-1}. In
79 addition, Soliva-Fortuny et al. (2017) noticed that the application of PEF treatments
80 produced significant greater concentration of phenolic compounds in apples. Nevertheless,
81 together with the stress-adaptive response to PEF, several changes in quality attributes
82 could be triggered (González-Casado et al., 2018a). It is known that PEF may strongly
83 affect the structural integrity of cell walls, and hence the firmness of fruits and vegetables
84 (Lebovka, Praporscic, & Vorobiev, 2004; Shayanfar, Chauhan, Toepfl, & Heinz, 2013).
85 This fact could lead to undesirable effects on the final quality of tomato fruits.

86 Stress response in plant tissues is thought to be affected by internal and external factors
87 (Hodges & Toivonen, 2008). On the one hand, the internal factors represent metabolic
88 responses and may include morphological, physiological and biochemical defence
89 mechanisms. On the other hand, the external factors, namely environmental and storage
90 conditions may intensify or inhibit the manifestation of the internal factors. It is well
91 established that proper control of postharvest storage conditions, mainly temperature, is
92 critical to maintain quality and to extend the self-life of tomatoes (Lana, Tijskens, & Van
93 Kooten, 2005). In this regard, previous studies have been aimed at evaluating the influence
94 of storage temperature on quality and metabolic behaviour of intact tomato fruits,
95 including the biosynthetic pathway of carotenoids (Javanmardi & Kubota, 2006; Vinha,
96 Barreira, Castro, Costa, & Oliveira, 2013). However, there is a lack of knowledge
97 regarding the effects of the storage conditions on the elicited biosynthesis of carotenoids in
98 tomato fruits subjected to different PEF treatments. The objective of this work was to study
99 the accumulation of carotenoids in tomato fruits as well as the main modifications in their

physicochemical properties as affected by PEF treatment intensity and the storage conditions, namely time and temperature.

2. MATERIAL AND METHODS

2.1. REAGENTS

Butylated hydroxytoluene (BHT) was acquired from Scharlau Chemie S.A. (Barcelona, Spain). Magnesium hydroxide carbonate was purchased from Sigma-Aldrich (St. Louis, MO, USA). Lycopene, γ-carotene, δ-carotene, β-carotene, lutein, phytofluene and phytoene standards were obtained from Carote-Nature (Ostermundigen, Switzerland).

2.2. TOMATO FRUITS

Tomato fruits (Solanum lycopersicum, cv. Raf) were obtained from a local supplier in Lleida (Spain). Unlike other tomato cultivars, Raf tomatoes can be found at different ripeness stages, from green-mature to red ripe. The fruits were acquired at turning stage, which means that more than a 10 % but not more than a 30 % of the surface showed a definite change in colour from green to red (USDA, 1991). Tomatoes were then stored at 12 ± 1 ºC until they reached a light-red stage (60 – 90 % of the surface showing red colour) (USDA, 1991). Prior to PEF treatments, tomatoes were rinsed with tap water and dried carefully with paper cloth.

2.3.PULSED ELECTRIC FIELD TREATMENTS

PEF treatments were carried out with a device manufactured by Physics International (San Leandro, CA, USA). The apparatus delivers monopolar exponential-wave pulses from a capacitor of 0.1 μF with a frequency of 0.1 Hz. Treatments were conducted in batch mode. The treatment chamber was a parallelepiped methacrylate container (0.2 x 0.08 m) with

two parallel stainless steel electrodes separated by a gap of 0.01 m. A batch of tomatoes (2 fruits; ca. 260 g/batch) was placed into the treatment chamber filled with tap water (conductivity of 0.03 S m^{-1}). Tomato fruits were subjected to either 5 pulses at 40 kV m^{-1} or 5 pulses at 200 kV m^{-1}, resulting in specific energy inputs of 0.02 kJ kg^{-1} and 0.38 kJ kg^{-1}, respectively. These PEF conditions were selected according to the results obtained in preliminary experiments. Each treatment was repeated fourfold and each replicate comprised two tomato fruits.

2.4. STORAGE CONDITIONS

Immediately after PEF processing, tomatoes were stored in darkness at 4, 12 or 20 ºC for different storage times (1, 3 and 5 days). Untreated tomatoes were used as a reference. Just after treatment and at specific storage times, both untreated and PEF-treated tomatoes were withdrawn from the storage chambers. Quality attributes (colour, texture, pH and total soluble solids) from each tomato fruits were then determined. Afterwards, tomatoes from each treatment batch were ground (Solac Professional Mixter BV5722, Spain). Homogeneous samples were then freeze-dried and stored at −40 °C until carotenoids extraction. The detailed methodologies to determine each parameter are described hereafter.

2.5.CAROTENOIDS

2.5.1. Extraction of carotenoids

Carotenoid extraction and quantification was carried out following the methodology proposed by Rodríguez-Roque, et al. (2013) with minor modifications. One gram of freeze-dried tomato sample was mixed with 0.1 % (w/w) magnesium hydroxide carbonate and 10 mL of 0.05 % (w/v) BHT in ethanol:hexane (4:3 v/v). The mixture was

145 homogenized using an Ultraturrax T-25 Basic (IKA®-Werke GmbH & Co., Staufen,
146 Germany) for 2 min in an ice-bath. Afterwards, it was filtered under vacuum through grade
147 1 Whatman paper. The residue was re-extracted once with 10 mL of ethanol:hexane (4:3
148 v/v) for 2 min with an Ultraturrax. Then, the mixture was again filtered and the residue was
149 washed twice with 5 mL of ethanol and once with 5 mL of hexane. All the filtrates were
150 combined in an amber round-bottom flask and evaporated (rotovapor R-3000, BUCH,
151 Switzerland) to dryness at 45 ºC for 15 min. The residue was then saponified under a N_2
152 atmosphere by adding 10 mL of methanolic KOH 0.5 M + 0.1 % BHT (v/w) and 10 mL of
153 diethyl ether for 30 min with continuous agitation. Afterwards, the extract was placed in an
154 amber decanting funnel and washed twice with 25 mL of 10 % NaCl solution and thrice
155 with 25 mL of distilled water. The aqueous phase was discarded each time. The organic
156 phase was collected and rotoevaporated to dryness at 45 °C for 20 min. The residue was
157 dissolved with 4 mL of diethyl ether and placed in an amber glass vial. Finally, the solvent
158 was evaporated under a N_2 flow and stored at −40 °C until analysis. Before injection into
159 the HPLC system, the carotenoid extract was reconstituted with 1 mL of methylene
160 chloride and filtered through a 0.45 µm filter. All the extractions were conducted in
161 duplicate.

