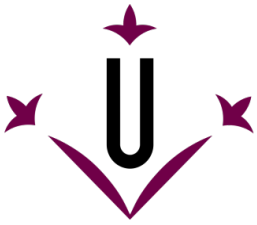

Changes of carotenoid content in carrots after application of pulsed electric field treatments

López-Gámez, Gloria., Elez-Martínez, Pedro, Martín-Belloso, Olga, Soliva-Fortuny, Robert*

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

* Corresponding author: Fax: +34 973 702596; e-mail: robert.soliva@udl.cat

Abstract

Under certain conditions, pulsed electric fields (PEF) can promote the accumulation of phytochemicals through a stress-mediated response in plant tissues. Likewise, electropermeabilization of cell membranes can also improve the extraction of intracellular compounds. The objective of this study was to evaluate the main causes driving the increase in carotenoids content in PEF-treated carrots and to study the impact of those treatments on their relevant quality attributes. Carrots were treated with PEF differing in electric field strength (E) (0.8, 2 and 3.5 kV/cm) and number of pulses (5, 12 and 30). Individual carotenoids content, colour, pH and total soluble solids (TSS) were determined after 0 h and 24 h after PEF treatment. Total and individual carotenoid contents (phytoene and β-carotene) increased just after applying $E \geq 2$ kV/cm, whereas lutein concentration decreased, and that of α-carotene remained similar to that found in untreated carrots. After applying 2 and 3.5 kV/cm, TSS and pH remained unaltered, but cortical browning index increased, which was correlated to carotenoid content. Increase in total carotenoid content is likely related to better extractability, but differences in the content of individual compounds suggest that PEF may also act by modulating their biosynthesis or causing their degradation.

Keywords: Carrot; carotenoids; pulsed electric fields; quality attributes

Abbreviations: PEF, pulsed electric fields; E, electric field strength; TSS, total soluble solids; ROS, reactive oxygen species; Y, luminance of a colour; x, derived parameter of colour tristimulus values; y, derived parameter of colour tristimulus values; L*, lightness; a*, green-red chromaticity; b*, blue-yellow chromaticity; BI, browning index; C*, chroma; h, hue angle; BHT, butyl hydroxytoluene; FW, fresh weight; *r*, Pearson coefficient; η_p^2, partial square eta value.

1. Introduction

Carrot is one of the most consumed root vegetables worldwide, containing ample amounts of carotenoids (e.g. α-carotene, β-carotene, lutein) (Arscott & Tanumihardjo, 2010). Provitamin A such as α-carotene and β-carotene play an important role in human health by acting as a natural antioxidant, protecting tissues from free radicals and against some types of cancer or cardiovascular diseases (Kopec & Failla, 2018; R. M. Schweiggert & Carle, 2017). Besides, lutein intake is essential for the visual system and prevents macular degeneration (Schweiggert & Carle, 2017). Thus, their consumption is highly beneficial for health. However, they are hardly detached from carrot tissues, as cell walls and chromoplast membranes act as barriers that limit their release during digestion (Schweiggert, Mezger, Schimpf, Steingass, & Carle, 2012). Thermal treatments (e.g. blanching, cooking, pasteurization) may be beneficial to facilitate their liberation from the food matrix (Cilla et al., 2018; Mehmood & Zeb, 2020). However, heat also promotes carotenoids degradation and changes in quality attributes such as colour and flavour, especially in low water activity food systems (Maiani et al., 2009), which critically determines consumers' acceptance. Therefore, the search for processing technologies that help to preserve carrots' fresh-like appearance while enhancing its bioactive content and accessibility is a current challenge.

