Electrical Impedance Spectroscopy insights into plant tissues treated by Pulsed Electric Fields

Jessica Genovese[1,2], Samo Mahnič-Kalamiza[2], Matej Kranjc[2], Pietro Rocculi[1], Damijan Miklavčič[2]

[1]*University of Bologna, Department of Agricultural and Food Sciences, P.zza Goidanich 60, 47521 Cesena, Italy*
[2]*Univerza v Ljubljani, Fakulteta za elektrotehniko, Tržarška cesta 25, 1000 Ljubljana*
E-pošta: jessica.genovese3@unibo.it

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Abstract. *Electroporation or pulsed electric field (PEF) treatment is known to cause an increase of cell membrane permeability and consequently an increase of the cell membrane conductivity. This is explained by the creation of aqueous pathways in the lipid domain of the cell membrane exposed to the external electric field. Since the cell membrane exhibits relatively high impedance, any permeabilization will result in a drop in impedance of the single cell and consequently of the tissue. Hence, the electroporation effect on biological matrices can be assessed by measurements of their electrical properties. The electrical impedance spectroscopy (EIS) has been suggested as a reliable method to estimate the extent of tissue damage due to high voltage treatment. This study reports on results of the bioimpedance measurements performed on different PEF-treated plant tissues (i.e. apples and potatoes). Furthermore, since bioimpedance depends on several physiological parameters, and changes in electrical properties can be masked by other processes, EIS was performed on a model system, i.e. an agarose phantom, lacking any cell structures and constituents. As expected, no changes of the measured electrical parameters were detected in the agarose samples. On the contrary, plant tissues showed a pronounced drop of the normalized impedance proportional to the electric field amplitude applied to the tissue.*

1 Introduction

The electrical impedance spectroscopy (EIS) is a powerful technique for material characterization by the investigation of its electrical properties. The majority of food matrices are composed of cells, and by the simplest electrical model of an individual cell, the cell is represented as an insulating membrane, while the intracellular and the extracellular media are modelled as ionic solutions (electrolytes). Electrolytes behave as a resistive (ohmic) load up to hundreds of MHz, while, in contrast, membranes exhibit high resistance but also considerable capacitance. Typically, passive electric properties (conductivity, σ, and permittivity, ε) are strongly dependent on the frequency [1]. Looking at one single cell or a suspension of cells between electrodes, several dispersions related to different polarization mechanisms can be identified. At low frequencies ($f = 1 - 10^4$ Hz), the α-dispersion is related to the lateral movement of ions along the insulating membrane; the β-dispersion ($f = 10^4 - 10^8$ Hz) reflects the polarization of the cell membrane; the rotation of molecules having a permanent dipole (water, proteins) cause the γ-dispersion ($f > 10^9$ Hz) [2-3]. Therefore, cells with their membranes will influence tissue impedance in a frequency range up to several MHz. Since the cell membrane acts as a capacitance, low-frequency currents cannot pass via the intracellular route, thus the magnitude of impedance will be higher across the low-frequency range. High-frequency currents are, on the contrary, free to flow through the cell membrane, i.e. the membrane is "invisible" to high-frequency fields. Typically, for most vegetable and animal tissues, the transition between low- and high-frequency behaviour arises at the spectral band starting from about 50 Hz and ending at about 10 MHz [4-5]. Being that the electrical impedance is very sensitive to the permeability of cell membranes, measurements of changes in the complex impedance have been suggested as a method to estimate the degree of tissue damage due to PEF treatment [6]. The utility of impedance measurement has already been demonstrated for various biological systems [7-9].

The aim of our study was to investigate the response of different plant tissues exposed to various amplitudes of the electric field by measuring changes in electrical properties of tissue occurring after pulse application. Additionally, bioimpedance measurements were performed on a model system, i.e. an agarose phantom, which lacks the cell structure, and therefore no changes in its impedance were expected to be seen.

2 Materials and Methods

Measurements of the electrical properties before and after electroporation were performed on different plant tissues and on an agarose phantom.