162 **2.5.2. Analysis of carotenoids**

163 Carotenoids were quantified by high-performance liquid chromatography (HPLC)
164 following the methodology reported by Odriozola-Serrano et al. (2009). The HPLC system
165 was equipped with a 600 controller and a diode array detector 2996 (Waters Corp.) set to
166 scan from 240 to 550 nm. Separations were performed on a reverse-phase C18 Spherisorb®
167 ODS2 (5 µm) stainless steel column (4.6 mm x 250 mm) at room temperature with a flow
168 rate of 0.7 mL min^{-1}. The gradient was as follows: 0 – 10 min, acetonitrile (85 %),

169 methanol (10 %), methylene chloride (3 %) and hexane (2 %); 10 – 40 min, acetonitrile (45
170 %), methanol (10 %), methylene chloride (23 %) and hexane (22 %); and 40 -60 min,
171 acetonitrile (85 %), methanol (10 %), methylene chloride (3 %) and hexane (2 %).
172 Carotenoids were identified on the basis of the retention times and absorption spectrum
173 characteristics. Their quantification was carried out by comparison with external standards
174 of lycopene, γ-carotene, δ-carotene, β-carotene, lutein, phytofluene and phytoene. The
175 content for each carotenoid compound was expressed as μg kg^{-1} on a fresh weight basis
176 (fw). Total carotenoid concentration was calculated as the sum of individual compounds
177 and also expressed as μg kg^{-1} (fw).

178 **2.6.PHYSICOCHEMICAL PROPERTIES**

179 **2.6.1. Colour**

180 Tomato surface colour was measured using a Minolta colorimeter (Minolta CR-400,
181 Konica Minolta Sensing, Inc., Osaka, Japan). The equipment was set up for a D65
182 illuminant and a 10º observer angle. A white standard plate (Y = 94.00, x = 0.3158, y =
183 0.3322) was used for calibration. The values of L^* (lightness), a^* (green to red colour), and
184 b^* (blue to yellow colour) were determined. Tomato colour was assessed by measuring the
185 lightness (L^*) and hue angle (hº), which was calculated using equation 1.

186 $$h^o = tan^{-1}\frac{b^*}{a^*} \quad (1)$$

187 **2.6.2. Firmness**

188 Firmness was evaluated by measuring the maximum penetration force for a 4-mm-
189 diameter steel probe using a TA-XT2 texture analyser (Stable Micro Systems Ltd., Surrey,
190 England). The fruits were placed so that the plunger penetrated the pericarp approximately

1 cm away from their geometric centre to a depth of 10 mm at a rate of 5 mm s^{-1}. Results were expressed in Newtons (N).

2.6.3. Total soluble solids

Total soluble solids (TSS) were determined by measuring the refraction index with an Atago RX-1000 refractometer (Atago Company Ltd., Tokyo, Japan) at 25 ºC. Results were expressed as %.

2.6.4. pH

A Crison 2001 pH-meter (Crison Instruments S.A., Alella, Barcelona, Spain) was used to measure the pH values of the fruit flesh at 25 ºC.

2.7. STATISTICAL ANALYSIS

Statistical analysis was performed using the JMP Pro v. 12.0.1 statistic software (SAS Institute, Cary, NC, USA). Results were expressed as mean ± standard deviation (n = 8). A multifactor analysis of variance (ANOVA) was performed at $p < 0.05$ in order to determine significant differences in carotenoid concentrations considering the factors studied in this research (energy input, storage time and storage temperature).

3. RESULTS AND DISCUSSION

3.1.CAROTENOIDS PROFILE

Changes in carotenoid concentrations as affected by energy input of PEF treatment, storage time and storage temperature can be observed in Figure 1. Pooled data indicate that the concentration of individual and total carotenoids in tomato fruits was affected by storage time and temperature, as well as by the interaction of these factors with the PEF processing

212 conditions (Figure 1). The delivered specific energy input influenced the initial
213 concentration of carotenoids in PEF-treated tomatoes. Total and individual carotenoid
214 concentrations were significantly enhanced by 23-171 % in just-treated tomato
215 fruitssubjected to 200 kV m^{-1}, which is related to the electroporation effect of PEF. The
216 loss of structural integrity, which was reflected in a dramatical loss of firmness in tomato
217 fruits (Figure 3), may favour the release and extraction of carotenoids located inside the
218 cells. However, by applying electric field treatments below the threshold of irreversible
219 electroporation (100 - 200 kV m^{-1} in plant tissues) (González-Casado et al., 2018a), no
220 significant differences on the initial content of each individual compound were noticed.
221 This is consistent with the results reported by Luengo, Condón-Abanto, Álvarez, & Raso,
222 (2014) who concluded that extraction yield of carotenoids from *Chlorella vulgaris* cells
223 increased immediately after PEF processing by increasing the electric field strength
224 applied.

225 As storage progressed, the concentration of carotenoids in tomatoes was differently
226 affected depending on the PEF energy input delivered and the post-treatment storage
227 temperature (Figure 1). A peak total carotenoid concentration of 14912 ± 845 µg kg^{-1} was
228 reached in those fruits subjected to an overall energy input of 0.02 kJ kg^{-1} and subsequently
229 stored during 5 d at 12 ºC. On the other hand, the application of the most intense PEF
230 treatment (0.38 kJ kg^{-1}) yielded a similar increase in the total carotenoids content, up to
231 13169 ± 747 µg kg^{-1}, only 24 h after the treatment application, to subsequently decrease,
232 when tomatoes were stored at 12 ºC. These results indicate that the application of higher
233 intense PEF treatments led to a faster accumulation of carotenoids in tomato fruits during
234 storage, which seems to point out that the changes in tomato metabolism can vary
235 depending on the intensity of the stress imposed. This is in accordance with the results
236 previously reported by Vallverdú-Queralt et al., (2012) and González-Casado, et al

237 (2018a), which allow concluding that the accumulation of carotenoids in tomato fruits
238 directly depends on the electric field strength applied.

239 With regard to individual carotenoid compounds, their concentration exhibited maximum
240 values in PEF-treated tomatoes stored at 12 ºC. The highest enhancement in phytoene (53
241 %) and phytofluene (60 %) was reached in tomatoes subjected to PEF treatments with an
242 energy input of 0.38 kJ kg^{-1} and subsequently stored at 12 ºC during 3 d. In a similar way,
243 lycopene content increased by 70 % in tomato fruits treated with the highest energy input
244 (0.38 kJ kg^{-1}) and subsequently stored at 12 ºC for 1 d. However, milder PEF treatments
245 (0.02 kJ kg^{-1}) led to a slower and higher increase in lycopene concentration, reaching its
246 maximum enhancement (150 %) in tomatoes stored at 12 ºC for 5 d. Although the increase
247 in carotenoid content in tomato fruits may seem difficult to explain due to the complexity
248 of biological systems, a well-establish explanation for these observations is the activation
249 of the secondary metabolism as a way to overcome the stressful conditions triggered by
250 PEF treatments (Vallverdú-Queralt et al., 2013; González-Casado et al, 2018b).

