



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 °C 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_{O₂} and R_{CO₂}, 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.

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

In concomitance with the acceleration of tomato metabolism after the application of PEF, several changes in quality attributes may be

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-diphenyl-1-picrylhydrazyl; R_{O₂}, oxygen consumption; R_{CO₂}, 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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affected. It is known that PEF can strongly affect the tissue firmness of fruit and vegetables, such as carrots, potatoes and apples, because of its action at the cell membrane level (Lebovka et al., 2004; Shayanfar et al., 2013). Moreover, plant secondary metabolites are known to contribute to colour, flavour and taste of the foods (Balasa et al., 2011). All these parameters determine the final quality of tomato fruit, and hence, their end use or even their acceptance by consumers. However, to the best of our knowledge, there are no previous studies aimed at evaluating the effect of the application of PEF treatments on quality attributes of whole fruit and vegetables.

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

2. Materials and methods

2.1. Reagents

Butyl hydroxytoluene (BHT) was acquired from Scharlau Chemie S.A. (Barcelona, 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 (*Lycopersicon 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 colour 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 colour 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 × 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 et al. (2014b) and is displayed in Table 1. Untreated and PEF-treated tomatoes were immediately stored at 4 °C for 24 h, as previously described by Queralt et al. (2013a, 2013b); . Respiratory activity and

Table 1
PEF-processing treatment 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

physicochemical properties of tomatoes were then measured. Afterwards, tomatoes were ground for 20 s in a blender (Solac Professional Mixer 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 Odrizola-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 xg for 15 min at 4 °C 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 and 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 Eq. (1):

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

where A_{470} is the absorbance at 470 nm, V is the total volume of extract (mL), $A_{1\text{cm}}^{1\%}$ 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 et al. (2002). The absorbance of the extracts was measured at 503 nm using hexane as a blank. LC was calculated according to Eq. (2).

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

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 (17.2×10^4 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 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 °C. Changes in the composition of the headspace were measured twice using a gas analyser

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