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Impedance and texture analysis techniques for detecting and characterising electroporation in plant tissues

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Duration of the experiments: 120 min
Max. number of participants: 4
Location: Tissue Laboratory
Level: Basic

PREREQUISITES

Participants should be familiar with Laboratory safety (S1). No prior knowledge of laboratory work is required. Basic skills of handling electronic instruments such as an oscilloscope and impedance analyser are an advantage, but not prerequisite.

The aim of this laboratory practice is to detect (and quantify) electroporation effects in plant tissues of disparate origin, structure, and water content & solute composition [1], by employing electrical impedance measurements and texture analysis (i.e., tissue's response to mechanic forces). Students will learn of the importance of plant tissue composition and structure, and how these properties impact detection and quantification of electroporation effects in fresh plant matrices.

THEORETICAL BACKGROUND

The application of PEF treatment in food processing is gaining momentum and seeing intensive research and development. New electroporation-based treatments are continuously put to the test and are optimized both at the laboratory and industrial scale processes. PEF treatment offers increasing benefits in terms of low energy use and minimization of food quality deterioration. For successful treatment, an appropriate choice of methods assessing changes due to electroporation occurring in biological matrices of alimentary interest is, however, crucial. Despite a considerable body of literature in the field, detailed information regarding the detection and quantification of the effects of electroporation in complex and highly inhomogeneous multicellular systems, such as real food systems (e.g., plant tissues), is still limited. Moreover, due to the unique characteristics and properties of the biological tissue processed, a case-by-case PEF treatment optimization protocol is often required.

In food-related PEF applications, measurements of the dielectric properties of the tissue are often used to determine the degree of cell membrane disruption by electroporation [2,3]. Electrical impedance spectroscopy (EIS) has been suggested as a reliable method to estimate the extent of tissue damage due to PEF treatment. EIS relies on the theory that, from an electrical point of view, an individual cell can be represented as an insulating membrane exhibiting relatively high resistance to electric current and considerable capacitance, and intra- and extra-cellular media (electrolytes) that behave as a resistive (ohmic) load up to hundreds of MHz. As electroporation affects the permeability (i.e., conductivity) of the cell membrane, multifrequency impedance measurements can be used to assess the degree of membrane permeabilization due to PEF treatment [4,5].

Another way of assessing changes in electroporated plant tissues is offered by texture analysis (texture in the sense of the response of a material to mechanical forces). Plant tissues in structures such as roots, fruits, and tubers, often exhibit considerable turgidity (high turgor pressure) when fresh and hydrated. Increasing membrane permeability of the cells by electroporation can result in release of the intracellular water that is filtered through the extracellular matrix [6]. From the analysis of tissue's response to external force at the exact moment of electroporation and within minutes after, it is possible to evaluate the extent to which the electroporated plant tissue has been affected by the treatment. Texture analysis thus offers an alternative method to evaluating the degree of cell membrane disruption in treatment protocol optimisation where impedance measurements are either unavailable or not practical.

EXPERIMENT

We will perform concurrent sample deformation analysis (at constant loading force) and impedance measurements (pre- and post-pulse delivery) on two plant tissues: an apple fruit sample, and a potato tuber sample. You will familiarize yourself with the principles of the two methods of analysis by experimenting on potato tuber and apple fruit sample, both at a given voltage, after which we will move to a computer and analyse the data that you obtained along with previously gathered data obtained in a voltage escalation study using both types of tissue. You will work with deformation and impedance data recorded for various treatment efficacies (correlating with the extent of changes in tissue caused by electroporation). Together with your data point you will have a total of five different voltages (and thus five different voltage-to-distance ratios) and the control (0 V) to work with. Altogether, you will analyse twelve sets of impedance and texture measurements.