251 Moreover, a maximum concentration in δ-carotene, lutein, γ-carotene and β-carotene was
252 attained in tomatoes subjected to the most intense PEF treatment (0.38 kJ kg^{-1}) and
253 subsequently stored at 12 ºC (Figure 1). The highest concentration of each individual
254 carotenoid was reached at different storage times. Thus, highest δ-carotene concentration
255 was reached after three days of storage (177 µg kg^{-1}), while lutein, γ-carotene and β-
256 carotene were enhanced by 238 %, 200 % and 77 %, respectively after just 1 d. Lycopene
257 is cyclized either to yield δ–carotene and lutein by lycopene ε-cyclase (LCYE) and
258 lycopene β-cyclase (LYCB) or to produce γ-carotene and β-carotene by lycopene β-cyclase
259 (LCYB) alone (Lu & Li, 2008). Therefore, the increased concentration of these minor
260 carotenoids of tomato could be linked to the activation of genes encoding both LCYB and

LYCE. Our results are in agreement with those previously reported by Vallverdú-Queralt,et al., (2013) who proposed that PEF treatments produce the activation of LYCB and LYCE, thus resulting in an increase in β-carotene, 9-*cis*-β-carotene and lutein in tomato fruits. However, the biochemical mechanism by which biosynthetic pathway is activated by PEF needs more profound studies.

Despite the influence of post-treatment storage temperature on the accumulation of carotenoids in PEF-treated tomatoes has not been studied yet, several studies have already concluded that the storage temperature is a critical parameter in the biosynthesis of carotenoids of intact tomato fruits (Vinha et al, 2013). The results obtained in this work evidenced that the accumulation of carotenoids in PEF-treated tomatoes was generally higher at 12 ºC than at 4 or 20 ºC. This indicates that the activation of the antioxidant defense mechanisms triggered by PEF processing, and hence the activation of the biosynthesis of carotenoids, was temperature dependent. Low storage temperatures (4 ºC) deleteriously affected the rate of carotenoids accumulation probably due to chilling injury associated phenomena. On the contrary, high storage temperature (20ºC) led to the acceleration of tomato metabolism which resulted in a fast accumulation of carotenoids in PEF-treated tomatoes and a subsequently decay of fruit quality over storage. Therefore, similarly to the reported data concerning postharvest storage temperature of non treated tomatoes (Tadesse et al, 2015; Vinha et al, 2013), post-PEF treatment storage temperature of 12 ºC could be established as optimal to maintain a regular biochemical activity, including carotenoids accumulation in tomato fruits.

3.2.PHYSICOCHEMICAL PROPERTIES

3.2.1. Colour

The effects of PEF treatments and post-treatment storage conditions on colour parameters (L^* and h^o) of tomato fruits are shown in Figure 2. Untreated tomatoes exhibited initial L^* and h^o values of 44.93 ± 0.96 and 79.9 ± 2.8, respectively. Immediately after PEF processing, tomatoes treated with an energy input of 0.38 kJ kg^{-1} showed a significant ($p < 0.05$) decrease in L^* values as well as a rise in h^o values. In contrast, the application of milder treatments (0.02 kJ kg^{-1}) did not produce any significant instant change in colour parameters. Changes in L^* values usually denote colour darkening of tomatoes, whereas h^o has been usually associated to modifications in their characteristic red colour, as a consequence of carotenoid biosynthesis. Nevertheless, as colour was measured immediately after the application of PEF, colour changes are likely to be due to structural modifications caused in the fruit tissue. The most intense treatment conditions probably induced the decompartmentalization of oxidative enzymes, thus allowing them to come into contact with substrates of oxidation and browning processes (Asavasanti, Ersus, Ristenpart, Stroeve, & Barrett, 2010; González-Casado et al., 2018a).

Colour changes over storage of tomato fruits were differently affected by the PEF treatment intensity and the storage temperature (Figure 2). Changes in colour parameters (L^* and h^o) in both untreated and PEF-treated tomatoes were found to be significantly influenced by the storage temperature. In this regard, tomatoes stored at 4 ºC exhibited lower rate of colour development in comparison to those kept at 12 or 20 ºC. Other authors have already observed that colour development in intact tomatoes is strongly related to the storage temperature (Žnidarčič & Požrl, 2006). As reported in their work, chilled storage reduces enzymatic activities and hence colour development of tomato, which is in accordance with our results. Nevertheless, to the best of our knowledge, no previous works have assessed the influence of storage temperature on the colour of tomato fruits as affected by PEF.

309 L^* significantly ($p < 0.05$) decreased in untreated tomatoes, reaching values of 41.85 ±
310 0.73 after 5 d of storage at 20 ºC (Figure 2A). The L^* values of tomato fruits were not
311 significantly ($p > 0.05$) influenced by the application of PEF treatments with 0.02 kJ kg^{-1} in
312 comparison to untreated tomatoes, regardless the assessed storage temperature. In contrast,
313 tomato fruits subjected to PEF treatments delivering an energy input of 0.38 kJ kg^{-1}
314 exhibited significantly ($p < 0.05$) lower L^* values (from 42 ± 2 to 40.69 ± 0.08) throughout
315 the storage period in comparison to those found in untreated tomatoes (from 45.6 ± 1.1 to
316 41.0 ± 1.3). The h^o values of untreated and PEF-treated tomatoes significantly ($p < 0.05$)
317 decreased throughout the storage period, especially in those fruits stored at 20 ºC (Figure
318 2B). PEF-treated tomatoes did not exhibit significant differences ($p > 0.05$) in h^o values in
319 comparison to untreated fruits, with the exception of those treated with 0.38 kJ kg^{-1} and
320 stored at 20 ºC. Under these treatment and storage conditions, h^o values were significantly
321 higher (from 85 ± 3 to 72 ± 7) than those observed in untreated fruits (from 80 ± 3 to 50.1
322 ± 1.3), thus denoting a delay in the development of red colour under those conditions. This
323 delay in the reddening of tomato tissues could be related to the concomitant effect of
324 intense PEF treatments and abusive storage temperatures. In this regard, the extent of
325 tissue electroporation and the associated loss of cell viability has been reported to be
326 tightly related with the intensity of the PEF treatments (Martín-Belloso & Soliva-Fortuny,
327 2011). Our results suggest that storage temperature below 12 ºC could compensate the
328 deleterious effect of intense PEF treatments on cell viability, thus favouring the normal
329 colour development in tomato fruits.

