Food Research International 77 (2015) 556–564

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Innovative approach to determine the effect of pulsed electric fields on the microstructure of whole potato tubers: Use of cell viability, microscopic images and ionic leakage measurements

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ARTICLE INFO

Article history:
Received 21 June 2015
Received in revised form 13 August 2015
Accepted 18 August 2015
Available online 24 August 2015

Keywords:
Pulsed electric fields
Potatoes
Electroporation
Ion migration
Cell permeability
Microstructure

ABSTRACT

The aim of this study was to gain an in-depth understanding of the effect of pulsed electric fields (PEF) on the microstructure of potato tubers. The effect of peeling prior to PEF was also studied. Whole potato tubers were subjected to PEF at a constant frequency of 50 Hz, over a range of electric field strengths (0.2 to 1.1 kV/cm) and energy levels (1 to 10 kJ/kg). To determine the uniformity of the PEF effect across the potato tuber, cell viability was assessed using tetrazolium salt staining. To evaluate the effectiveness of PEF processing the leakage of ionic species from the tubers was measured using atomic absorption spectrophotometry and FESEM-EDS analysis. In addition the influence of PEF on cell disruption and microstructural damage was assessed using scanning electron microscopy (Cryo-SEM). As the electric field strength and energy increased potassium ion leakage and electrical conductivity of the medium increased. The orientation of the tuber towards the electrodes and the presence of the skin greatly affected the impact of PEF on cell disruption and viability. At electric field strengths of 0.3 kV/cm and above, potato cells located in the pith (inner medulla) showed more damage and had a higher proportion of cell death compared to cells located in the outer medulla. This is the first study to provide visual evidence that the application of electric fields to solid, living foods, such as potato tubers, results in an uneven distribution of cell damage and death owing to the presence of vascular bundles and cells that vary in their resistance to electric fields.

1. Introduction

Pulsed electric field (PEF) processing involves subjecting food, placed between two electrodes, to pulsed high voltage electric fields for a very short time (μs to ms), leading to permeabilization of cell membranes (Toepfl, Heinz, & Knorr, 2005). PEF processing at electric field strengths between 0.2 and 1 kV/cm for 0.1 to 10 ms can effectively disrupt plant tissues without a significant increase in temperature (Fincan & Dejmek, 2002; Lebovka, Bazhal, & Vorobiev, 2002; Lebovka, Shynkaryk, & Vorobiev, 2007). Previous studies have demonstrated that electroporation induced by moderate electric fields (0.5–5 kV/cm) conserves the cell wall network (Corrales, Toepfl, Butz, Knorr, & Tauscher, 2008; Toepfl, Mathys, Heinz, & Knorr, 2006; Vorobiev & Lebovka, 2010), increasing juice extraction yields (Praporscic, Ghnimi, & Vorobiev, 2005), drying efficiency (Janositz, Noack, & Knorr, 2011; Lebovka et al., 2007) and extractability of metabolites (Aguiló-Aguayo et al., 2014; Donsì, Ferrari, & Pataro, 2010). Most PEF research on food has used liquid, semi-solid (puree) or solid plant materials that have been mechanically fragmented prior to PEF treatment, such as plant tissue slices (Janositz et al., 2011; Mhemdi, Grimi, Bals, Lebovka, & Vorobiev, 2013), cylinders (Ammar, Lanoisellé, Lebovka, Van Hecke, & Vorobiev, 2010; Boussetta, Grimi, Lebovka, & Vorobiev, 2013), strips (Shayanfar, Chauhan, Toepfl, & Heinz, 2013), cubes (Bazhal, Lebovka, & Vorobiev, 2003; Ignat, Manzocco, Brunton, Nicoli, & Lyng, 2015) or discs (Lebovka, Praporscic, & Vorobiev, 2004). Due to the requirement to pre-process plant materials prior to PEF treatment the tissues will have already experienced considerable damage which is likely to impact on the nature of the PEF induced changes.