2.1 Plant tissues

Three plant tissues were used for this study:
- Apples (*Malus domestica*, cv 'Golden Deliciuos');
- Apples (*Malus domestica*, cv 'Idared');
- Potatoes (*Solanum tuberosum*, cv 'Liberta').

The raw materials were purchased at a local supermarket and, before trials, they were manually cut

using a sharp cork-borer to obtain cylindrical-shape samples of 26 mm in diameter and 6 mm in height.

2.2 Agarose phantom

The agarose phantom was made of agarose powder (Thermo Scientific, USA) and distilled deionized water (Braun, Germany). The electrical conductivity of the agarose mixture was adjusted (with the addition of NaCl saline solution) to the initial electrical conductivity of the vegetable tissues used in this study.

The agarose samples were cut into cylindrical-shape of 26 mm in diameter and 6 mm in height.

2.3 Electroporation protocol

Cylindrical samples were placed in a treatment chamber consisted of two stainless-steel parallel plate electrodes spaced at 6 mm from each other, and used to apply 8 rectangular monopolar pulses of 100 µs duration to the tissue with a pulse repetition rate of 1 Hz. For each treatment, different pulse amplitudes were used (from 50 V to 1500 V), resulting in different electric field strengths (V/cm, calculated as the ratio between the applied voltage and the electrodes distance). The delivered voltage and current in the chamber were measured and recorded by a high-voltage probe (mod. HVD3206A, LeCroy USA) and a current probe (mod. CP031A, LeCroy USA) connected to a sequencing digital storage oscilloscope (mod. HDO6104A-MS, LeCroy USA).

2.4 Impedance measurements

The bioimpedance measurement system was assembled by connecting the electroporation chamber that holds the sample and the electrodes to a precision LCR meter (mod. E4980A, Keysight Inc. USA). The same electrodes that were employed to deliver the high-voltage electroporation pulses were also used for the impedance measurements. Switching relays were adopted to alternate the electrical connections of the electrodes between the generator and the LCR meter. Multi-frequency parallel capacitance (C_p) and parallel resistance (R_p) measurements of untreated and treated samples (immediately, i.e. between 3-5 seconds, after the pulse application) were performed in the frequency range of 50 Hz to 1 MHz by applying a 100 mV peak potential difference to the electrodes. Data were acquired using an in-house developed software for the LCR meter control and data capture (based on the Arduino and National Instruments LabVIEW software platforms). The magnitude of impedance was calculated according to equation 1.

$$|Z| = \sqrt{R^2 + X^2} \quad (1)$$

where R is the real component (resistance) and X the imaginary component (reactance) of impedance.

3 Results

For various applied voltage magnitudes and different materials analysed, the ratio between the absolute impedance value after the electroporation ($|Z_a|$) and before the electric treatment ($|Z_b|$) is reported.

As expected, no changes in the magnitude of impedance were observed when agarose phantoms were exposed to pulses of 500 V, 1500 V, or 2000 V amplitude (Fig.1).

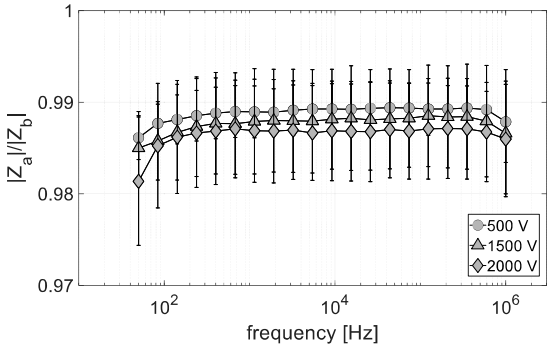

Figure 1. Normalised absolute impedance versus frequency at different applied voltage magnitudes for agarose phantom. Results are expressed as means ± standard deviations (error bars) of n = 4