330 **3.2.2. Firmness**

331 Changes in firmness of tomato fruits as affected by PEF treatments and storage conditions
332 are shown in Figure 3. Tomato fruits subjected to the most intense PEF treatment (0.38 kJ

333 kg^{-1}) instantly lost a 44 % of their initial firmness. Conversely, the application of
334 treatments delivering an energy input of 0.02 kJ kg^{-1} did not significantly ($p > 0.05$) affect
335 tomato firmness. The impact of PEF treatments on the texture and structure of plant tissues
336 is well described in literature (Vorobiev & Lebovka, 2009). Structural integrity is strongly
337 related to the intensity of PEF treatments. Thus, treatments carried out at 40 kV m^{-1} did not
338 appear to produce significant modifications in tomato at the cell membrane level. In
339 contrast, treatments conducted at 200 kV m^{-1} apparently caused major damage to cell
340 membranes, thus leading to evident signs of softening. These results are in agreement with
341 literature data, which report critical field strengths exceeding the threshold of irreversible
342 electroporation of membranes in plant tissues within the range of 100 - 200 kV m^{-1}
343 (González-Casado et al., 2018a; Soliva-Fortuny, Balasa, Knorr, & Martín-Belloso, 2009).

344 Firmness was scarcely affected by storage under low temperature conditions. Thus, for
345 both untreated and PEF-treated tomatoes, major firmness changes did not occur over
346 storage at 4 and 12 ºC. However, storage at 20 ºC led to significant ($p < 0.05$) tissue
347 softening over the storage period, regardless the applied PEF treatment. During tomato
348 softening, pectins typically undergo solubilisation and depolymerisation, which contribute
349 to cell wall disintegration (Požrl, Žnidarčič, Kopjar, Hribar, & Simčič, 2010). In line with
350 our results, this process has been shown to be strongly inhibited over chill storage
351 (Tadesse, Ibrahim, & Abtew, 2015).

352 **3.2.3. Total soluble solids (TSS) and pH**

353 The effects of PEF treatments and post-treatment storage conditions on TSS and pH of
354 tomato fruits are shown in figure 4 and 5, respectively. Untreated tomatoes exhibited initial
355 TSS and pH values of 5.05 ± 0.48 % and 3.935 ± 0.005, respectively. TSS content was not
356 found to be immediately affected after PEF processing. In contrast, the application of PEF

357 treatments with energy inputs of 0.02 and 0.38 kJ kg^{-1} caused an instant increase ($p < 0.05$)
358 in pH of tomatoes in comparison to the values observed in untreated fruits. Changes in
359 intracellular pH have been previously associated to the role of cytosolic ions, such as Ca^{2+},
360 which act as secondary messengers in the response of plants under stress conditions (Kader
361 & Lindberg, 2010). Rakhmankulova and others (2003) reported that respiration plays a
362 special role in plant adaptation to stressful conditions as the crossroads of total metabolism.
363 Hence, the increase in pH values is likely to be related to the acceleration of tomato
364 respiration, which may involve the degradation of organic acids (González-Casado et al,
365 2018a) .

366 As storage progressed, TSS and pH in tomato fruits differed depending on the energy input
367 delivered and the post-treatment storage temperature (Figure 4 and 5). Untreated tomatoes
368 exhibited a continuous rise in TSS and pH values during storage, which may be associated
369 to the accumulation of sugars, such as glucose and fructose, and a decrease in organic
370 acids, respectively (Anthon, Lestrange, & Barrett, 2011; Fanciullino, Bidel, & Urban,
371 2014). PEF treatments with an energy input of 0.02 kJ kg^{-1} did not exert any change in pH
372 values of tomato during storage in comparison to untreated fruits. In contrast, treatments
373 delivering a higher energy input (0.38 kJ kg^{-1}) led to a significant increase in pH values,
374 especially in tomatoes stored at 20 ºC. At such storage temperature, the rise in pH values
375 was much greater and faster than that observed in tomato fruits stored at chilling
376 temperatures (4 and 12 ºC), thus reaching pH values of 4.150 ± 0.032 on the third storage
377 day. On the other hand, PEF treatments with an energy input of 0.38 kJ kg^{-1} also led to a
378 maximum 37 % increase in TSS in tomatoes stored at 12 ºC for 3 d. These observations
379 could be related to the acceleration of tomato metabolism induced by PEF, which may lead
380 to a faster accumulation of sugars and a sharper loss of organic acids. All these processes
381 have a biochemical nature, and hence are temperature-dependent (Lana et al., 2005).

382 Consequently, the variation of TSS and pH values was more pronounced when tomatoes
383 were stored at higher temperatures. Therefore, the post-treatment storage temperature
384 would condition the accumulation of sugars and degradation of organic acids in PEF-
385 treated tomatoes.

386 **4. CONCLUSIONS**

387 The electric field strength applied and post-treatment storage conditions significantly
388 affected the accumulation of carotenoids in tomato fruits as well as their physicochemical
389 properties. The concentration of the major carotenoids of tomato reached their maximum
390 values when fruits were stored at 12 ºC. Under this storage temperature, tomatoes
391 subjected to 0.38 kJ kg^{-1} exhibited the fastest accumulation of individual carotenoids.
392 However, this treatment produced irreversible damage to tomato tissues, thus leading to
393 deleterious changes in their physicochemical properties. On the other hand, mild PEF
394 treatment conditions (0.02 kJ kg^{-1}) led to a slower but maximum accumulation of total
395 carotenoids (58 %) and lycopene (150 %) without negatively affecting the quality
396 attributes of tomato fruits. These findings allow for a better understanding of the changes
397 in carotenoid concentrations in tomato fruits using PEF treatments by controlling post-
398 treatment storage temperatures.

399 The results obtained in this study evidence that PEF may be effectively applied to stimulate
400 the biosynthesis and accumulation of carotenoids in tomato fruits, thus enhancing their
401 health-related properties. However, the accurate control of both electric field strength and
402 post-treatment storage conditions is necessary for a feasible application of PEF. These
403 findings will be of value to especially for the development of higher-quality tomato
404 products as they can be used as a strategy to enhance the nutritional and functional quality
405 attributes of tomato derivatives.

