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Electric field distribution in relation to cell membrane electroporation in potato tuber tissue studied by magnetic resonance techniques

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ABSTRACT

Magnetic resonance electrical impedance tomography (MREIT) enables determination of electric field distribution during electroporation in which cell membrane permeability is increased by application of an external high electric field. In this study, MREIT was performed for the first time to predict electroporated areas in a pulsed electric field (PEF) treated vegetable tissue. The study was performed on potato tubers using different amplitudes of electric pulses and results were evaluated also by means of multiparametric MRI. MREIT determined regions of electric field distribution corresponded to visible darkened areas of the treated potatoes, as well to the results of multiparametric MRI. Results of this study suggest that MREIT could be used as an efficient tool for improving the effectiveness of PEF treatment applications.

Industrial relevance: This study presents a method capable of determining electric field distribution during PEF treatment using magnetic resonance electrical impedance tomography. The method has a practical value as it can potentially enable monitoring of the outcome of PEF applications which strongly depends on local electric field. Measurement of electric field distribution would enable detection of insufficient electric field coverage before the end of the PEF treatment, thus increasing and assuring its effectiveness.

1. Introduction

In recent years, pulsed electric field (PEF) has been recognized as an efficient alternative to conventional approaches in numerous food processing applications (Barbosa-Canovas, Pierson, Zhang, & Schaffner, 2000; Mahnič-Kalamiza, Vorobiev, & Miklavčič, 2014; Raso & Heinz, 2006; Vorobiev & Lebovka, 2010). PEF is based on electroporation, i.e. biological phenomena that increase permeability of a cell membrane when exposed to an electric field (Kotnik, Kramar, Pucihar, Miklavcic, & Tarek, 2012; Tsong, 1991; Yarmush, Golberg, Serša, Kotnik, & Miklavčič, 2014). In general, electroporation occurs when electric field strength exceeds a certain value, also known as electroporation threshold. If the field strength remains under irreversible electroporation threshold and the exposure time is sufficient, a cell membrane remains in a state of higher permeability for a period of time (Rols & Teissié, 1990). However, if the field strength exceeds irreversible electroporation threshold, irreversible electroporation occurs and the cell loses its homeostasis which leads to cell death (Jiang, Davalos, & Bischof, 2015). Consequently, applied electric field mostly determines the outcome and the efficiency of electroporation applications, including food processing applications. Electric field strength in the range from several 100 V/cm to up to 1–2 kV/cm, i.e. moderate electric fields, are employed for extraction of water or solute out of plant tissues in applications such as juice extraction (Vorobiev & Lebovka, 2010), dehydration (Jaeger, Buechner, & Knorr, 2012), valuable compound recovery (Boussetta et al., 2011) and cryopreservation (Phoon, Galindo, Vicente, & Dejmek, 2008). Exposing treated plant tissues to high pulsed electric field, i.e. from 5 kV/cm to up to 50 kV/cm, is likely to cause irreversible damage of cells and for that reason can be used in applications such as liquid food product preservation (Buckow, Ng, & Toepfl, 2013; Raso, Calderón, Góngora, Barbosa-Cánovas, & Swanson, 1998; Toepfl, 2011).

A method capable of determining electric field distribution during the pulse delivery has a practical value as it can potentially enable monitoring of the outcome of PEF applications which strongly depends on local electric field (Miklavčič et al., 1998). Measurement of electric field distribution would enable detection of insufficient electric field coverage before the end of either reversible or irreversible PEF treatment, thus increasing and assuring its effectiveness. As the electric field distribution cannot be measured directly, we proposed an indirect approach. Magnetic resonance electrical impedance tomography (MREIT) proved to be an excellent candidate for determining an electric field distribution during electroporation (Kranjc, Bajd, Serša, & Miklavčič, 2011). The method enables reconstruction of the electric

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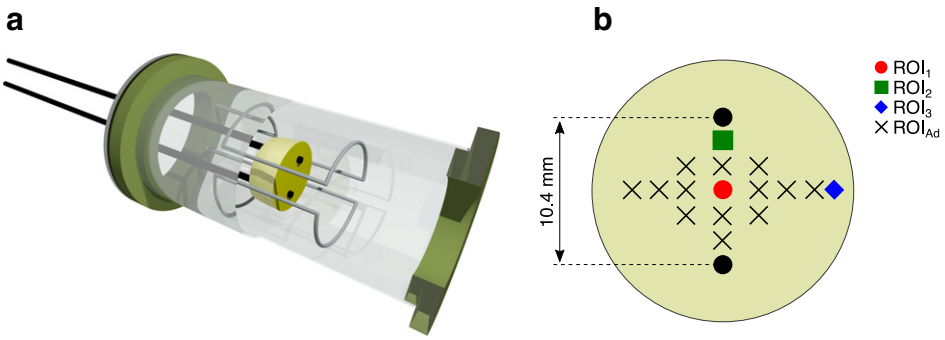

Fig. 1. Potato tuber sample with inserted needle electrodes placed in a MR microscopy probe (a), schematic axial cross-section through the potato sample with indicated three ROIs where multiparametric analysis was performed (b).

field distribution by measurement of an electric current density distribution and electrical conductivity of the treated subject during the application of electric pulses using MRI followed by numerical data analysis. MREIT has advanced rapidly in the last decade, especially in electrical conductivity imaging of biological tissues (Kim et al., 2009; Seo & Woo, 2014). MREIT enables determination of an electric field distribution in situ while taking into account changes that occur in the tissue due to electroporation. We demonstrated a successful reconstruction of the electric field distribution during electroporation in an agar phantom (Kranjc et al., 2011), ex vivo animal tissue (Kranjc, Bajd, Serša, & Miklavčič, 2014; Kranjc, Bajd, Serša, Woo, & Miklavčič, 2012), in silico (Kranjc et al., 2012) and in mouse tumor in vivo (Kranjc et al., 2015).

