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“Cold” electroporation in potato tissue induced by pulsed electric field

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ABSTRACT

This work compares PEF-induced effects in potato tissue at temperatures below and above ambient (T = 2–45 °C). The potato (Agata) was selected for investigation. The PEF treatment using electric field strength E = 200–800 V/cm and bipolar pulses of near-rectangular shape with pulse duration t_p (=100 μs) was applied. The PEF experiments were done under non-isothermal conditions with temperature increase owing to the effect of ohmic heating. The linear temperature dependencies of electrical conductivity of potato tissue with different values of the electrical conductivity disintegration index, Z, were observed. However, the values of the conductivity temperature coefficient, α, at the reference temperature T_r = 25 °C were noticeably different for the intact ($\alpha_i = 0.0255 \pm 0.0003$ °C^{-1}) and completely damaged ($\alpha_d = 0.031 \pm 0.009$ °C^{-1}) potato tissues. This difference was explained by the impact of temperature on the structure of the damaged tissue. The non-isothermal PEF treatment was shown to be an effective tool for electroporation at low temperatures (below ambient). For initial temperature T_i = 2 °C, the most power saving was the PEF treatment at E = 200 V/cm ($W \approx$ 20–30 kJ/kg), and the PEF treatment at E = 400–800 V/cm required more power consumption ($W \approx$ 50–80 kJ/kg). The PEF treatment at the fixed value of E (=400 V/cm) showed that the total power consumptions (accounting for PEF treatment and thermal heating), required for high level of tissue disintegration, $Z \approx 0.9$, were comparable for initial temperatures T_i = 2 °C ($W \approx$ 50–80 kJ/kg) and T_i = 20 °C ($W \approx$ 80 kJ/kg) and were noticeably higher for initial temperature T_i = 40 °C ($W \approx$ 150 kJ/kg).

1. Introduction

Nowadays the electroporation is a recognized tool for enhancing the expression, drying, extraction and diffusion processes in plant tissues (Barbosa-Cánovas and Cano, 2004; Raso and Heinz, 2006; Vorobiev and Lebovka, 2008). The application of pulsed electric fields (PEFs) of 300–1000 V/cm electric field strength, E, and of 100–1000 μs pulse duration, t_i, allows rather efficient plant tissue permeabilisation without noticeable ohmic heating. PEF treatment of different vegetable and fruit tissues (apple, potato, cucumber, aubergine, pear, banana, and carrot) at ambient or moderately high temperatures (T = 20–50 °C) was studied previously in details (Vorobiev and Lebovka, 2011).

The experimentally estimated values of power consumption, W, in PEF-treated tissues were found to be rather low and typically lying within 1–20 kJ/kg. For example, they were 6.4–16.2 kJ/kg (E = 0.35–3.0 kV/cm) for potato (Angersbach et al. 1997), 0.4–6.7 kJ/kg (E = 2–10 kV/cm) for grape skin (López et al. 2008), 2.5 kJ/kg (7 kV/cm) for red beetroot (López et al. 2009a), 3.9 kJ/kg (7 kV/cm) for sugar beet (López et al. 2009b), and 10 kJ/kg (400–600 V/cm) for chicory root (Loginova et al. 2010). Preheating positively affects the PEF induced effect and the enhanced electroporation was observed at temperatures, T, between 40 °C and 50 °C that may be explained by the increased fluidity of cell membranes (Lebovka et al., 2005a,b). However, preheating requires supplementary power consumption and the PEF treatment of cold materials at temperatures below ambient ($T \sim$ 0–5 °C) may be useful in industrial applications. However, the mechanism of PEF-induced changes al low temperatures (below ambient) is still unclear and requires further investigations.

In this work, the potato was used as a model system for testing electroporation effects at temperatures below and above ambient. Note that potato is a popular model material commonly used for the PEF treatment experiments (Angersbach et al. 1997; Lebovka et al. 2005a,b, 2006, 2007, 2008; Praporscic et al. 2006; Galindo et al. 2008a,b; Pereira et al. 2009).

The main aim was to study the temperature effects on damage efficiency and power consumption during the PEF experiments

Abbreviation: PEF, pulsed electric field.

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Nomenclature

C	specific heat (kJ kg/°C)
E	electric field strength (V/cm)
I	current intensity (A)
n	total number of pulses
m	mass of sample (kg)
r^2	coefficient of determination
t_{PEF}	time of PEF treatment (s)
t_p	pulse duration (µs)
Δt	pulse repetition time (ms)
T	temperature (°C)
T_r	(=25 °C) reference temperature (°C)
ΔT	temperature increase (°C)
U	voltage (V)
ΔU	activation energy (kJ/mol)
W	specific energy consumption (kJ/kg)
Z	electrical conductivity disintegration index

Greek symbols

α	electrical conductivity temperature coefficient (°C^{-1})
σ	electrical conductivity (S cm^{-1})
τ	characteristic damage time (s)

Subscripts

d	damaged
i	initial, intact
r	reference

under the condition of simultaneous temperature increase owing to the effect of ohmic heating. The initial temperature of materials was varied between 2 °C and 40 °C. The effects of PEF-induced damage on temperature dependence of potato electrical conductivity were also investigated and discussed.

