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

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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 °C) 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 °C than in those fruits stored at 4 or 20 °C. 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 °C 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 °C 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.

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 and 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 et al., 2011; Vallverdú-Queralt et al., 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 et al., 2012; Martín-Belloso and Elez-Martínez, 2005), extraction of intracellular compounds (Luengo et al., 2014; Vorobiev and Lebovka, 2006), preservation of certain food components (Odriozola-Serrano et al., 2009a), 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 et al., 2018a,b; Soliva-Fortuny et al., 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

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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conditions (Balasa et al., 2011; Fanciullino et al., 2014). Preliminary studies have demonstrated the feasibility of applying PEF treatments to trigger the accumulation of some phytochemicals. González-Casado et al. (2018a) observed a maximum 1.5-fold increase in the carotenoids content of tomato fruits stored at 4 °C for 24 h after the application of 30 pulses at 200 kV m^{-1}. In addition, Soliva-Fortuny et al. (2017) noticed that the application of PEF treatments produced significant greater concentration of phenolic compounds in apples. Nevertheless, together with the stress-adaptive response to PEF, several changes in quality attributes could be triggered (González-Casado et al., 2018a). It is known that PEF may strongly affect the structural integrity of cell walls, and hence the firmness of fruits and vegetables (Lebovka et al., 2004; Shayanfar et al., 2013). This fact could lead to undesirable effects on the final quality of tomato fruits.

Stress response in plant tissues is thought to be affected by internal and external factors (Hodges and Toivonen, 2008). On the one hand, the internal factors represent metabolic responses and may include morphological, physiological and biochemical defence mechanisms. On the other hand, the external factors, namely environmental and storage conditions may intensify or inhibit the manifestation of the internal factors. It is well established that proper control of postharvest storage conditions, mainly temperature, is critical to maintain quality and to extend the self-life of tomatoes (Lana et al., 2005). In this regard, previous studies have been aimed at evaluating the influence of storage temperature on quality and metabolic behaviour of intact tomato fruits, including the biosynthetic pathway of carotenoids (Javanmardi and Kubota, 2006; Vinha et al., 2013). However, there is a lack of knowledge regarding the effects of the storage conditions on the elicited biosynthesis of carotenoids in tomato fruits subjected to different PEF treatments. The objective of this work was to study 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 °C 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 °C 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 homogenized using an Ultraturrax T-25 Basic (IKA®-Werke GmbH & Co., Staufen, Germany) for 2 min in an ice-bath. Afterwards, it was filtered under vacuum through grade 1 Whatman paper. The residue was re-extracted once with 10 mL of ethanol:hexane (4:3 v/v) for 2 min with an Ultraturrax. Then, the mixture was again filtered and the residue was washed twice with 5 mL of ethanol and once with 5 mL of hexane. All the filtrates were combined in an amber round-bottom flask and evaporated (rotovapor R-3000, BUCH, Switzerland) to dryness at 45 °C for 15 min. The residue was then saponified under a N_2 atmosphere by adding 10 mL of methanolic KOH 0.5 M + 0.1% BHT (v/w) and 10 mL of diethyl ether for 30 min with continuous agitation. Afterwards, the extract was placed in an amber decanting funnel and washed twice with 25 mL of 10% NaCl solution and thrice with 25 mL of distilled water. The aqueous phase was discarded each time. The organic phase was collected and rotoevaporated to dryness at 45 °C for 20 min. The residue was dissolved with 4 mL of diethyl ether and placed in an amber glass vial. Finally, the solvent was evaporated under a N_2 flow and stored at −40 °C until analysis. Before injection into the HPLC system, the carotenoid extract was reconstituted with 1 mL of methylene chloride and filtered through a 0.45 μm filter. All the extractions were conducted in duplicate.

2.5.2. Analysis of carotenoids

Carotenoids were quantified by high-performance liquid chromatography (HPLC) following the methodology reported by Odriozola-Serrano et al. (2009b). The HPLC system was equipped with a 600 controller and a diode array detector 2996 (Waters Corp.) set to scan from 240 to 550 nm. Separations were performed on a reverse-phase C18 Spherisorb® ODS2 (5 μm) stainless steel column (4.6 mm x 250 mm) at room temperature with a flow rate of 0.7 mL min^{-1}. The gradient was as follows: 0–10 min, acetonitrile (85%), methanol (10%), methylene chloride (3%) and hexane (2%); 10–40 min, acetonitrile (45%), methanol (10%), methylene chloride (23%) and hexane (22%); and 40 −60 min, acetonitrile (85%), methanol (10%), methylene chloride (3%) and hexane (2%). Carotenoids were identified on the basis of the