406 **REFERENCES**

407 Anthon, G. E., Lestrange, M., & Barrett, D. M. (2011). Changes in pH, acids, sugars and
408 other quality parameters during extended vine holding of ripe processing tomatoes.
409 *Journal of the Science of Food and Agriculture, 91*(7), 1175–1181.
410 http://doi.org/10.1002/jsfa.4312

411 Asavasanti, S., Ersus, S., Ristenpart, W., Stroeve, P., & Barrett, D. M. (2010). Critical
412 electric field strengths of onion tissues treated by pulsed electric fields. *Journal of*
413 *Food Science, 75*(7). http://doi.org/10.1111/j.1750-3841.2010.01768.x

414 Balasa, A., Janositz, A., & Knorr, D. (2011). Electric field stress on plant systems.
415 *Encyclopedia of Biotechnology in Agriculture and Food* (pp. 208–211). DOI:
416 10.1081/E-EBAF-120045407

417 Elez-Martínez, P., Sobrino-López, Á., Soliva-Fortuny, R., & Martín-Belloso, O. (2012).
418 Pulsed electric field processing of fluid foods. In Cullen,P.J, Tiwari, K.B., and
419 Valdramidis , V. (Eds.), Novel Thermal and Non-Thermal Technologies for Fluid
420 Foods. Elsevier. http://doi.org/10.1016/B978-0-12-381470-8.00004-9

421 Fanciullino, A. L., Bidel, L. P. R., & Urban, L. (2014). Carotenoid responses to
422 environmental stimuli: Integrating redox and carbon controls into a fruit model. *Plant,*
423 *Cell and Environment*, *37*(2), 273–289. http://doi.org/10.1111/pce.12153

424 González-Casado, S., Martín-Belloso, O., Elez-Martínez, P., & Soliva-Fortuny, R. (2018).
425 Enhancing the carotenoid content of tomato fruit with pulsed electric field treatments:
426 Effects on respiratory activity and quality attributes. *Postharvest Biology and*
427 *Technology*, *137*(July 2017), 113–118.
428 http://doi.org/10.1016/j.postharvbio.2017.11.017

429 González-Casado, S., Martín-Belloso, O., Elez-Martínez, P., & Soliva-Fortuny, R. (2018).

Application of pulsed electric fields to tomato fruit for enhancing the bioaccessibility of carotenoids in derived products. *Food and Function*, DOI: 10.1039/c7fo01857f.

Hedges, L. J., & Lister, C. E. (2005). Nutritional attributes of tomatoes. *Crop & Food Research Confidential Report No. 1391 1-94*,

Hodges, D. M., & Toivonen, P. M. A. (2008). Quality of fresh-cut fruits and vegetables as affected by exposure to abiotic stress. *Postharvest Biology and Technology*, *48*(2), 155–162. http://doi.org/10.1016/j.postharvbio.2007.10.016

Ilahy, R., Hdider, C., Lenucci, M. S., Tlili, I., & Dalessandro, G. (2011). Antioxidant activity and bioactive compound changes during fruit ripening of high-lycopene tomato cultivars. *Journal of Food Composition and Analysis*, *24*(4–5), 588–595.

Javanmardi, J., & Kubota, C. (2006). Variation of lycopene, antioxidant activity, total soluble solids and weight loss of tomato during postharvest storage. *Postharvest Biology and Technology*, *41*(2), 151–155. http://doi.org/10.1016/j.postharvbio.2006.03.008

Kader, A., & Lindberg, S. (2010). Cytosolic calcium and pH signaling in plants under salinity stress. *Plant Signaling & Behavior*, *5(3)*, 233–238.

Lana, M. M., Tijskens, L. M. M., & Van Kooten, O. (2005). Effects of storage temperature and fruit ripening on firmness of fresh cut tomatoes. *Postharvest Biology and Technology*, *35*(1), 87–95. http://doi.org/10.1016/j.postharvbio.2004.07.001

Lebovka, N. I., Praporscic, I., & Vorobiev, E. (2004). Effect of moderate thermal and pulsed electric field treatments on textural properties of carrots, potatoes and apples. *Innovative Food Science and Emerging Technologies*, *5*(1), 9–16.

Lu, S., & Li, L. (2008). Carotenoid metabolism: Biosynthesis, regulation and beyond. *Journal of Integrative Plant Biology*, *50*(7), 778–785.

454 Luengo, E., Condón-Abanto, S., Álvarez, I., & Raso, J. (2014). Effect of pulsed electric
455 field treatments on permeabilization and extraction of pigments from Chlorella
456 vulgaris. *The Journal of Membrane Biology*, *247*(12), 1269–1277.

457 Martín-Belloso, O., & Elez-Martínez, P. (2005). Enzymatic inactivation by pulsed electric
458 fields. In Da-Wen Sun (Eds.), *Emerging Technologies for Food Processing*, 155–181.
459 http://doi.org/10.1016/B978-012676757-5/50009-8

460 Martín Belloso, O., & Soliva Fortuny, R. (2011). Pulsed electric fields processing basics.
461 In Zhang, H. Q., Barbosa-Cánovas, G. V., Balasubramaniam V. M et al (Eds.),
462 *Nonthermal Processing Technologies for Food*. Blackwell Publishing Ltd., 157–175.

463 Odriozola-Serrano, I., Soliva-Fortuny, R., Hernández-Jover, T., & Martín-Belloso, O.
464 (2009). Carotenoid and phenolic profile of tomato juices processed by high intensity
465 pulsed electric fields compared with conventional thermal treatments. *Food*
466 *Chemistry*, *112*(1), 258–266.

467 Odriozola-Serrano, I., Soliva-Fortuny, R., & Martín-Belloso, O. (2009). Impact of high-
468 intensity pulsed electric fields variables on vitamin C, anthocyanins and antioxidant
469 capacity of strawberry juice. *LWT - Food Science and Technology*, *42*(1), 93–100.
470 http://doi.org/10.1016/j.lwt.2008.05.008

471 Požrl, T., Žnidarčič, D., Kopjar, M., Hribar, J., & Simčič, M. (2010). Change of textural
472 properties of tomatoes due to storage and storage temperatures. *Journal of Food,*
473 *Agriculture and Environment*, *8*(2), 292–296.

474 Rakhmankulova, Z. F., Fedyaev, V. V, Podashevka, O. A., & Usmanov, I. Y. (2003).
475 Alternative respiration pathways and secondary metabolism in plants with different
476 adaptive strategies under mineral deficiency. *Russian Journal of Plant Physiology*,
477 *50*(2), 206–212.

478 Rodríguez-Roque, M. J., Rojas-Graü, M. A., Elez-Martínez, P., & Martín-Belloso, O.
479 (2013). Changes in vitamin C, phenolic, and carotenoid profiles throughout in vitro
480 gastrointestinal digestion of a blended fruit juice. *Journal of Agricultural and Food*
481 *Chemistry*, *61*(8), 1859–1867. http://doi.org/10.1021/jf3044204

482 Shayanfar, S., Chauhan, O., Toepfl, S., & Heinz, V. (2013). The interaction of pulsed
483 electric fields and texturizing - antifreezing agents in quality retention of defrosted
484 potato strips. *International Journal of Food Science and Technology*, *48*(6), 1289–
485 1295.