2. Materials and methods

2.1. Materials

The potato (Agata) was selected for the present investigation. The fresh potatoes of good and uniform quality purchased from a local market (Compiègne, France) were used for experiments within one week after purchase. They were stored in a polyethylene bag in a laboratory refrigerator at 280 K. All the experiments were carried out during the period from March to May. Tissue replicates with different orientations in the tuber were sampled from the central part of the tuber.

The cell used in the PEF treatment experiments consisted of a polypropylene cylindrical glass with the inner diameter of 29 mm and an electrode at its bottom. It was filled with fresh potato juice that was chosen as a natural medium in order to reduce the sample degradation and for improvement of the electric contacts between the electrodes and the sample. The cylinder-shaped sample (29 mm in diameter and 5 mm in height) was placed inside the cell, then the second electrode was installed on the top of the sample, and the electric field was applied straightforwardly to the tissue sample. For measurements of the temperature dependences (T = 2–40 °C) of electrical conductivity, the treatment cell was placed in the cryostat with silicon oil working medium (HUBER CC2, Offenburg, Germany).

The PEF treatment was applied to samples at selected electric field strengths E (=200, 400 and 800 V/cm). The PEF generator (400 V, 38 A, Service Electronique UTC, Compiegne, France) provided bipolar pulses of near-rectangular shape with pulse duration t_p (=100 µs) and pulse repetition time Δt (=200 ms).

The total time of electrical treatment during the PEF experiments, t_{PEF}, was calculated as the total number of pulses (n) multiplied by the pulse duration, t_p, *i.e.*, $t_{PEF} = n\, t_p$ and was not longer than 1 s. The specific power consumption of the PEF treatment, W, was estimated by summation of power consumptions during each pulse, i.e.,

$$W = \sum_{i=1}^{nn_t} UIt_p/m. \tag{1}$$

Here, U is the PEF voltage, I is the current intensity, and m is the mass of the sample.

The initial temperature of the sample (T_i = 2–40 °C) was maintained with help of a fluid (silicone oil) thermostat (Huber, France) (the temperature stability was ±0.1 K). The temperature of the sample, T, continuously increased during the PEF treatment owing to the ohmic heating. So, the PEF treatment was done under non-isothermal conditions. The temperature elevation was controlled in the experiments with a Teflon coated K-type thermocouple (±0.1 K) introduced into the centre of the sample.

The electrodes were connected to the PEF generator and the electrical conductivity of samples was measured during the inter-train period at the frequency 0.5 kHz, which was selected as an optimal for purposes of removing the polarizing effects on the electrodes and tissue sample (Miklavcic et al., 2006). All the output data (current, voltage, electrical conductivity, and temperature) were collected using a data logger and special software adapted by Service Electronique UTC, Compiegne, France.

The degree of tissue damage was estimated from the electrical conductivity disintegration index, Z, (Vorobiev and Lebovka, 2008):

$$Z = (\sigma - \sigma_i)/(\sigma_d - \sigma_i) \tag{2}$$

where σ is the measured electrical conductivity value and the subscripts i and d refer to the conductivities of the untreated (initial) and completely damaged tissue, respectively.

Application of the Eq. (2) gives $Z = 0$ for an intact tissue and $Z = 1$ for a maximally damaged material. The maximum tissue damage degree ($Z = 1$) was obtained at strong PEF treatment of plant tissues at the electric field strength E = 800 V cm^{-1} and reaching a temperature up to 60–70 °C. After such treatment, the electrical conductivity of all the studied plant tissues attained the maximal value σ_d.

All experiments were repeated, at least, three times. One-way analysis of variance was used for statistical analysis of the data using the Statgraphics plus (version 5.1, Statpoint Technologies Inc., Warrenton, VA). For each analysis, significance level of 5% was assumed. The error bars presented on the figures correspond to the standard deviations.

3. Results and discussion

3.1. Temperature dependence of electrical conductivity of PEF treated tissues

Fig. 1 presents electrical conductivity, σ, versus temperature, T, for potato tissues with different electrical conductivity disintegration indexes, Z. The different levels of $Z = Z_r$ were obtained by PEF treatment of potato tissue at reference temperature T_r = 25 °C with electric field strength E = 400 V/cm.