Pulsed electric fields (PEF) is a non-thermal processing technology that involves the delivery of electric energy in the form of short pulses to a food product placed between two electrodes. Its application causes a transmembrane potential difference in biological tissues, which entails the increase in cell permeability through an electropermeabilization mechanism. PEF has been applied on solid food products with the purpose of extracting intracellular metabolites (e.g. pigments or antioxidants) (G. Pataro et al., 2020; Wiktor et al., 2015), enhancing juice yield (El Kantar et al., 2018), as a pre-treatment to soften tissues and facilitate further processing (e.g. cutting or peeling) (Fauster et al., 2018), among others. Despite being a non-thermal technology, structural changes caused by electropermeabilization are likely to impact carrot quality attributes. Therefore, treatments must be optimized in order to minimize these changes and avoid the degradation of bioactive compounds.

Apart from improving the extraction of bioactive compounds, PEF has been recently proposed as a tool to induce stress in plant products or cell cultures, thus stimulating the biosynthesis of bioactive compounds (Jacobo-Velázquez et al., 2017). Some studies have shown that PEF may cause the accumulation of flavonoids and flavan-3-oles in apples (Ribas-Agustí et al., 2019; Soliva-Fortuny et al., 2017), phenolic compounds in grapes (Balaša, 2014) and carrots (López-Gámez et al., 2020a) or carotenoids in tomato (González-Casado et al., 2018b; Vallverdú-Queralt et al., 2013). However, free radical generation during PEF treatment would also be involved in the oxidative degradation of some nutrients (Gianpiero Pataro & Ferrari, 2020). Although the mechanism that triggers PEF bioactive compounds accumulation is not yet fully understood, some hypotheses suggest that disruption of cell membranes, either reversible or irreversible, as well as the presence of free radicals, may elicit a defence response as other types of abiotic stressors (e.g. wounding) (Jacobo-Velázquez et al., 2017). This response would trigger an immediate burst of reactive oxygen species (ROS) (Baxter et al., 2014; Carmody et al., 2016), which act as signals to activate defence mechanisms (Fanciullino et al., 2014). This ultimately would enable obtaining commodities with higher nutritional value. Hence, PEF may be implemented with a dual purpose: 1) to obtain derived products from a commodity with enhanced antioxidant content; 2) to improve the extractability of bioactive compounds, thus enhancing their bioaccessibility.

The main aim of this study was to investigate the feasibility of PEF as a pre-treatment to improve carotenoids content and their extraction and to elucidate the main causes underpinning these changes. Additionally, colour, pH, and total soluble solids (TSS) were evaluated to assess the feasibility of obtaining fresh-like derived products from PEF-treated carrots.

2. Material and methods

2.1. Chemicals and reagents

Phytoene and β-carotene standards were acquired from Carote-Nature (purity ≥ 95 %) (Ostermundigen, Switzerland), α-carotene was purchased from Supelco-Merck (purity ≥ 98 %) (Darmstadt, Germany) and lutein from Acros Organics (purity 90%) (New Jersey, USA).

2.2. Carrot samples

Carrots (*Daucus carota* cv. Nantes) were purchased in a local supermarket (Lleida, Spain). They were selected based on their uniform size and shape (caliber 25/35 mm and length 17 ± 2 cm), avoiding those with visual defects. Carrots were stored in darkness at 4 ºC, within a week until processing. Before treatments, carrots were washed with tap water to remove any soil residues and debris, and the excess of water was removed with a paper cloth.

2.3. Pulsed electric field (PEF) treatments

PEF treatments (**Table 1**) were conducted in a batch PEF system (Physics International, San Leandro, CA, USA) equipped with a TG-70 gas control unit and a PT55 pulse generator (Pacific Atlantic Electronics Inc., El Cerrito, CA, USA). The equipment delivers exponential-wave pulses of 4 μs from a capacitor of 0.1 μF at a fixed frequency of 0.1 Hz. Since 0, 5, 12 and 30 pulses were applied, treatment times were 0, 20, 48 and 120 μs, respectively. Whole carrots (0.11 ± 0.01 kg) were placed individually in the treatment chamber, which consists of a parallelepiped methacrylate container with two parallel stainless-steel electrodes (20 × 5 cm) separated by a gap of 5 cm. The chamber was filled with an aqueous solution containing NaCl (conductivity of 10 μS cm^{-1}), in which the ratio carrot:aqueous solution was 1:3 (w:v). The specific energy input (Ws), expressed in kJ/kg, was calculated according to Eq. (1) (Wiktor et al., 2015):