486 Soliva-Fortuny, R., Balasa, A., Knorr, D., & Martín-Belloso, O. (2009). Effects of pulsed
487 electric fields on bioactive compounds in foods: a review. *Trends in Food Science &*
488 *Technology*, *20*(11–12), 544–556.

489 Soliva-Fortuny, R., Vendrell-Pacheco, M., Martín-Belloso, O., & Elez-Martínez, P. (2017).
490 Effect of pulsed electric fields on the antioxidant potential of apples stored at different
491 temperatures. *Postharvest Biology and Technology*, 132, 195-201,
492 http://doi.org/10.1016/j.postharvbio.2017.03.015

493 Tadesse, T. N., Ibrahim, A. M., & Abtew, W. G. (2015). Degradation and formation of
494 fruit color in tomato (Solanum lycopersicum L.) in response to storage temperature.
495 *American Journal of Food Technology*, *10*(4), 147–157.
496 http://doi.org/10.3923/ajft.2015.147.157

497 USDA. (1991). United States Standards for Grades of Fresh Tomatoes.

498 Vallverdú-Queralt, A., Oms-Oliu, G., Odriozola-Serrano, I., Lamuela-Reventós, R. M.,
499 Martín-Belloso, O., & Elez-Martínez, P. (2012). Effects of pulsed electric fields on
500 the bioactive compound content and antioxidant capacity of tomato fruit. *Journal of*
501 *Agricultural and Food Chemistry*, *60*, 3126–3134.

Vallverdú-Queralt, A., Oms-Oliu, G., Odriozola-Serrano, I., Lamuela-Raventós, R. M., Martín-Belloso, O., & Elez-Martínez, P. (2013). Metabolite profiling of phenolic and carotenoid contents in tomatoes after moderate-intensity pulsed electric field treatments. *Food Chemistry*, *136*(1), 199–205.

Vinha, A. F., Barreira, S. V. P., Castro, A., Costa, A., & Oliveira, M. B. P. P. (2013). Influence of the storage conditions on the physicochemical properties, antioxidant activity and microbial flora of different tomato (Lycopersicon esculentum L.) cultivars. *Journal of Agricultural Science*, *5*(2), 118–129. http://doi.org/10.5539/jas.v5n2p118

Vorobiev, E., & Lebovka, N. I. (2006). Extraction of intercellular components by pulsed electric fields. In Raso, J. and Heinz, V. (Eds.), *Pulsed Electric Fields Technology for the Food Industry*, 153–193.

Vorobiev, E., & Lebovka, N. (2009). Pulsed-electric-fields-induced effects in plant tissues: fundamental aspects and perspectives of applications. *Food Biotechnology*. http://doi.org/10.1007/978-0-387-79374-0 2

Žnidarčič, D., & Požrl, T. (2006). Comparative study of quality changes in tomato cv. Malike (Lycopersicon esculentum Mill .) whilst stored at different temperatures. *Acta Agriculturae Slovenica*, *87*(september), 235–243.

FIGURE CAPTIONS

Figure 1. Individual carotenoids in tomatoes as affected by PEF treatments and subsequent storage temperature. A: phytoene; B: phytofluene; C: lycopene; D: β-carotene; E: γ-carotene; F: δ-carotene; G: lutein; H: total carotenoids. Values are means ± standard deviation (n = 8).

Figure 2. Colour (A: lightness and B: hue angle) of tomato fruits as affected by PEF treatments and subsequent storage temperature. Values are means ± standard deviation (n = 8).

Figure 3. Firmness of tomato fruits as affected by PEF treatments and subsequent storage temperature. Values are means ± standard deviation (n = 8).

Figure 4. Total soluble solids content (%) of tomato as affected by PEF processing and subsequent storage temperature. Values are means ± standard deviation (n = 8).

Figure 5. pH of tomato as affected by PEF processing and subsequent storage temperature. Values are means ± standard deviation (n = 8).

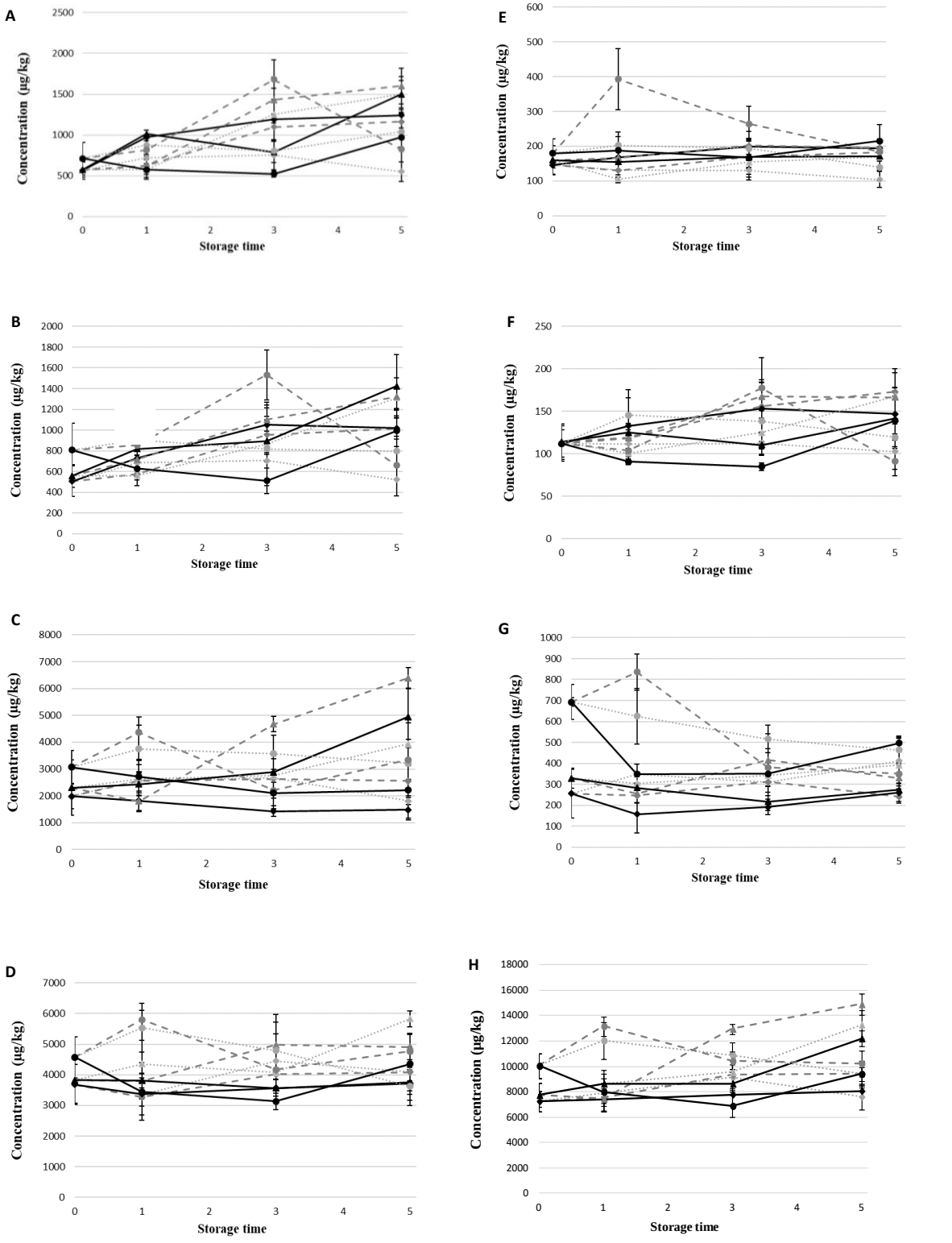
A
Concentration (µg/kg)
Storage time
B
Concentration (µg/kg)
Storage time
C
Concentration (µg/kg)
Storage time
D
Concentration (µg/kg)
Storage time
E
Concentration (µg/kg)
Storage time
F
Concentration (µg/kg)
Storage time
G
Concentration (µg/kg)
Storage time
H
Concentration (µg/kg)
Storage time
Untreated - 4ºC
Untreated - 12ºC
Untreated - 20ºC
0.02 kJ/kg - 4ºC
0.02 kJ/kg - 12ºC
0.02 kJ/kg - 20ºC
0.38 kJ/kg - 4ºC
0.38 kJ/kg - 12ºC
0.38 kJ/kg - 20ºC

