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Metabolic response of fresh-cut apples induced by pulsed electric fields

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ABSTRACT

Pulsed electric field (PEF) treatments can induce metabolic stress responses in plant tissue as a function of the applied conditions. This study highlighted the metabolic effects of reversible and irreversible electroporation in fresh-cut apple tissue, by adjusting the electric field strength to 100, 250 and 400 V/cm (100 μs pulse width, 60 pulses, 100 Hz). Metabolic heat, O_2 and CO_2 gas analysis, along with metabolomics, were employed to jointly evaluate the PEF-induced effects after 24 h at 10 °C. Marked metabolic changes were registered when the threshold of irreversible electroporation was exceeded, at 250 and 400 V/cm. With such treatments, a drop of metabolic heat and respiration rate was observed, as a probable consequence of the loss of the cell viability, anaerobic respiration pathways were noticeably lowered, while γ-aminobutyric acid metabolism was activated. Conversely, minimal modifications of the metabolism heat and metabolites concentrations were noticed when 100 V/cm was applied.

Industrial relevance: Metabolic response of fresh-cut fruit and vegetables as function of the manufacturing process is a fundamental aspect directly related to the quality of the final products. Pulsed electric fields (PEF), as well as other innovative technologies, can induce undesired effects on tissue metabolism that might limit the industrial application. Furthermore, the analytical methods used in the present work provide useful tools for the optimization of the PEF treatment conditions for fresh-cut manufacturers.

1. Introduction

Pulsed electric field (PEF) technology is a non-thermal process, which has recently stimulated an increasing interest in the food field. The application of high electric fields between two electrodes can be exploited for different goals, for instance to enhance mass transfer phenomena (Donsì, Ferrari, & Pataro, 2010; Puértolas, Luengo, Álvarez, & Raso, 2012; Taiwo, Angersbach, & Knorr, 2002) or to inactivate microorganisms (González-Arenzana, Portu, López, López, Santamaría, Garde-Cerdán, & López-Alfaro, 2015; Timmermans, Groot, Nederhoff, van Boekel, Matser, & Mastwijk, 2014). The mechanism of action includes the creation of pores due to the application of electric fields high enough to induce a potential difference of approximately 0.2 V across the cell membrane (Teissie, Eynard, Gabriel, & Rols, 1999). In a second step, pores can expand, aggregate and, once the external electric field is removed, even reseal (Vorobiev & Lebovka, 2009). The extent of the process, also known as electroporation, strongly depends on the applied process parameters, such as electric field strength, number and shape of pulses, their width and frequency. Indeed, different goals and industrial applications can be achieved by adjusting the treatment conditions (Barba, Parniakov, Pereira, Wiktor, Grimi, Boussetta, Saraiva, Raso, Martin-Belloso, & Witrowa-Rajchert, 2015).

The effect of PEF in plant tissues has been studied by several techniques according to the desired objective: examples are the release of valuable compounds (Carbonell-Capella, Buniowska, Esteve, & Frígola, 2015; Luengo, Álvarez, & Raso, 2013), extraction yield (Bazhal, Lebovka, & Vorobiev, 2001), changes in colour and texture (Lebovka, Praporscic, & Vorobiev, 2004; Wiktor, Schulz, Voigt, Witrowa-Rajchert, & Knorr, 2015). Moreover, methods have been developed to indirectly evaluate the extent of electroporation based on electrical impedance (Angersbach, Heinz, & Knorr, 2002; Ivorra, 2010; Lebovka, Bazhal, & Vorobiev, 2002), microscopy (Fincan & Dejmek, 2002) and time domain nuclear magnetic resonance (TD-NMR) (Dellarosa, Ragni, Laghi, Tylewicz, Rocculi, & Dalla Rosa, 2016).