In this study, MREIT was performed for the first time to predict electroporated areas in a PEF treated vegetable tissue. MREIT was followed by multiparametric MR imaging including ADC and T_2 mapping that enabled dynamical follow-up of tissue changes after the PEF treatment. Our study was performed on potato tubers since PEF treatment is already well established in potato industry for reducing cutting forces, oil uptake and browning during frying (Ignat, Manzocco, Brunton, Nicoli, & Lyng, 2014). Besides apple tissue (Grimi, Mamouni, Lebovka, Vorobiev, & Vaxelaire, 2011), potato tuber is found to be appropriate for studying electroporation effects due to a possible additional visual discern of electroporated areas that become distinctively darker hours after the treatment (Hjouj & Rubinsky, 2010; Ivorra, Mir, & Rubinsky, 2009). As the applied electric field often results in non-uniform changes of cell viability due to potato tuber microstructure (Faridnia, Burritt, Bremer, & Oey, 2015) a method that would allow monitoring of the electric field distribution in the treated tubers during the PEF treatment would be of a great value.

2. Material and methods

2.1. Raw material handling

Yellow-fleshed potato tubers (*Solanum tuberosum*) cultivar "Agata" were purchased at the local supermarket (Ljubljana, Slovenia) and stored at 4 °C in the dark closed refrigerated chamber until used, i.e. less than 2 days. All of the potato tubers used in this study were from the same batch and free from any external damage.

2.2. Experimental setup

From the potato a disc-like sample measuring 21 mm in diameter and 2 mm in height was sliced and then placed in an acrylic glass container. As in our previous ex vivo studies (Kranjc et al., 2014) two cylindrically shaped, i.e. needle electrodes, were inserted in the potato sample. The electrodes were made of platinum–iridium, their diameter was 1 mm and they were inserted at a distance of 10.4 mm (see Fig. 1b). After the insertion, the electrodes were connected to an electric pulse generator, which was triggered by an MRI spectrometer synchronously with the Current Density Imaging (CDI) pulse sequence. The sample was then inserted in a 25 mm MR microscopy probe inside a horizontal-bore superconducting MRI magnet (Fig. 1a). Each PEF treatment experiment was performed on a different fresh potato sample to ensure identical initial conditions in all experiments.

The feasibility study of monitoring electric field distribution during the application of electric pulses was performed on 15 potato tubers that were divided in two groups as shown in Table 1. Potatoes from group 1 and 2 were subjected to the electric pulses and to MREIT for reconstruction of electric field distribution inside the tubers. Electroporated areas in the potatoes from group 1 were evaluated by digital photographs taken 18 h after the PEF treatment, while the potatoes from group 2 were evaluated by dynamical multiparametric MRI. The photographs were taken by a digital camera Olympus XZ-1 (Olympus Corporation, Tokyo, Japan) with settings for exposure time (1/125 s) and aperture (f/2.5) kept the same for all samples. The PEF treated potatoes of both groups were compared by electric field distributions and the corresponding electroporated areas as obtained by MREIT analysis. In potatoes from group 2 regions of interest were used for assessment of electroporation treatment effects. The regions measure 5 × 5 pixels, i.e. 2.3 × 2.3 mm, and were placed: ROI_1 in the center between the electrodes, ROI_2 in proximity of the electrodes and ROI_3 in the outer region (Fig. 1b). Additional regions of interests (ROI_{Ad}) were introduced in determination of correlation between T_2 values and values of electric field.

2.3. Electroporation protocol

Electroporation treatment of potatoes was performed by applying two sequences of four high voltage electric pulses with a duration of 100 μs, a pulse repetition frequency of 5 kHz and with an amplitude of 500 V, 750 V and 1000 V for samples group 1 and 750 V for samples from group 2. The electric pulses were delivered between the electrodes by an electric pulse generator Cliniporator Vitae (IGEA, Carpi, Italy).

2.4. Magnetic resonance imaging: current density imaging complemented by multiparametric MRI

The MR imaging was performed on a MRI scanner consisting of a 2.35 T (100 MHz proton frequency) horizontal bore superconducting

Table 1
Two groups of potato tubers used in the study.

	Group 1	Group 2
Number of samples	8	7
Names of samples	C1.1, C2.1 (control) P1.1–P1.6	C2.1–C2.4 (control) P2.1, P2.2, P2.3
Amplitude of applied el. pulses	500 V (P1.1, P1.2) 750 V (P1.3, P1.4) 1000 V (P1.5, P1.6)	750 V (P2.1, P2.2, P2.3)
Evaluation of electroporated area	Digital photography	Multiparametric MRI