Conversely, as a result of β-dispersion, the frequency dependence of the impedance can be observed in plant tissues (Fig. 2,3,4 A). Considering that low-frequency currents cannot penetrate the intact cell membrane and are restricted to pass via the extracellular space; when electroporation occurs, it will cause an increase of cell membrane permeability, resulting in a drop in the magnitude of electrical impedance (i.e. low-frequency currents will be able to pass through the intracellular space). Hence, for a better visualization of the dependency of the impedance drop on the magnitude of electric field applied, the normalized absolute impedance at sampling frequency of 5 kHz has been selected (Fig. 2,3,4 B). The indicated frequency has been selected considering that two-electrode impedance measurements suffer from electrode polarization effects, thus a high enough frequency has to be used for comparison.

For both apple cultivars (Golden Delicious and Idared), the curves show a pronounced drop in the normalized impedance when 500 V/cm voltage-to-distance ratio pulses were applied (Fig. 2,3). Dissimilarly, in the potato tissue, a consistent drop of the normalized impedance from ~ 1 (i.e. no differences in the impedance measured after electroporation compared to the value before electroporation) to 0.53 ± 0.02 has been attained after applying 250 V/cm (Fig.4). Electric fields above 500 V/cm did not result in a further decrease in impedance, suggesting the achievement of maximum cell membrane permeabilization using this particular experimental protocol (i.e. number of pulses and pulse duration) (Fig.4 B).

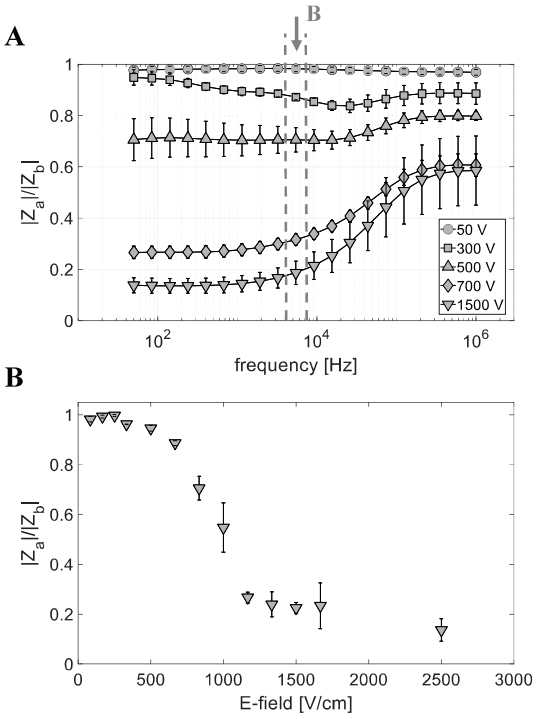

Figure 2. (A) Normalized absolute impedance versus frequency at different applied voltage magnitudes for apple cv. Golden Delicious (not all the data are shown for clarity of presentation). (B) Normalized absolute impedance at sampling frequency of 5 kHz versus the electric field magnitude. Results are expressed as means ± standard deviations (error bars) of n = 4