Pulsed electric fields, by acting at the level of membranes, can also deeply affect cell activities. As a consequence, metabolic stress responses of cells can be induced and lead to undesired effects on the

Abbreviations: GA, glutamic acid; GABA, γ-aminobutyric acid; HMDB, human metabolome database; HR-NMR, high resolution nuclear magnetic resonance; LD, linear discriminant; LDA, linear discriminant analysis; MOSFET, metal-oxide-semiconductor field-effect transistor; PC, principal component; PCA, principal component analysis; PEF, pulsed electric fields; R_A, resistance of apple sample; R_{WA}, resistance (in series) of water between the apple samples and the electrodes; R_{WF}, resistance (in parallel) of water which is parallel to the apple cylinders; RQ, respiration quotient; sPLSDA, sparse partial least squares discriminant analysis; TD-NMR, time domain nuclear magnetic resonance; VIP, variable importance in projection.

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quality of the final products. This might limit the application of PEF in fresh-cut products. Generally, fresh-cut fruit and vegetables undergo minimal processing, such as peeling, cutting or pre-treatment with different solutions (Mauro, Dellarosa, Tylewicz, Tappi, Laghi, Rocculi, & Dalla Rosa, 2016; Santagapita, Laghi, Panarese, Tylewicz, Rocculi, & Dalla Rosa, 2013) which, nevertheless, provokes metabolic responses (Rocculi, Panarese, Tylewicz, Santagapita, Cocci, Gómez Galindo, Romani, & Dalla Rosa, 2012). In this contest, the application of PEF, on one side can ease the mass exchange between the outer solution and the tissue, on the other side can trigger further stress responses. To the best of our knowledge, few works have been focused on the metabolic aspects induced by PEF in postharvest fruit and vegetable products (Galindo, Dejmek, Lundgren, Rasmusson, Vicente, & Moritz, 2009; Galindo, Wadsö, Vicente, & Dejmek, 2008).

Fresh-cut products are metabolic active tissues, that can therefore produce heat as a function of both normal cell activities, involving tens of metabolic pathways, and further technological processes applied. Thermal power and heat can be continuously monitored by isothermal calorimetry and this gives rise to gross values of the cell metabolisms where many metabolic pathways account for the overall thermal development (Galindo, Rocculi, Wadsö, & Sjöholm, 2005; Wadsö & Galindo, 2009). Of greater importance, when the sample conditions are standardized, a direct evaluation of the effects of the technologies can be carried out (Panarese, Laghi, Pisi, Tylewicz, Dalla Rosa, & Rocculi, 2012; Tappi, Berardinelli, Ragni, Dalla Rosa, Guarnieri, & Rocculi, 2014). Moreover, the measurement of the heat is often coupled with the analysis of the consumed O_2 and produced CO_2, which allow clarifying whether other non-aerobic metabolisms are activated (Cortellino, Gobbi, Bianchi, & Rizzolo, 2015).

A trait that all the above-mentioned methods have in common is their ability to easily estimate the gross stress response, with reduced possibilities to investigate how the different technological treatments fine-tune the physiology of the samples cells. In this respect, metabolomics, the comprehensive analysis of the soluble low weight metabolites by means of high-throughput techniques like mass spectrometry (MS) of high resolution nuclear magnetic resonance (HR-NMR), is considered the election approach (Fiehn, 2002; Laghi, Picone, & Capozzi, 2014). This has been successfully applied for food quality control, health and nutritional purposes, fingerprinting, including traceability and authenticity, and, recently, to assess and backwardly adjust technological processes (Trimigno, Marincola, Dellarosa, Picone, & Laghi, 2015). To the purpose, specific multivariate analytical tools need to be developed and tailored to discriminate the effects of the applied technologies on precursors, intermediates and products of different metabolic pathways (Laghi, Picone, & Capozzi, 2014).

The objective of the present work was to assess the metabolic response of fresh-cut apples upon pulsed electric field treatments, so as to obtain a deeper understanding of such promising technological treatment and outline instruments allowing its tuning. Three different levels of electric field strength, 100, 250 and 400 V/cm with fixed pulse width (100 μs), number of pulses ($n = 60$) and frequency (100 Hz) were studied, because they are known to produce both reversible and irreversible electroporation effects on cell membranes in apple tissue (Dellarosa, Ragni, Laghi, Tylewicz, Rocculi, & Dalla Rosa, 2016). A comprehensive evaluation by means of a multianalytical approach based on calorimetry, gas analysis and metabolomics was chosen to complementarily describe gross alteration on metabolic activities and specific fine changes in metabolites composition. High resolution ^{1}H nuclear magnetic resonance (HR-NMR) was employed for the analysis of the metabolic profiling, together with a novel non-targeted statistical tool based on sparse partial least square discriminant analysis (sPLSDA) and linear discriminant analysis (LDA). The investigation was conducted 24 h after PEF treatment, so to give to the fruit's tissue time to put in place strategies to defend themselves from possible damages.