$$Ws = \frac{V^2 Cn}{2m} \quad (1)$$

where V, C, n, and m are the voltage, capacitance of the energy storage capacitor, number of pulses and mass of the sample in the treatment chamber, respectively. The temperature of the treatment water was measured before and immediately after PEF application and it did not significantly increase (18.0 ± 1.0 ºC).

Untreated and PEF-treated carrots were characterized in terms of carotenoid contents and quality attributes just after processing (0 h) or after 24 h at 4 °C in darkness. These conditions were based

on previous studies which determined that phenolic content peaked at such time (López-Gámez et al., 2020a). For the analysis of carotenoids, carrots were frozen at -40 ºC at their corresponding post-treatment times (0 h or 24 h). Before freezing carrots, their top ends (2.5 ± 0.5 cm) were removed, and the rest was cut in 1 cm-thick slices. Just before carotenoid extraction, carrots were crushed (Moulinex, 700 W) to overcome heterogeneity generated by different locations of the product within the PEF treatment chamber.

2.4. Quality attributes

Colour. The colour of peel, cortical and vascular tissues was measured by using a colorimeter (CR-400, Konica Minolta, Osaka, Japan). A white standard plate (Y = 94.00, x = 0.3158, y = 0.3322) was used for calibration. Three measurements of each specific tissue were taken, and each measurement represents the average of three readings. The apparatus had an aperture size of 10 mm and it was set up for a D65 illuminant, 10° observer angle. CIEL a* and b* parameters were used to calculate chroma (C*) [Eq. (2)], hue angle (h) [Eq. (3)] and browning index (BI) [Eq. (4)] according to Huang et al., (2019).

$$C^* = (a^{*2} + b^{*2})^{1/2} \quad (2)$$

$$h = \arctan \frac{b^*}{a^*} \quad (3)$$

$$BI = \frac{100\,(X - 0.31)}{0.17} \quad (4)$$

$$\text{Where } X = \frac{a^* + 1.75L^*}{5.645L^* + a^* - 3.012b^*}$$

pH. Previous to pH measurements, whole carrots were cut into 1 cm-thick slices and ground in a blender to obtain a homogeneous sample, which was used for determining the pH values. A pH-meter (Crison 2001, Crison Instruments S.A., Alella, Barcelona, Spain) was used.

Total soluble solids (TSS). The same homogenate obtained for pH determination was used for measuring total soluble solids. TSS was determined by refractometry (RX-1000, Atago, Tokyo, Japan) at 25 ºC and results were expressed as % of total soluble solids.

2.5. Carotenoids extraction

146 Total carotenoids were extracted according to the method described by Sadler, Davis, & Dezman
147 (1990), with slight modifications. Homogenized carrot tissue (1 g) was stirred for 20 minutes with
148 50 mL of hexane:acetone:ethanol (50:25:25) solution containing 1 $g \cdot L^{-1}$ BHT. Then, 15 mL of
149 NaCl [10 % (w/v)] solution was added and the samples were stirred for 10 additional minutes.
150 Samples were left to stand for ≥ 3 minutes, and the upper organic phase was analyzed by high-
151 performance liquid chromatography (HPLC). All extractions were performed in duplicate, and
152 samples were protected from light throughout extraction and analysis to avoid carotenoid
153 degradation and isomerization.