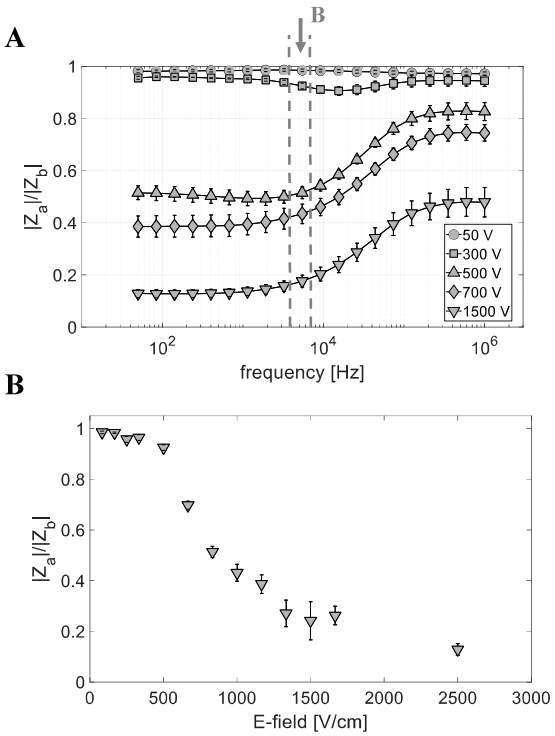

Figure 3. (A) Normalized absolute impedance versus frequency at different applied voltage magnitudes for apple cv. Idared (not all the data are shown for clarity of presentation). (B) Normalized absolute impedance at sampling frequency of 5 kHz versus the electric field magnitude. Results are expressed as means ± standard deviations (error bars) of n = 4

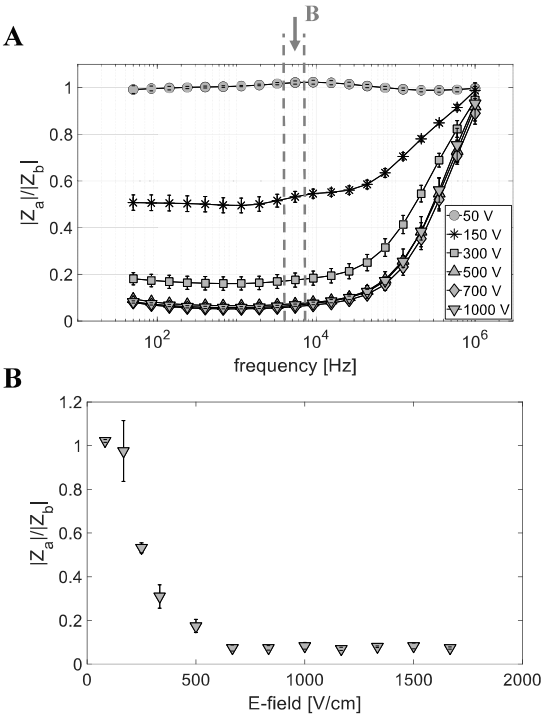

Figure 4. (A) Normalized absolute impedance versus frequency at different applied voltage magnitudes for potato (not all the data are shown for clarity of presentation). (B) Normalized absolute impedance at sampling frequency of 5 kHz versus the electric field magnitude. Results are expressed as means ± standard deviations (error bars) of n = 4

4 Discussions

The aim of this study was to assess the response of plant tissues subjected to pulsed electric field treatments of various pulse amplitudes by measuring the tissue electrical properties. The bioimpedance analysis of PEF-treated plant tissues has shown to be strongly related with the magnitude of the applied voltage.

In general, it can be observed that all vegetable samples exhibit a consistent decrease in their impedance following PEF treatment and the trend is non-linearly related to the magnitude of the electric field applied, suggesting the thresholding nature of the phenomenon. The decrease in impedance can be attributed to the permeabilization of the cell membrane, leading to an increase in the membrane conductivity. On the contrary and as expected, no matter the applied electric field amplitude, in agarose phantom the pulses did not cause any decrease in electrical impedance. The agarose was chosen deliberately as a control/model system, as it is not affected by electroporation due to lack of presence of any of the biological cell structures. Therefore, the changes detected in the select biological tissues by bioimpedance measurements must be related to changes that have occurred due to the application of pulses of certain electric field amplitude. In fact, as it can be discerned from Fig. 2,3,4 (B), a dramatic decrease of the normalized impedance is observed above a certain electric field strength threshold. The latter has shown different values in relation to the different plant tissues analysed in this study (E ~ 500 V/cm for apples; E ~

250 V/cm for potatoes). This could be explained by the different characteristics of the biological material that could affect the electrical parameters, such as the size of cells, the packing density, the spatial distribution of air, the overall tissue moisture content, and initial cell turgor pressure. For this reason, further studies should focus on the relation between the changes of the electrical parameters and changes of other physical characteristics (e.g. mechanical properties) of the biological material subject to PEF treatment.

Acknowledgments

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