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Effect of pulsed electric fields on the antioxidant potential of apples stored at different temperatures

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ABSTRACT

The effects of pulsed electric fields (PEF, 0.008–1.3 kJ kg^{-1}) on the total phenolic, flavonoid and flavan-3-ol contents, as well as on the antioxidant capacity of apples stored at different temperatures (4 and 22 °C) along 48 h were studied. Contents of phenolic compounds observed in PEF-treated apples were higher than those of untreated. The mildest PEF treatment (0.008 kJ kg^{-1}) produced the maximum increases of total phenolics (13%) and flavan-3-ol (92%) contents in apples stored during 24 h at 22 °C, while it was observed at 4 °C for flavonoids (58%). On the other hand, the antioxidant capacity of apples was enhanced by 43% respect to that of untreated with the mildest PEF treatment after 12 h at 4 °C and by 15% after 24 h at 22 °C. Therefore, PEF technology could be used to increase the antioxidant potential of apples by controlling treatment and storage conditions.

1. Introduction

Consumers are more and more concerned about the nutritional and health-related characteristics of fruits and vegetables. Evidence suggests that a diet high in fruits and vegetables may decrease the risk of chronic diseases because of their high content in phytochemicals (Boyer and Liu, 2004). Apples are among the most popular and frequently consumed fruits in the world, because of their availability throughout the year and the general perception that apples are good for health. Epidemiological studies support the view that frequent apple consumption is associated with a reduced risk of chronic pathologies such as cardiovascular disease, specific cancers, and diabetes (Koutsos et al., 2015). The health benefits of apple consumption are mainly related with phenolic compounds content (Hyson, 2011). Moreover, there is a strong correlation between phenolic content of apples and antioxidant activity (Kalinowska et al., 2014).

The antioxidant compounds in some fruits and vegetables can be lost during handling after harvest, even during minimal processing and storage. In this sense, postharvest treatments are needed to preserve the quality and antioxidant potential of fresh produce (Villa-Rodriguez et al., 2015). The application of postharvest abiotic stresses (i.e., wounding, UV-light radiation, modified atmospheres, exogenous phytohormones) has been proposed in recent years as an effective strategy to activate the secondary metabolism of fruits and vegetables leading to the accumulation of antioxidant compounds with health-promoting benefits (Becerra-Moreno et al., 2015). Some reports suggest that pulsed electric fields (PEF) could act as abiotic stressor when applied during postharvest affecting the metabolism of vegetables (Galindo et al., 2008, 2009).

PEF technology has been extensively studied as preservation technique of foods. Numerous studies have demonstrated the ability of PEF to obtain shelf-stable plant-based liquid foods with high nutritional and sensory value (Odriozola-Serrano et al., 2013; Saldaña et al., 2014). Moreover, PEF may also be used as a pretreatment of solid vegetable matrices to improve processes such as extraction by pressing or solvent diffusion, osmotic dehydration, drying, and freezing (Donsi et al., 2010).

Recently, PEF has been proposed as a promising new abiotic elicitor for stimulating the secondary metabolites biosynthesis and accumulation in plant cell cultures (Cai et al., 2011; Gueven and Knorr, 2011; Saw et al., 2012). Little information has been found in the scientific literature regarding the use of PEF as possible treatment to enhance or stimulate the production of sencondary plant metabolites, such as phenolics, in fruits and vegetables. Vallverdú-Queralt et al. (2012) observed a maximum increase in total phenolics content (36.6%) when tomato fruits were stored at 4 °C for 24 h after a PEF processing of 1 kV cm^{-1} and 16 pulses, contributing to an increase in the antioxidant capacity of tomato fruit by more than 20%. Vallverdú-Queralt et al. (2013) reported that 24 h at 4 °C after PEF treatments (0.4–2.0 kV cm^{-1} and 5–30 pulses) led to an increase in hydroxycinnamic acids and flavanones contents in tomato fruits, whereas flavonols, coumaric and ferulic acid-*O*-glucoside were not affected. Moreover, the increases of phenolic compounds concentrations depended on the PEF treatment intensity. However, as far as we know, no informa-

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tion is available regarding the effects of PEF on the antioxidant potential of fruits and vegetables stored at different temperatures. Therefore, the aim of this work was to evaluate the impact of PEF treatment intensity (0.008–1.3 kJ kg^{-1}) on the phenolic compounds content and the antioxidant capacity of apples stored at different temperatures (4 and 22 °C) during 48 h.