In addition to PEF enhancing cell disruption, this technique has been reported to induce stress responses resulting in changes in plant metabolism and an increase in the levels of bioactive compounds (Balasa, Janositz, & Knorr, 2011). Thereby raising the question of how PEF affects the viability of plant cells, there is however no published data on the impact of PEF on plant cell viability in intact solid plant tissues/organs.

In this study, whole potato tubers were chosen as a model system because PEF has been used commercially in the potato industry to reduce cutting forces, lower oil uptake and reduce browning during frying (Ignat et al., 2015), and improved diffusion processes (Angersbach, Heinz, & Knorr, 2000; Vorobiev & Lebovka, 2006). Also, other studies

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http://dx.doi.org/10.1016/j.foodres.2015.08.028
0963-9969/

have investigated some aspect of the impact of PEF on potatoes (Boussetta et al., 2013; Galindo, Vernier, Dejmek, Vicente, & Gundersen, 2008; Janositz et al., 2011; Lebovka, Praporscic, Ghnimi, & Vorobiev, 2005a, 2005b; Lebovka et al., 2007; Pereira, Galindo, Vicente, & Dejmek, 2009) thereby allowing comparisons between the present and earlier studies. A potato tuber is a living plant organ composed of cells of different sizes and functions and it has a unique vascular system (Fig. 1). How PEF affects the whole potato tuber, with respect to current distribution and the concomitant effects on microstructure, cell viability, ion leakage, and how the potato skin influences the PEF effect is not well understood.

The main objective of this study was to gain an in-depth understanding of the effect of PEF on the microstructure of whole potato tubers. Cell viability tests were conducted using tetrazolium salt staining to evaluate the uniformity of the PEF effect across the potato tuber. The leakage of ions from tubers and changes in electrical conductivity were also measured in the surrounding solution, to evaluate the effectiveness of PEF processing. Measuring the degree of cell disintegration index (Z) (Barba, Brianceau, Turk, Boussetta, & Vorobiev, 2015; Barba, Grimi, & Vorobiev, 2015; Parniakov, Barba, Grimi, Lebovka, & Vorobiev, 2014) and changes in conductivity as a result of the release and transport of ionic species due to pore formation has been traditionally used to confirm the effectiveness of PEF induced membrane permeabilization (Angersbach, Heinz, & Knorr, 1999, Angersbach et al., 2000; Lebovka, Bazhal, & Vorobiev, 2000, Lebovka, Bazhal, & Vorobiev, 2001, Lebovka et al., 2002). However, since this technique does not give any indication whether the release of ionic species uniformly occurs after PEF treatment, scanning electron microscopy (Cryo-SEM) was used to visualize the effect of PEF on microstructural damage, cell structure and Cryo-SEM combined with energy dispersive spectroscopy (EDS) was used to observe ion migration. In addition, the effect of peeling the tubers prior to PEF was also studied.

2. Material and methods

2.1. Potato samples

A single batch of medium size (~70 g, 60 mm length and 50 mm width) potato tubers (*Solanum tuberosum* L. cv. Nadine) from the same harvest was purchased from a local grower (Dunedin, New Zealand) and stored in the dark at 7–8 °C under controlled relative air humidity (90–95%). During the study, the moisture content of the potato tubers was monitored by drying 20 g of fresh potato tissue at 105 °C until a constant weight was achieved. The moisture content of the potatoes was 81.91% on average. Only tubers of a similar size, shape and weight, without any external damage were selected for use in the PEF trials.

