Impedance and texture analysis techniques for detecting and characterising electroporation in plant tissues L14

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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.

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 requirements 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 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 for the determination of the degree of cell membrane disruption by electroporation. 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.

Another possibility 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 not dehydrated. Disrupting the selectively permeable membrane of the cells by electroporation can result in release of the intracellular water that is filtered out through the extracellular matrix. 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 offers an alternative method to evaluating the degree of cell membrane disruption in treatment protocol optimisation where impedance measurements are either unavailable or impractical.

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, 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.

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. To vary the treatment efficacy, and thus the extent of changes in tissue caused by electroporation, we will perform a voltage escalation study at three different voltages (and thus three different voltage-to-distance ratios), and repeat every experiment twice to ensure we have a stable set of data to work with (we would opt for a higher number of repetitions in a non-learning environment, the limit to two is due to time constraints). Altogether, we will perform 12 sets of impedance and texture measurements. In addition to recording the impedance and piston displacement, we will also be monitoring the pulse voltage and current with an oscilloscope (Teledyne LeCroy HDO6104A-MS).

<This labwork is conducted by Samo Mahnič-Kalamiza (placeholder for banner)>

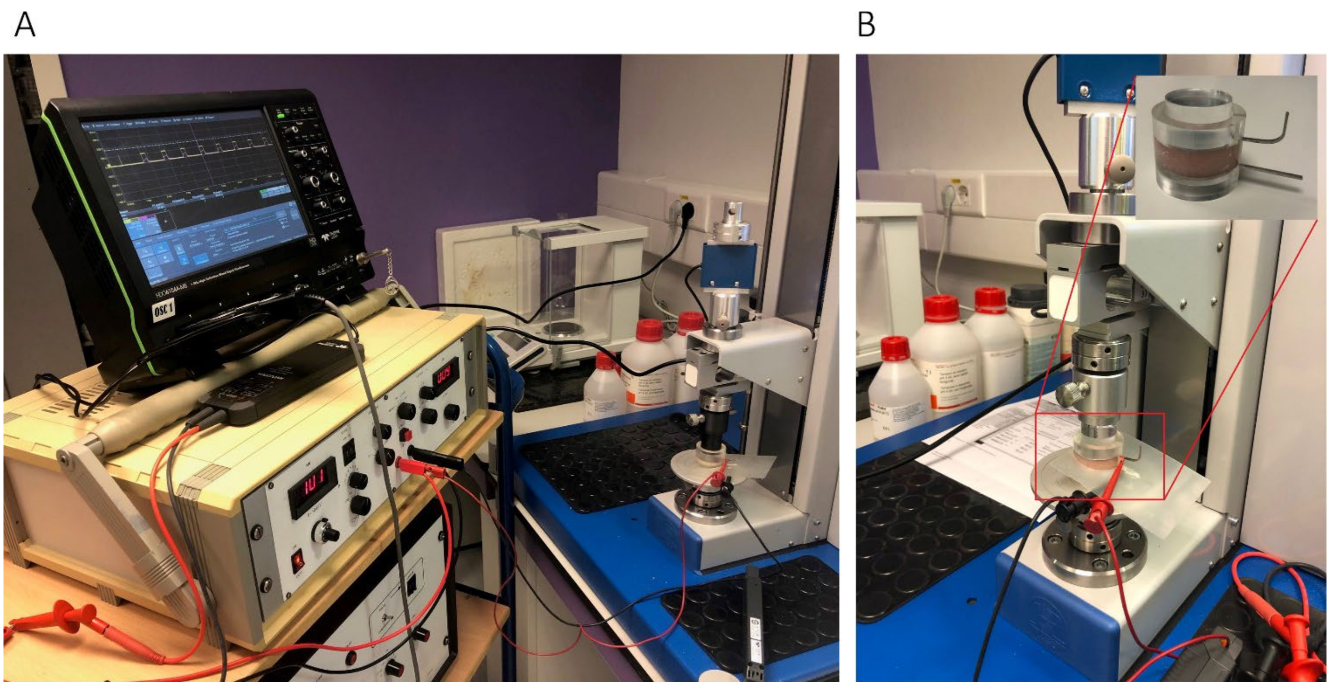

Figure 1: (A) Experimental setup showing the texture analyser, generator, and oscilloscope; and (B) A detailed look at the treatment chamber as set up under the texture analyser piston and of the treatment chamber setup on its own.

Protocol:
We will prepare six samples of apple fruit (cultivar depending on availability) cut into 6 mm thick cylinders of 25 mm in diameter. We will also prepare six samples of a potato tuber (cultivar depending on availability) cut into cylinders of identical dimensions as for the apple fruit.
Samples will be placed into a cylindrical treatment chamber with plate electrodes at the top and bottom of the sample (see Figure 1), 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 (in short – 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).
The force will be applied for a total of 2 minutes. After 30 seconds under load, you will measure the pre-pulse impedance, and then immediately deliver 8 pulses of 100 us at 1 Hz repetition frequency, then immediately measure the post-pulse impedance. The loading of the sample will then continue for another minute or so (until 2 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 workbooks (under NOTES & RESULTS to the right).

FURTHER READING:

Lebovka, N., & Vorobiev, E. (2017). Techniques to detect electroporation in food tissues. In Handbook of electroporation. https://doi.org/10.1007/978-3-319-32886-7_150.

Angersbach, A., Heinz, V., & Knorr, D. (1999). Electrophysiological model of intact and processed plant tissues: Cell disintegration criteria. Biotechnology Progress 15/4, 753-762. https://doi.org/10.1021/bp990079f.

Grimi, N., Lebovka, N., Vorobiev, E., Vaxelaire, J. (2009). Compressing Behavior and Texture Evaluation for Potatoes Pretreated by Pulsed Electric Field. Journal of Texture Studies 40, 208–224. https://doi.org/10.1111/j.1745-4603.2009.00177.x.

Mahnič-Kalamiza, S., Vorobiev, E. (2014). Dual-porosity model of liquid extraction by pressing from biological tissue modified by electroporation. Journal of Food Engineering 137, 76–87. https://doi.org/10.1016/j.jfoodeng.2014.03.035.

NOTES & RESULTS