2. Material and methods

2.1. Reagents

Methanol (HPLC grade), Folin–Ciocalteu reagent (2 N), hydrochloric acid 37% and sodium hydroxide were purchased from Scharlab S.L (Sentmenat, Spain). Sodium carbonate was obtained from POCH S.A (Poland). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), gallic acid, sodium nitrite 97% and (+)-catechin were purchased from Sigma Aldrich Co. (St. Louis, MO, EUA). Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) 97% was supplied by Sigma Aldrich Chemie GmbH & Co. KG (Steinheim, Germany). Vanillin 99% and aluminum chloride were purchased from Acros Organics (New Jersey, USA). High-purity water (Milli-Q water) was produced in the laboratory (Millipore Corporation, Bedford, MA, USA).

2.2. Sample preparation

Commercially mature apples (*Malus domestica*, var. Golden delicious) were purchased from a local supermarket (Lleida, Spain). The fruits were kept under regular cold storage until processing without applying any postharvest treatment. Apple fruits were selected according to uniformity in maturity and sanity. The pH (Crison 2001 pH-meter; Crison Instruments SA, Alella, Barcelona, Spain), the titratable acidity, the soluble solids content (Atago RX-1000 refractometer; Atago Company Ltd., Japan), the colour (Minolta CR-400, Konica Minolta Sensing, Inc., Osaka, Japan) and the firmness (TA-XT2 Texture Analyzer, equipped with a 4 mm rod, Stable Micro Systems Ltd., Surrey, England, U.K.) of apples were determined. The physic-chemical characteristics of apples were: pH = 3.90 ± 0.11, titratable acidity = 4.55 ± 0.85 g L^{-1} malic acid, soluble solids = 13.35 ± 0.07%, colour: L* = 73.63 ± 2.01, a* = −15.20 ± 2.47 and b* = 43.49 ± 0.50, and firmness = 7.52 ± 0.54 N. Apples were washed with chlorinated water (200 mg L^{-1}) for 5 min before use.

2.3. PEF processing of apples

PEF treatments were conducted in a batch equipment (Physics International, San Leandro, CA, USA) which delivers pulses from a capacitor of 0.1 μF with an exponential decaying waveform. A stainless steel parallel plate (20 × 8 cm) treatment chamber with a distance between plates of 10 cm was employed, using tap water as conductive medium. Whole apple fruits (two per batch) were treated at 0.4–2 kV cm^{-1}, using 5–35 monopolar pulses of 4 μs at a frequency of 0.1 Hz, which correspond to an specific energy input of 0.008–1.3 kJ kg^{-1}. PEF-treated and untreated apples were stored at different temperatures (4 and 22 °C) and times (0, 12, 24, 36 and 48 h). After each storage time, samples were freeze dried and kept at −30 °C until analysis.

2.4. Phenolics and antioxidant capacity analysis

2.4.1. Phenolics extraction

The extraction of phenolics was based on the methodology followed by Patras et al. (2009) with some modifications. Methanolic extracts were prepared by adding 1 g of freeze dried samples to 5 mL of 80% methanol and homogenizing for 2 min at 13,600 rpm using an Ultra-Turrax T 25 (IKA® WERKE, Germany). The samples were then centrifuged for 20 min at 4020 × *g* and 4 °C (Hettich® EBA 21 centrifuge, Andreas Hettich GmbH & Co.KG., Tuttlingen, Germany) and filtered through Whatman No 1 filter paper. The supernatant was transferred into a volumetric flask. The extraction of the residue was repeated adding 5 mL of 80% methanol, sonicating for 5 min and centrifuging for 20 min at 4020 × g and 4 °C. Both supernatants were combined into the same volumetric flask. The resulting methanolic extract was used to determine the total phenolic, flavonoid and flavan-3-ol contents as well as the total antioxidant capacity.