2.2. PEF treatment

PEF treatments were performed using a ELCRACK® HVP 5 PEF system (DIL, German Institute of Food Technologies, Quakenbruck, Germany) in a batch treatment configuration. The treatment chamber (100 mm length × 80 mm width × 50 mm height, 400 mL capacity) consisted of two parallel stainless steel electrodes (5 mm thick), separated by a distance of 80 mm. Pulse shape (square wave bipolar) was monitored on-line using an oscilloscope (Model UT2025C, Uni-Trend Group Ltd, China), during the treatment. The treatment time was calculated by multiplying the pulse width (τ) by the number of pulses (n) applied. The pulsed electrical energy, also known as specific input energy (W_{spec}) was applied to the potato tubers as a square-wave pulse and was calculated according to Zhang, Barbosa-Cánovas, and Swanson (1995) using Eq. (1).

$$\text{Specific energy input, } Wspec\ \left(\frac{kJ}{kg}\right) = \frac{V^2 \times (n.\tau)}{\mathrm{R} \times \mathrm{w}} \quad (1)$$

V is the voltage peak value of pulses (in kV), n is the number of pulses applied (dimensionless), τ is the width of the square-wave pulses (in μs), R is the effective load resistance (in ohm) calculated based on the cross-sectional area of chamber, pulse current and the conductivity of the sample to be treated, and w is the weight of sample (potato and buffer) (in gram) in the PEF treatment chamber. In this study, different operating variables were used resulting in effective electric field strengths between 0.2 and 1.1 kV/cm, pulse width of 20 μs, fixed pulse frequency of 50 Hz, total pulse number of 540 and specific energy between 1 and 10 kJ/kg. Experiments were carried out at 20 °C. Temperature changes due to PEF treatments were found to be negligible. For each PEF processing condition, at least 5 independent experiments using independent whole potato tubers were conducted ($n = 5$) for each evaluation i.e. cell permeability, cell viability, microstructure and ion migration.

For each treatment a potato tuber was weighed, positioned in the middle of PEF chamber (the distance between electrodes and the position of the potato tuber was standardised during PEF treatments as depicted in Fig. 2) and completely immersed in phosphate buffer (0.05 M NaH_2PO_4, pH 7.0), ensuring the absence of air bubbles and allowing uniform electric field strength across the two electrodes. The weight of the tuber and buffer used was standardised and measured. After PEF treatment, the tuber was removed from the chamber and sliced using a stainless steel Mandoline vegetable slicer with a 1.5–2 mm fixed gap (Fig. 2). The electrical conductivity and temperature of the phosphate buffer (0.05 M NaH_2PO_4, pH 7.0) prior to and after PEF treatment was monitored using a conductivity/temperature meter (CyberScan CON 11, Eutech Instruments, Singapore).

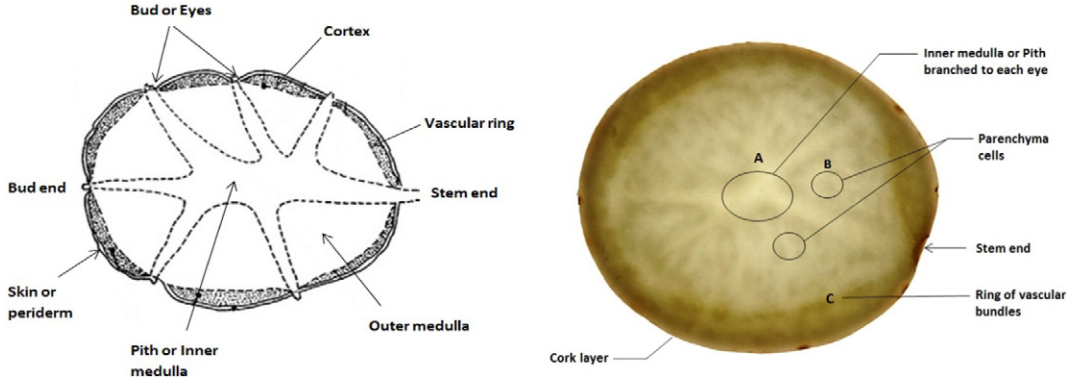

Fig. 1. Cross section of the potato tuber showing internal structure.