154 **2.6. Identification and quantification of individual carotenoids**

155 Carotenoids were quantified by HPLC, following a procedure validated by Cortés, Esteve,
156 Frígola, & Torregrosa (2004). An aliquot of 20 µL of the extracted samples was injected into the
157 HPLC system, which was equipped with a 600 Controller, a 486 Absorbance Detector, a
158 thermostatic column compartment, and a 717 Plus Auto Sampler with a cooling system (Waters,
159 Milford, MA). Carotenoids were separated using a reverse-phase C18 Spherisorb ODS2 (5 µm)
160 stainless steel column (4.6 mm x 250 mm). The mobile phase consisted of methanol/ammonium
161 acetate 0.1 M, milli-Q water, methyl tert-butyl ether, and methanol. The flow rate was fixed at 1
162 mL/min and the total run time was 60 min. The column was set at 30 ºC, while sample amber
163 vials on the autosampler were preserved at 4 ºC. Carotenoids were identified by UV–vis spectral
164 data and their retention times (Cortés et al., 2004) and quantified by integration of the peak areas.
165 Data were compared to calibration curves and results were expressed on a fresh weight basis as
166 mg of carotenoid compound in 100 g of carrot.

167 **2.7. Statistical analysis**

168 All experiments were conducted in triplicate, and results were reported as the mean ± standard
169 deviation (SD). Statistical analysis was carried out using IBM SPSS Statistics 21 software (SPSS
170 Inc., Chicago, IL) and SigmaPlot 11.0 (Systat Software Inc, Chicago, IL, USA). Results were
171 subjected to an analysis of variance (ANOVA) followed by Tukey *post hoc* test to establish
172 statistical differences among mean values. In order to establish differences between carotenoid

content at 0 h and 24 h, a two-way ANOVA was performed. The dependent variable was carotenoid content, whereas time and PEF treatments were those independent. The relationship between variables was determined using the Pearson (r) coefficient and the calculation of the partial square eta value (η_p^2) was reported to indicate the effect size of each processing parameter. The significance of differences was defined at $p < 0.05$.

3. Results and discussion

3.1. Carotenoid profile of carrots

The effect of PEF on total and individual carotenoid content is displayed in **Fig. 1** and **Fig. 2**, respectively. The total carotenoid contents of carrots were significantly affected by PEF treatment as a function of processing parameters. Statistical analysis indicated that electric field strength was the main parameter affecting total carotenoid concentrations ($\eta_p^2 = 0.727$; $p < 0.001$). Total content increased just after applying $E \geq 2$ kV/cm (**Fig. 1**). Hence, the highest carotenoid content (83.8 %) compared to untreated carrots, was obtained just after applying 5 pulses of 3.5 kV/cm (0.61 kJ/kg). After 24 h of treatments, carotenoid content increased 0.72- and 1.13-fold in carrots treated with 12 pulses of 3.5 kV/cm (1.92 kJ/kg) and 5 pulses of 2 kV/cm (0.22 kJ/kg), respectively. Nevertheless, carrots subjected to other PEF conditions had similar carotenoid contents to untreated carrots.

Statistical analyses showed that changes in individual carotenoid content were mainly due to applied electric field strength [lutein ($\eta_p^2 = 0.914$; $p < 0.001$), phytoene ($\eta_p^2 = 0.718$; $p < 0.001$), β-carotene ($\eta_p^2 = 0.674$; $p < 0.001$) and α-carotene ($\eta_p^2 = 0.442$; $p < 0.01$)]. Phytoene and β-carotene increased just after treatments of $E \geq 2$ kV/cm. PEF-treated carrots had similar α-carotene content as those untreated, excepting carrots treated with 5 pulses of 3.5 kV/cm (0.61 kJ/kg), where a 63.3 % increase was observed (**Fig. 2**). On the other hand, lutein content decreased between 15.1 % and 96.4 % when electric field strengths of 0.8 kV/cm and 2 kV/cm were applied. Furthermore, carotenoid content considerably changed 24 h after PEF treatments and differed depending on the individual compound. Carrots treated with 3.5 kV/cm or 2 kV/cm (0.22 kJ/kg

