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Metabolite profiling of phenolic and carotenoid contents in tomatoes after moderate-intensity pulsed electric field treatments

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A metabolite profiling approach was used to study the effect of moderate-intensity pulsed electric field (MIPEF) treatments on the individual polyphenol and carotenoid contents of tomato fruit after refrigeration at 4 °C for 24 h. The MIPEF processing variables studied were electric field strength (from 0.4 to 2.0 kV/cm) and number of pulses (from 5 to 30).

Twenty four hours after MIPEF treatments, an increase was observed in hydroxycinnamic acids and flavanones, whereas flavonols, coumaric and ferulic acid-*O*-glucoside were not affected. Major changes were also observed for carotenoids, except for the 5-*cis*-lycopene isomer, which remain unchanged after 24 h of MIPEF treatments. MIPEF treatments, conducted at 1.2 kV/cm and 30 pulses, led to the greatest increases in chlorogenic (152%), caffeic acid-*O*-glucoside (170%) and caffeic (140%) acids. On the other hand, treatments at 1.2 kV/cm and 5 pulses led to maximum increases of α-carotene, 9- and 13-*cis*-lycopene, which increased by 93%, 94% and 140%, respectively. Therefore, MIPEF could stimulate synthesis of secondary metabolites and contribute to production of tomatoes with high individual polyphenol and carotenoid contents.

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1. Introduction

Tomatoes (*Solanum lycopersicum* L.) contain some valuable bioactive components, including antioxidants such as carotenoids and phenolic compounds. Recently, the high content of phenolic compounds, such as flavonoids and hydroxycinnamic acids in tomatoes has been attracting interest because of their apparent multiple biological effects (Crozier, Jaganath, & Clifford, 2009). Moreover, tomatoes contain carotenoids such as β-carotene and α-carotene, which possess provitamin A activity (Olson, 1989). The consumption of β-carotene, lycopene and other carotenoids decreases the risk of certain cancers and cardiovascular diseases (Das, Otani, Maulik, & Das, 2005; Djuric & Powell, 2001; Giovannucci, 1999; Giovannucci et al., 1995). *Trans*-lycopenes represents the most abundant lycopene isomer in tomato, with 5-, 9-, 13-, and 15-*cis*-lycopene being the main *cis*-isomers detected (Frohlich, Conrad, Schmid, Breithaupt, & Bohm, 2007). Human studies have demonstrated the nutritional benefits of *cis* isomers in tomato products, because these compounds seem to be better absorbed in the intestine than are *trans* isomers (Takeoka et al., 2001).

The application of pulsed electric fields at high field strength has been proposed as an alternative to conventional food preservation techniques. Several studies have demonstrated the ability of high intensity pulsed electric fields to produce shelf-stable liquid foods with high nutritional value by inactivating microorganisms and enzymes (Soliva-Fortuny, Balasa, Knorr, & Martin-Belloso, 2009). Other applications of pulsed electric fields, at moderate field strength, are currently under development. Moderate-intensity pulsed electric fields (MIPEF) permeabilise tissue structures, thus improving intracellular metabolite extraction (Soliva-Fortuny et al., 2009) and enhancing drying efficiency (De Vito, Ferrari, Lebovka, Shynkaryk, & Vorobiev, 2008). Metabolic responses of plant cells (Cai et al., 2011) and tissues (Galindo et al., 2009; Gomez Galindo, Wadso, Vicente, & Dejmek, 2008) upon the application of MIPEF have been studied. The analysis of potato demonstrated that, 24 h after the application of MIPEF, tissue metabolism showed MIPEF-specific stress responses, characterised by changes in the hexose pool that may involve starch and ascorbic acid degradation (Galindo et al., 2009). MIPEF-induced stresses could include a burst of ROS, which are endogenous signal components required for synthesis of secondary metabolites, such as polyphenols or carotenoids, which are known to be part of the defence response of plants to stress (Shohael et al., 2006).

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Metabolic analysis was used to characterise stress responses of vegetable tissues subjected to MIPEF, providing insights of how potato tissue responds to treatment (Galindo et al., 2009; Gomez Galindo et al., 2008). However, as far as we know, this is the first time that a targeted metabolite profiling of tomato polyphenols and carotenoids has been used to study the effects of MIPEF on the biosynthesis of secondary metabolites in whole fruits. The objective of this research was to characterise the biosynthesis of individual polyphenols and carotenoids in tomato 24 h after MIPEF treatments at different electric field strengths and numbers of pulses.

2. Materials and methods

2.1. Standards and reagents

All samples and standards were handled without exposure to light. Caffeic, ferulic, *p*-coumaric and chlorogenic acids, rutin and quercetin, β-carotene, β-apo-8'-caortenal, α-carotene, *trans*-lycopene, lutein and methyl tert-butyl ether (MTBE) were purchased from Sigma® (Madrid, Spain); naringenin, naringenin-7-*O*-glucoside, eriodictyol and hexane were from Extrasynthèse (Genay, France). Ethanol, methanol and formic acid (HPLC grade) were obtained from Scharlau (Barcelona, Spain) and ultrapure water (Milli-Q) from Millipore (Bedford, USA).

2.2. Tomatoes

Commercially mature tomato fruit (c.v. Daniella) were purchased from a local supermarket (Lleida, Spain). The pH (Crison 2001 pH-metre; Crison Instruments SA, Alella, Barcelona, Spain), soluble solids content (Atago RX-1000 refractometer; Atago Company Ltd., Japan), firmness (TA-XT2 Texture Analyzer; Stable Micro Systems Ltd, Surrey, England) and colour (Minolta CR-400, Konica Minolta Sensing, Inc.,Osaka, Japan) of the tomato fruit were determined. The physicochemical characteristics of tomatoes were: pH = 4.45 ± 0.01, firmness = 20.4 ± 2.51 N·s, soluble solids = 3.8 ± 0.1 Brix, colour: L^* = 38.5 ± 0.4, a^* = 18.1 ± 1.9, b^* = 24.6 ± 1.8.