2. Material and methods

2.1. Raw material

Apples (*Malus domestica*, cv Cripps Pink) were purchased at a local market and stored at 2 ± 1 °C for three weeks, during which all the experiments were conducted. Before experiments, apples were kept at room temperature for 2 h. Raw material had an average moisture content of 83.5 ± 0.5 g and was at commercial maturity, characterized by soluble solid content of 13.5 ± 0.5 g and titrable acidity of 0.36 ± 0.02 g of malic acid per 100 g of fresh product (corresponding to an average ripening index of 37.5). Cylindrical samples (8 mm diameter and 20 mm length with an average weight of 1 g) were obtained from apple parenchyma by cutting with a manual cork borer and a scalpel. Eight cylinders from each fruit were obtained and used for the experiments.

2.2. Pulsed electric field (PEF) treatments and monitoring

PEF treatments were applied to apple samples using an in-house developed pulse generator equipment based on capacitors as energy tank and controlled by a STW9N150 MOSFET (metal-oxide-semiconductor field-effect transistor) (STMicroelectronics, Geneve, Swiss). Briefly, 60 monopolar pulses of near-rectangular shape, fixed pulse width of 100 ± 2 μs and repetition time of 10.0 ± 0.1 ms (100 Hz) were chosen, according to the experimental conditions used by Dellarosa, Ragni, Laghi, Tylewicz, Rocculi, and Dalla Rosa (2016). PEF treatments were conducted at 20 °C in a 30 × 20 × 20 mm (length × width × height) chamber equipped with two stainless steel electrodes with an active contact surface of 20 × 20 mm^2. For each trial, six apple cylinders were arranged with the two circle sides parallel to the electrodes and the chamber was filled up with tap water (conductivity at 25 °C of 328 ± 1 μS cm^{-1}) with a final product-to-water ratio around 1:1 (v/v) (Fig. 1). Applied current and voltage values were measured at the electrodes by a digital oscilloscope (PicoScope 2204a, Pico Technology, UK) connected to the equipment and a personal computer.

Four samples groups, including control, were obtained by treating apple cylinders with a voltage of 300 V, 750 V and 1200 V to the electrodes (pulse width = 100 μs, number of pulses = 60, frequency = 100 Hz). These conditions led to the nominal electric field strength of 100, 250 and 400 V/cm in the chamber and the energy input of 19, 151 and 424 J/kg of sample, respectively. However, the presence of tap water between the samples and the electrodes (two different materials with different resistivity between the electrodes) gave rise to inhomogeneous distributions of the electric fields within the chamber. Taking into account the known resistivity (3.05 kΩ × cm) of the tap water and the geometries of the cylindrical samples within the chamber, the specific voltage values applied to the apple tissue could be calculated according to the Ohm's law. Fig. 1 shows the chamber arrangement and the equivalent electrical circuit used for the calculation, where tap water acts as resistor both in series and in parallel. Consequentially, treatments at the nominal field strength of 100, 250 and 400 V/cm gave rise to values of 115, 245 and 275 V/cm^3 of sample in the chamber. As commonly accepted throughout the literature, in the present work the treatments and the sample names were referred to the nominal electric field strength. Furthermore, the same approach based on equivalent circuits and the Ohm's law was employed to calculate possible changes of the electric resistivity of apple samples during the pulsation. To this respect, current and voltage values of the first and the last pulse of the 60-pulses train series (frequency = 100 Hz, pulse width = 100 μs) were monitored at each applied electric field.

2.3. Metabolic heat

Three fresh cylindrical samples (about 3 g) were placed in 20 mL glass ampoule and sealed with a teflon coated rubber seal and an