2.4.2. Determination of total phenolic content

Total phenolic content was determined using the Folin–Ciocalteu reagent according to the method of Odriozola-Serrano et al. (2008). A portion of 0.5 mL of methanolic extract was mixed with 0.5 mL of Folin-Ciocalteu reagent and 10 mL of saturated Na_2CO_3 solution. Samples were mixed and stored at room temperature in darkness for 60 min. Absorbance was measured at 725 nm using a CECIL 2021 spectrophotometer (Cecil Instruments Ltd., Cambridge, UK). Calibration curve was built with gallic acid (0–300 mg L^{-1}). Results were expressed as grams of gallic acid equivalents (GAE) per kilogram.

2.4.3. Determination of flavonoid content

Flavonoid content was determined based on the method described by Dávila-Aviña et al. (2012) with some modifications. One milliliter of the methanolic extract, 4 mL of deionized H_2O and 0.3 mL of $NaNO_2$ (5%) were mixed in a volumetric flask (10 mL). After 5 min, 0.3 mL of $AlCl_3$ (10%) were added and stored in the darkness for 1 min. Two milliliters of NaOH (1 mol L^{-1}) were added and the volumetric flask was adjusted by adding deionized H_2O. The absorbance was determined at 478 nm using a CECIL 2021 spectrophotometer (Cecil Instruments Ltd., Cambridge, UK). Calibration curve was built with (+)-catechin (0–300 mg L^{-1}). Results were expressed as grams of (+)-catechin equivalents (CE) per kilogram.

2.4.4. Determination of flavan-3-ol content

Flavan-3-ol content determination method was based on the vanillin assay described by Carbone et al. (2011) with some modifications. A volume of 1 mL of the methanolic extract and 5 mL of vanillin (1%) in methanol were mixed and rested for 5 min, and then, 5 mL of HCl (4%) were added. The absorbance was measured at 494 nm after 20 min of reaction time at room temperature using a CECIL 2021 spectrophotometer (Cecil Instruments Ltd., Cambridge, UK). Flavan-3-ol content were calculated from a calibration curve, using (+)-catechin (0–1500 mg L^{-1}) as standard. Results were expressed as grams of (+)-catechin equivalents (CE) per kilogram.

2.4.5. Determination of antioxidant capacity

The method used to measure the total antioxidant capacity was based on the DPPH assay described by De Ancos et al. (2002). Briefly, 0.05 mL of the methanolic extract or trolox standard, 0.05 mL of Milli-Q water and 3.9 mL of methanolic DPPH (0.025 g L^{-1}) were mixed, shaken and left in the dark for 30 min at room temperature. The absorbance was measured at 515 nm using a CECIL 2021 spectrophotometer (Cecil Instruments Ltd., Cambridge, UK) against a blank of methanol without DPPH. A calibration curve was obtained with the percentage of inhibition of the DPPH as a function of trolox standard concentration (0–0.4 mg mL^{-1}). Results were expressed as mmol of trolox equivalents (TE) per kilogram.

2.5. Statistics and experimental design

Two replications of each treatment were carried out and samples were analysed in triplicate. A multifactor analysis of variance (ANOVA) was performed at $p < 0.05$ in order to assess phenolic compounds content and antioxidant capacity changes among the PEF treatment intensities (0, 0.008, 0.3 and 1.3 kJ kg^{-1}), storage temperatures (4 and 22 °C) and times (0, 12, 24, 36 and 48 h). This statistical analysis was