and 1.19 kJ/kg) exhibited 1.05 - 1.57 times more phytoene than those untreated. Carrots treated with 12 pulses of 3.5 kV/cm (1.92 kJ/kg) showed twice as much β-carotene content than those untreated, although other treatments did not cause any significant change in their concentration (**Fig. 2**). Besides, α-carotene contents increased 24 h after applying 5 pulses of 0.8 kV/cm (0.14 kJ/kg) or 2 kV/cm and 3.5 kV/cm (1.92 kg/kg or 3.93 kJ/kg), whereas lutein decreased when applying electric field strengths of 0.8 kV/cm or 2 kV/cm (0.22 kJ/kg and 0.5 kJ/kg).

PEF treatment promotes different reactions in carrot tissue that could result in carotenoid content modifications. On the one hand, pore formation and structural rearrangements caused by PEF application may enhance the diffusion of solutes located inside the cells (Barba et al., 2015). Carrot carotenoids are usually stored in crystalline form in chromoplasts. Hence, the main barriers limiting their release are cell walls, membranes, and chromoplasts (Schweiggert et al., 2012). PEF-treated carrots ($E \geq 2$ kV/cm) showed higher levels of total carotenoids than untreated carrots immediately after treatment, which suggests an enhancement of their extractability. These results would be in accordance with those reported by Wiktor et al., (2015), in which carrots subjected to 3 and 5 kV/cm treatments had higher conductivity than those untreated, suggesting increased cell permeability. Likewise, other authors attributed the instant increase of total carotenoids to the mechanical disruption of carrot cell walls (Cuéllar-Villarreal et al., 2016; Nowacka & Wedzik, 2016).

On the other hand, electropermeabilization could also cause ROS generation, a secondary signal belonging to the defence response of plants to stress (Elez-Martínez et al., 2017), which are necessary to synthesize secondary metabolites (e.g. carotenoids) (Fanciullino et al., 2014). The literature has shown that stresses such as drought (Sarker & Oba, 2018d, 2018c, 2018e) and salinity (Sarker et al., 2018; Sarker & Oba, 2018a, 2018f, 2020) increased the β-carotene and carotenoids content in amaranth since carotenoids scavenge singlet oxygen and lipid peroxy-radicals, and inhibit lipid peroxidation and superoxide generation under stress (Sarker & Oba, 2018c, 2018b). Some authors have also reported an accumulation of carotenoid content after submitting tomatoes to PEF treatments and attributed this increment to stress response (González-

Casado et al., 2018a; Vallverdú-Queralt et al., 2013). In addition, López-Gámez et al., (2020b) reported changes in phenylpropanoid metabolism and modifications of respiration rate in PEF-treated carrots, which are indicatives of stress induction. As stress signals are detected, the carotenoid biosynthetic pathway is upregulated, thus triggering carotenoids accumulation (Nisar et al., 2015). If a better extractability was the main reason behind the observed increment in total content, all individual compounds would also be better extracted. However, lutein content decreased whereas that of β-carotene increased for the same treatment conditions (e.g., 5 and 12 pulses of 2 kV/cm) and α-carotene content remained unaltered (**Fig. 2**). Furthermore, significant correlations were found between the applied specific energy and some carotenoid contents, such as phytoene (r = 0.761; p < 0.001), lutein (r = 0.648; p < 0.001) or α-carotene (r = 0.402; p < 0.05). The carotenoid biosynthetic pathway in plants has a bifurcation step after lycopene, the two branches are distinguished by different cyclic end-groups. Two beta rings lead to the β, β branch (β-carotene and its derivatives), whereas one beta and one epsilon ring define the β, ε branch (α-carotene and its derivatives). As lutein is generated in the β, ε- branch and β-carotene in β, β-branch (Cunningham & Gantt, 1998), results could suggest modulation of lycopene β-cyclase (βLCY) and lycopene ε-cyclase (εLCY) enzyme activities. In this line, Ramos-Parra et al., (2019) reported that after the application of another non-thermal technology, high hydrostatic pressure (HHP), papaya fruit accumulated carotenoids during storage (4 ºC for 2 days) and supported these results with gene expression of enzymes involved in the carotenoid biosynthetic pathway.