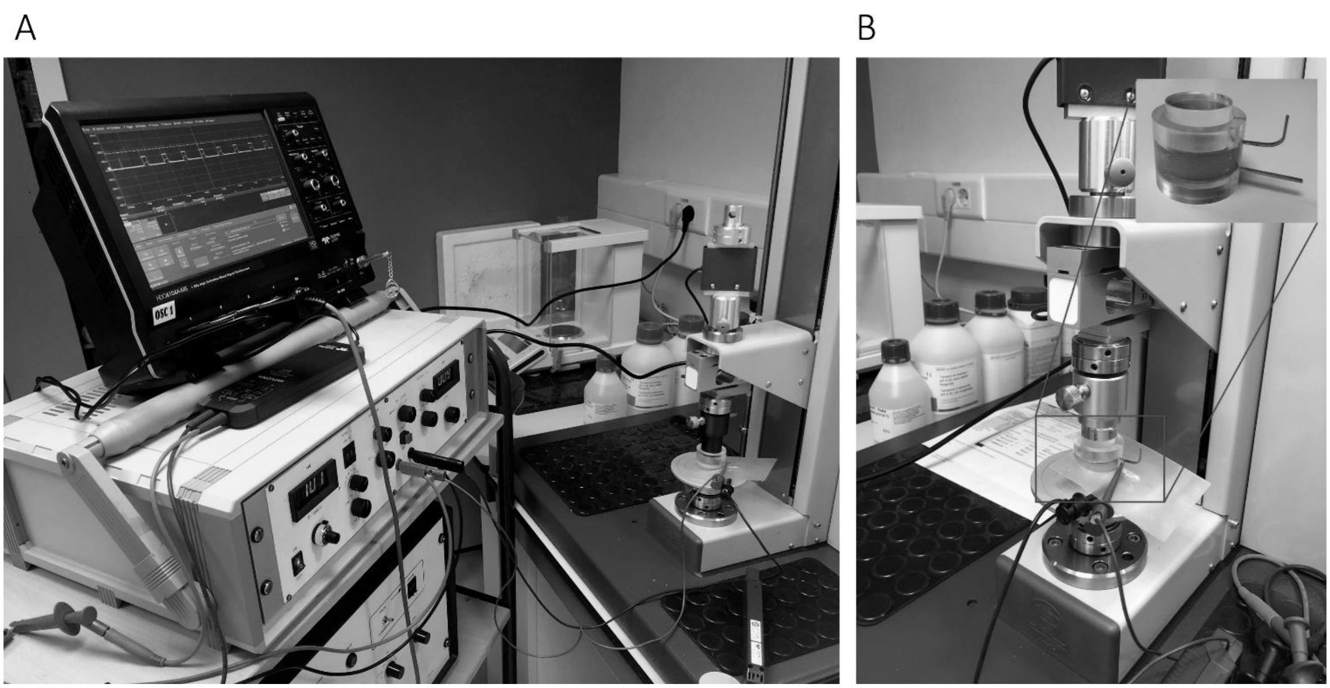

Figure 1: (A) Experimental setup showing the texture analyser, generator, and oscilloscope; and (B) A detailed view of the treatment chamber as set up under the texture analyser piston and of the treatment chamber setup on its own.

Protocol:

You will prepare a sample of apple fruit and of a potato tuber (cultivar depending on availability) cut into a 6 mm thick cylinder of 25 mm in diameter. Sample will then be placed into a cylindrical treatment chamber with plate electrodes at the top and bottom of the sample (see Figure 2), the entire setup will then be placed under the piston of a texture analyser (Hegewald & Peschke Inspect solo 1 kN-M) and subjected to a constant force of 5 N and 10 N for apple fruit and potato tuber, respectively. Electrodes will be connected both to a pulse generator (prototype device), as well as an impedance analyser (an LCR meter, Keysight E4980A), and a switching circuit that will switch between the pulse generator and the impedance analyser to protect the LCR instrument from high-voltage pulses (prototype device).

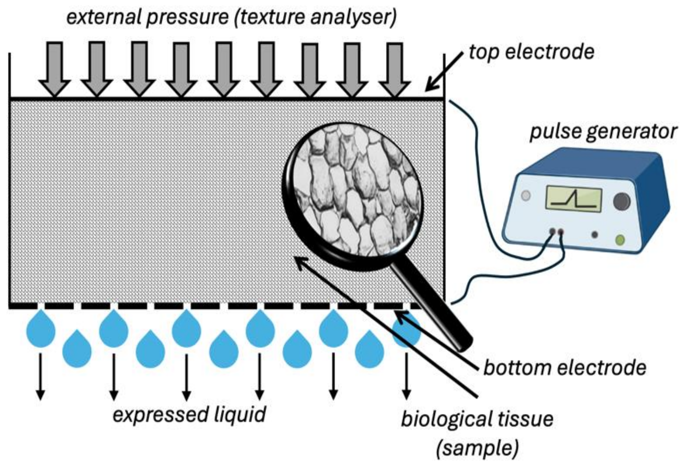

Figure 2: A schematical illustration of a sample placed into a cylindrical treatment chamber with plate electrodes at the top and bottom.

The force will be applied for a total of 3 minutes. After 10 seconds under load, you will measure the pre-pulse impedance, and after 30 seconds under load, you will deliver 8 pulses of 100 µs at 1 s-1 repetition rate, and then immediately measure the post-pulse impedance. The loading of the sample will then continue for another two and a half minutes (until 3 minutes total loading time is reached).

Deformation curves obtained from the texture analyser and impedance measurements will then be imported into MATLAB using scripts prepared in advance for further analysis, during which you will:
Calculate the ratio of post- to pre-pulse electrical impedance of the sample at 5 kHz frequency and plot it versus the applied voltage.
Calculate the total deformation of the sample from the moment of pulse delivery and up to the end of the constant force application and plot this deformation versus the applied voltage.

We will then compare the two functions/plots for both plant tissues and we will discuss the interpretation. The lab work concludes with a printout of graphs that you will paste into your workbook (under NOTES & RESULTS to the right).

REFERENCES:

[1] Lebovka N., Vorobiev E. Techniques to detect electroporation in food tissues. In *Handbook of Electroporation* (ed. Miklavcic, D.) Springer, 2017.

[2] Angersbach. A., Heinz V., Knorr, D. Electrophysiological model of intact and processed plant tissues: Cell disintegration criteria. *Biotechnol Prog,* 15/4:753-762, 1999.

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[4] Pavlin M., Miklavčič D. Effective conductivity of a suspension of permeabilized cells: a theoretical analysis. *Biophys J*, 85:719-729, 2003.

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NOTES & RESULS