Figure 1.

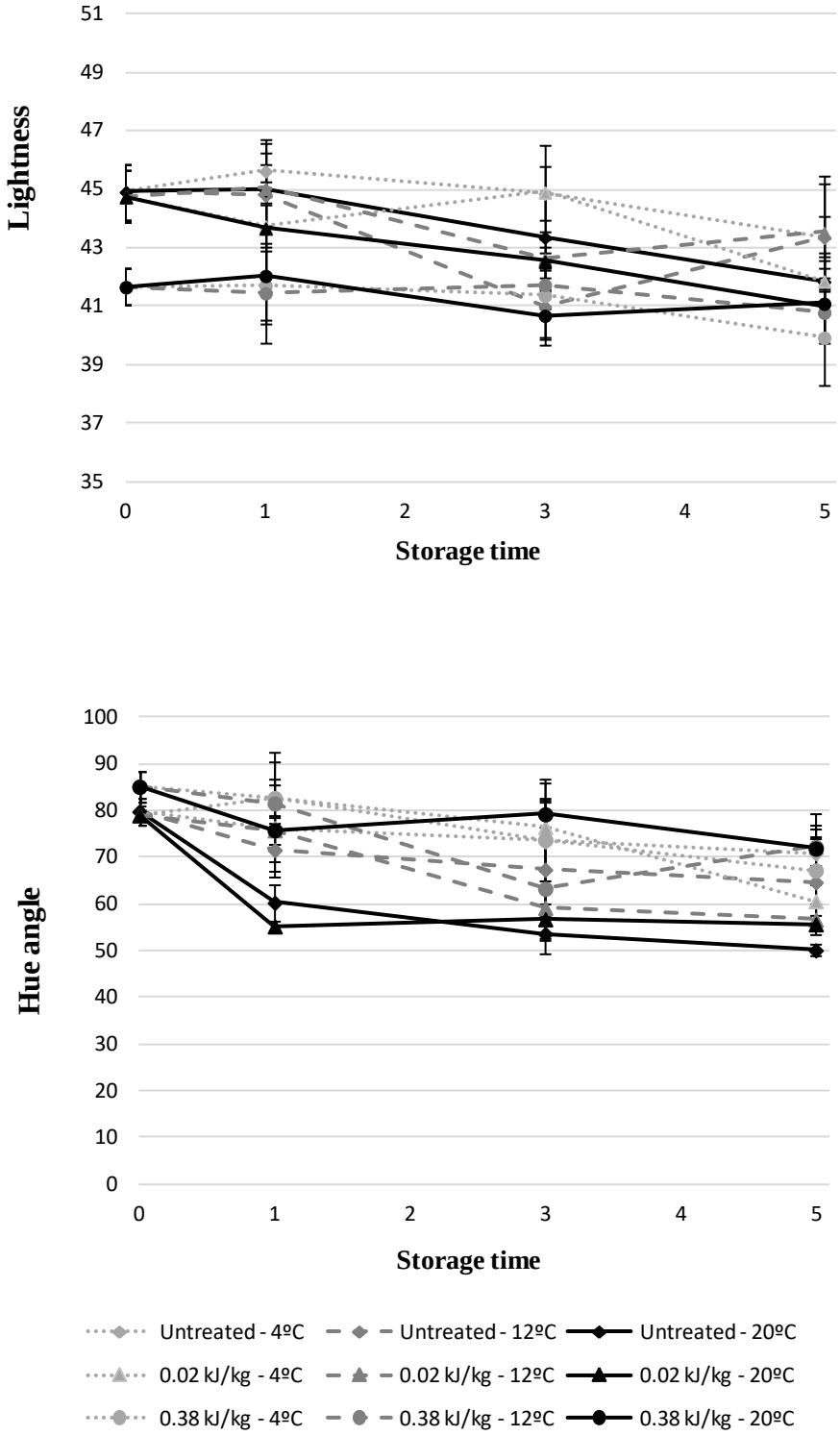

Figure 2.