The differences observed in the accumulation trend of individual carotenoids together with negligible softening (López-Gámez et al., 2020a), indicate that changes in the extractability of carotenoids cannot be the only reason explaining carotenoid accumulation. Carotenoid losses observed, for example, after applying 30 pulses of 3.5 kV/cm, could also occur as a result of their release throughout irreversible formed pores or their sensitivity to oxidative species generated (Fratianni et al., 2019; Wiktor et al., 2015). Therefore, carotenoid content obtained in PEF-treated carrots is the result of triggered events that enhance their content and extractability and the factors involving their degradation. This would explain that similar carotenoid contents were obtained

after applying treatments differing in their intensity (e.g., 1.92 kJ/kg and 0.22 kJ/kg). A high intensity would probably cause significant structural changes in cell walls or chromoplasts location leading to improve carotenoids extractability, whereas a lower intensity could trigger a stress defence response that triggers their biosynthesis to a greater extent than extractability.

The increment of carotenoid content could be related to a stress defence response induced by PEF. Further studies on gene expression of enzymes involved in carotenoid biosynthesis [e.g. phytoene synthase (PSY)] (Ramos-Parra et al., 2019) would be required for confirmation of present findings.

3.2. Carrot quality-related parameters and their correlation with carotenoid content

Colour changes in three different tissues (peel, cortical and vascular tissues), TSS, and pH of untreated and PEF-treated carrots are shown in **Table 2**. PEF processing did not trigger severe changes regarding pH and TSS. Only just after the application of 30 pulses of 2 kV/cm (1.19 kJ/kg) and 24 h after applying 12 pulses of 3.5 kV/cm (1.92 kJ/kg) a slight TSS decrease (11 %) was observed. Moreover, pH did not significantly vary between untreated (pH 6.2) and PEF-treated (pH 5.9 - 6.4) carrots. Conflicting information about these parameters in PEF-treated solid food is available. For instance, Wang et al., (2020) reported an increase in º Brix of PEF-treated apple peels (1.2 kV/cm). These results are in agreement with González-Casado et al., (2018), who disclosed increases in TSS (0.83 – 2.31 kJ/kg) and pH (0.09 – 2.31 kJ/kg) of PEF-treated tomatoes. TSS changes were attributed to cell membrane breakage, which may indirectly cause an osmotic imbalance and entail the increment of solids in tissues. Alternatively, they also proposed a faster ripening triggered by an increase in ethylene synthesis. Conversely, Ribas-Agustí et al., (2019) reported no alteration in TSS of PEF-treated apples, excepting those treated with the lowest energy (0.01 kJ/kg) and stored 24 h, which was attributed to the metabolic accumulation of organic acids and sugars. In these examples, changes in TSS might be related to the acceleration in their metabolism and climacteric ripening. Modifications in TSS of carrots, being non-climacteric vegetables, maybe due to changes in membrane permeability, which may entail solid gain or water loss during PEF treatments or a better extraction of sugars and other

280 water-soluble compounds. Regarding pH, Ribas-Agustí et al., (2019) reported an increase in
281 apples subjected to treatments of 1.8 and 7.3 kJ/kg. On the other hand, Leong and Oey, (2014)
282 did not observe any remarkable alteration in carrot puree treated by applying different specific
283 energies (1.08 – 516.28 kJ/kg) neither Balaša, (2014) in PEF-treated (< 66.5 kJ/kg) *Vitis vinifera*
284 cell cultures.