2.3. MIPEF processing

MIPEF treatments were conducted in a bath apparatus manufactured by Physics International (San Leandro, CA, USA), which can deliver pulses from a capacitor of 0.1 µF with an exponential decaying waveform. A stainless steel parallel plate treatment chamber was used. A batch of tomato fruit was placed in the treatment chamber filled with tap water. Tomato fruit were treated at 0.4–2 kV/cm, using 5–30 monopolar pulses of 4 µs at a frequency of 0.1 Hz. Two replicates of each treatment were carried out.

MIPEF-treated tomato fruit were collected and immediately refrigerated at 4 °C during 24 h, as previously described by Galindo et al. (2009). Untreated tomatoes were stored separately, also at 4 °C during 24 h. Then, both untreated and MIPEF-treated tomatoes were lyophilised and frozen at –20 °C prior to analysis.

2.4. Extraction and analysis of polyphenols

2.4.1. Extraction of polyphenols

Each replicate of the treatment was extracted and analysed three times in a darkened room with a red safety light to avoid oxidation of the analytes, following the procedure of Vallverdú-Queralt, Jáuregui, Medina-Remón, Andrés-Lacueva, and Lamuela-Raventós (2010) with some modifications.

Lyophilised tomato fruits (0.2 g) were weighed and homogenised with 80% ethanol in Milli-Q water (4 ml); the homogenate was sonicated for 5 min and centrifuged (4000 rpm at 4 °C) for 15 min. The supernatant was transferred into a flask and extraction was repeated. Both supernatants were combined and evaporated under nitrogen flow; finally, the residue was reconstituted with up to 2 ml of Milli-Q water containing 0.1% formic acid and filtered through a 13 mm, 0.45 µm Polytetrafluoroethylene (PTFE) filter from Waters (Milford, USA) into an insert-amber vial.

Solid phase extraction (SPE) was carried out to eliminate interferents, such as ascorbic acid, amino acids and reducing sugars. For this procedure, Oasis® MAX cartridges, with 30 mg of mixed-mode anion-exchange and reversed-phase sorbent from Waters (Milford, USA), following the procedure of Vallverdu-Queralt et al. (2011). The eluted fractions were evaporated under nitrogen flow and the residue was reconstituted with up to 500 µl of Milli-Q water containing 0.1% formic acid and filtered through a 13 mm, 0.45 µm PTFE filter into an insert-amber vial for HPLC analysis.

2.4.2. Analysis of polyphenols

HPLC–ESI-MS/MS was used to evaluate the content of flavonols, flavanones and hydroxycinnamic acids, following the procedures of other studies. (Vallverdu-Queralt, Jauregui, Medina-Remon, & Lamuela-Raventos, 2012; Vallverdu-Queralt et al., 2011). Method parameters appear in these studies of the same authors.

Liquid chromatography was performed with an Agilent series 1100 HPLC instrument (Agilent, Waldbronn, Germany) equipped with a quaternary pump, an autosampler and a column oven set to 30 °C. Mobile phase consisted of Milli-Q water containing 0.1% formic acid (A) and acetonitrile containing 0.1% formic acid (B). The injection volume was 20 µl and the flow rate was 0.4 ml/min. Separation was carried out in 20 min under the following conditions: 0 min, 5% B; 16 min, 40% B; 17 min, 95% B; 19 min, 95% B; 19.5 min, 5 % B. The column was equilibrated for 5 min prior to each analysis.

An API 3000 (PE Sciex, Concord, Ontario, Canada) triple quadrupole mass spectrometer, equipped with a Turbo Ionspray source in negative-ion mode, was used to obtain MS/MS data. Turbo Ionspray source settings were as follows: capillary voltage, −3500 V; nebuliser gas (N_2), 10 a.u. (arbitrary units); curtain gas (N_2), 12 a.u.; collision gas (N_2), 4 a.u.; focusing potential, −200 V; entrance potential, −10 V; drying gas (N_2), heated to 400 °C and introduced to a flow rate of 6000 cm^3/min. The declustering potential and collision energy were optimised for each compound in infusion experiments: individual standard solutions (10 µg/ml) dissolved in 1:1 (v/v) mobile phase were infused at a constant flow rate of 5 µl/min, using a model syringe pump (Harvard Apparatus, Holliston, MA, USA). Full-scan data acquisition was performed scanning from *m*/*z* 100 to 800 in profile mode and using a cycle time of 2 s with a step size of 0.1 u and a pause between scans of 2 ms. Identification of some compounds was carried out through neutral loss scan and precursor ion scan experiments. For quantification purposes, data were collected in the multiple reaction monitoring (MRM) mode, tracking the transition of parent and product ions specific for each compound.

Quantification of polyphenols was performed by the internal standard method. Polyphenols were quantified, related to their corresponding standard, and results were expressed as µg/g dry weight. When standards were not available, as in the case of caffeic acid-*O*-glucoside, *p*-coumaric acid-*O*-glucoside and ferulic acid-*O*-glucoside, polyphenols were quantified related to the corresponding hydroxycinnamic acid (caffeic, *p*-coumaric and ferulic acids). Cryptochlorogenic and dicaffeoylquinic acids were quantified with respect to the chlorogenic acid.

The total polyphenol (TP) content was obtained by the sum of all individual polyphenols.

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