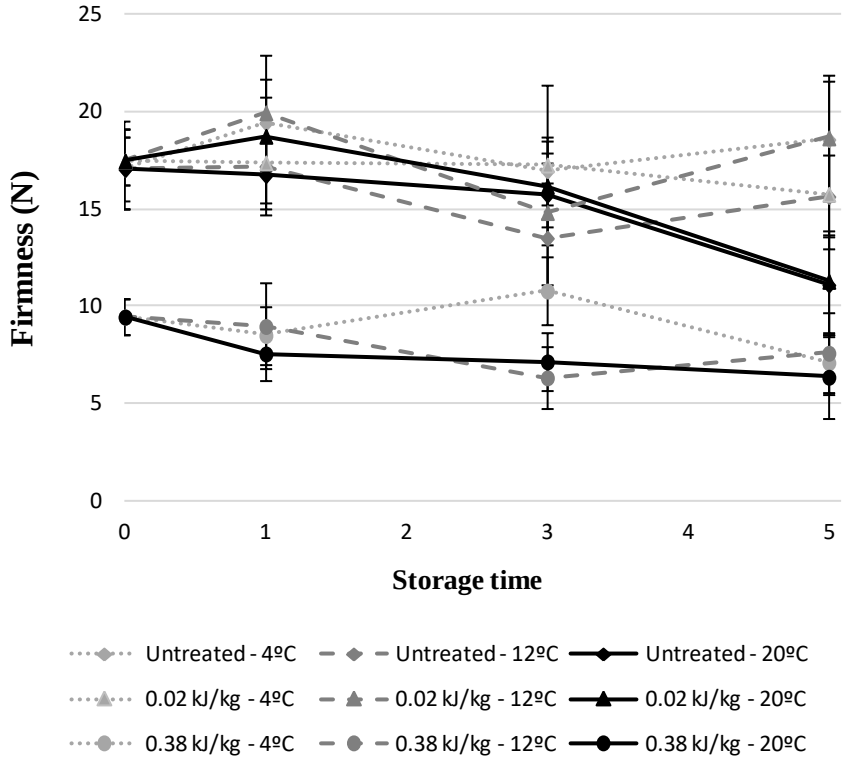
25
20
15
10
5
0
Firmness (N)
0
1
2
3
4
5
Storage time
Untreated - 4ºC
Untreated - 12ºC
Untreated - 20ºC
0.02 kJ/kg - 4ºC
0.02 kJ/kg - 12ºC
0.02 kJ/kg - 20ºC
0.38 kJ/kg - 4ºC
0.38 kJ/kg - 12ºC
0.38 kJ/kg - 20ºC

Figure 3.

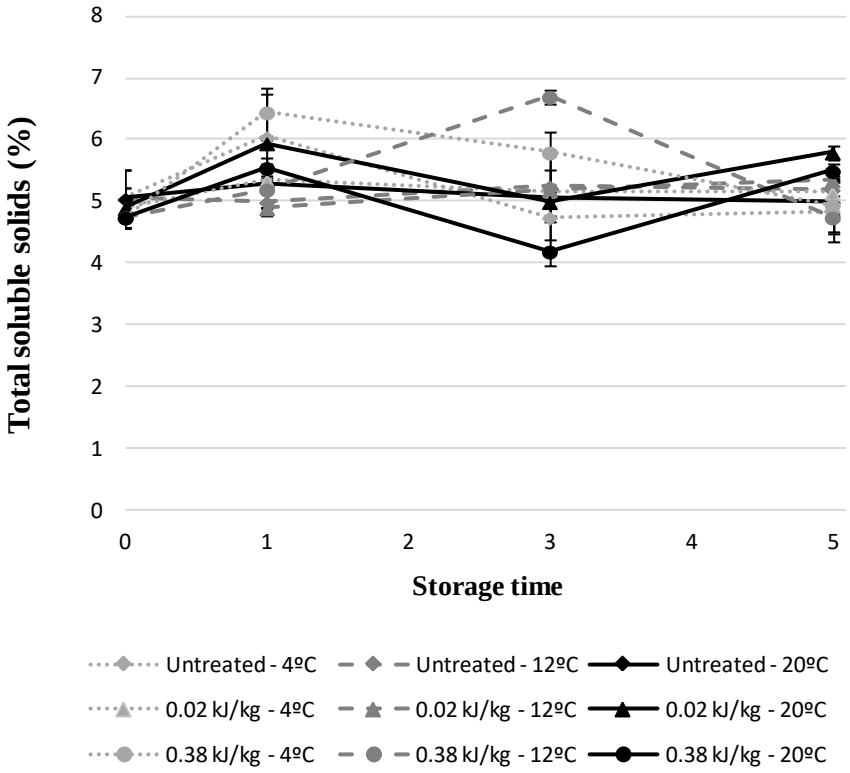
Total soluble solids (%)
8
7
6
5
4
3
2
1
0
0
1
2
3
4
5
Storage time
Untreated - 4ºC
Untreated - 12ºC
Untreated - 20ºC
0.02 kJ/kg - 4ºC
0.02 kJ/kg - 12ºC
0.02 kJ/kg - 20ºC
0.38 kJ/kg - 4ºC
0.38 kJ/kg - 12ºC
0.38 kJ/kg - 20ºC

Figure 4.

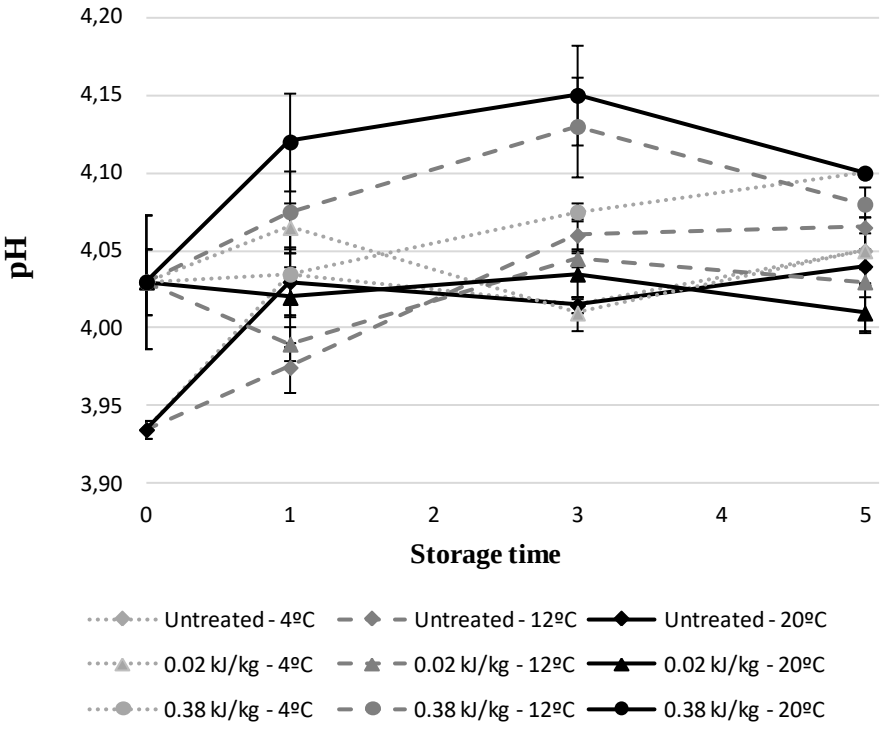
4,20
4,15
4,10
4,05
4,00
3,95
3,90
pH
0
1
2
3
4
5
Storage time
Untreated - 4ºC
Untreated - 12ºC
Untreated - 20ºC
0.02 kJ/kg - 4ºC
0.02 kJ/kg - 12ºC
0.02 kJ/kg - 20ºC
0.38 kJ/kg - 4ºC
0.38 kJ/kg - 12ºC
0.38 kJ/kg - 20ºC

Figure 5.