285 In general, colour differences among treated and untreated carrots were not significant, although
286 some differences in BI of cortical and vascular tissues were noticed in carrots treated with
287 different intensities. For instance, carrots treated by 0.8 kV/cm (0.38 kJ/kg and 0.87 kJ/kg)
288 showed lower BI than those treated by 2 kV/cm or 3.5 kV/cm. Statistical analysis showed that
289 electric field strength ($\eta_p^2 = 0.437$; $p = 0.006$), number of pulses ($\eta_p^2 = 0.335$; $p = 0.025$) and their
290 interaction ($\eta_p^2 = 0.493$; $p = 0.012$) exerted a significant effect on the BI of vascular tissues,
291 whereas cortical BI was mainly affected by electric field strength applied ($\eta_p^2 = 0.679$; $p < 0.001$).
292 Some authors have reported browning increase after submitting products to PEF (González-
293 Casado et al., 2018b; Ribas-Agustí et al., 2019) and they attributed these changes to cell
294 membrane disruption and greater contact between released phenolic substances and their
295 oxidative enzymes (e.g. polyphenol oxidase (PPO), peroxidase (POD)). Since PPO is mostly
296 present in carrot peel than in inner tissues (Alegria et al., 2016), changes in cortical BI may be
297 likely related to carotenoid content, which is mainly stored in secondary phloem (Ahmad et al.,
298 2019). These results are consistent with statistical correlations found between cortical BI and
299 phytoene ($r = 0.638$; $p = 0.047$) and β-carotene ($r = 0.683$; $p = 0.029$) contents. Besides, a
300 correlation was also found between cortical h and lutein contents ($r = 0.701$; $p = 0.024$).

301 On the other hand, C* and h were not affected by PEF treatments in any of the studied tissues.
302 However, a downward trend was observed in peel C* ($r = -0.467$; $p = 0.014$) and h ($r = -0.434$;
303 $p = 0.024$) values when increasing the applied specific energy. Changes in C* may suggest more
304 dullness in tissue because of electropermeabilization and leakage of intracellular content whereas
305 modifications in h could be associated with an increment in redness, supported by the increase in
306 carotenoids (**Fig. 1** and **Fig. 2**). Some authors have also reported maintenance of C* and h

parameters in PEF-treated blueberries (Jin et al., 2017), pumpkins (García-Parra et al., 2018), and juices obtained from just PEF-treated grapes (Leong et al., 2016). However, other studies found significant decreases in C* and increases in h of PEF-treated apples or alterations in a* and b* values of PEF-treated carrots depending on electric field strength applied (Wiktor et al., 2015). Discordant results are probably related to the intrinsic characteristics of each matrix and differences in processing parameters to which they are subjected. In this line, more intense colour changes may occur when PEF causes the irreversible formation of pores, but they can be reversed if pores are resealed. Furthermore, it also depends on the content of coloured pigments, considering that they may be enhanced or degraded because of the treatment intensity. Finally, the extent of activation of enzymes related to browning (Odriozola-Serrano et al., 2013) could also be decisive in colour modification.

4. Conclusions

PEF treatments positively influenced carrots quality, since carotenoid contents were enhanced without major changes in colour, pH, or TSS for at least 24 h. Just after PEF treatments ($E \geq 2$ kV/cm) total carotenoids (39.3 – 81.3 %), phytoene (45.5 – 95.4 %) and β-carotene (36 – 91.2 %) increased, whereas lutein was decreased (15.1 – 96.4 %). Additionally, significant correlations were found between applied specific energy input and lutein and phytoene contents. Obtained results suggest that PEF treatments could enhance their extractability due to electropermeabilization. On the other hand, ROS generation could also promote the degradation of certain compounds or trigger a stress defence response that affects the carotenoid biosynthesis pathway. In order to confirm this alternative, further studies related to enzyme activity involved in their synthesis are necessary. Obtained results demonstrate that PEF can be effectively exploited to enhance carotenoids extractability and/or content without altering carrot quality attributes. Furthermore, PEF stands as a potential pre-treatment to obtain derived products (juices or purees) with enhanced antioxidant content.

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