L11

Techniques of 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 must be familiar with basic laboratory safety procedures (see S1). While no prior laboratory experience is required, basic proficiency in handling electronic instruments such as oscilloscopes and impedance analysers is beneficial, though not essential.

The aim of this lab session is to demonstrate select methods of detecting and quantifying the effects of electroporation in plant tissues of various origin, structure, water content, and composition [1]. Using electrical impedance measurements and texture analysis (i.e., tissue response to mechanical force), participants will explore how plant tissue properties influence electroporation detection and its quantification in fresh plant matrices.

THEORETICAL BACKGROUND

Pulsed Electric Field (PEF) treatment is increasingly used in food processing due to its low energy consumption and minimal impact on food quality. Successful PEF treatment, however, depends on accurate detection and assessment of electroporation effects in biological materials.

Despite substantial research, the detection and quantification of electroporation in complex, inhomogeneous systems—such as real food matrices—remains challenging. PEF protocols often require optimization tailored to specific tissue types due to their unique structural and compositional properties.

One common method of assessment is electrical impedance spectroscopy (EIS), which evaluates changes in the dielectric properties of tissues. In electrical terms, a biological cell can be modelled as an insulating membrane with high resistance and significant capacitance, surrounded by conductive intracellular and extracellular fluids. Electroporation alters membrane permeability, and thus conductivity, making EIS a useful tool for estimating the extent of membrane disruption [2,3,4,5].

An alternative or complementary method is texture analysis, which examines the mechanical response of plant tissues (e.g., fruits, tubers, roots). Fresh, hydrated plant tissues often maintain high turgor pressure. Electroporation-induced membrane permeability can cause intracellular fluid to leak into the extracellular space [6]. By analysing tissue deformation in response to mechanical loading—particularly at and shortly after pulse delivery—we can evaluate the extent of electroporation-induced structural changes. Texture analysis is particularly valuable when impedance measurement equipment is unavailable or impractical.

EXPERIMENT

In this laboratory session, we will perform simultaneous mechanical deformation and electrical impedance measurements on potato (tuber) tissue. The goal is to investigate how electroporation affects the tissue by applying a pulsed electric field and observing changes in its mechanical and electrical properties.

Participants will first familiarize themselves with the analytical principles by conducting initial measurements on untreated samples. These measurements will be followed by data analysis, where participants will evaluate experimental results across a range of applied voltages. The dataset will include both the measurements obtained during the session and pre-recorded data from a voltage escalation study conducted on a different type of tissue (apple fruit).

In total, you will analyse 6 sets of paired impedance and deformation data. Each set corresponds to a different voltage treatment level, i.e. five distinct voltages plus a control (0 V).

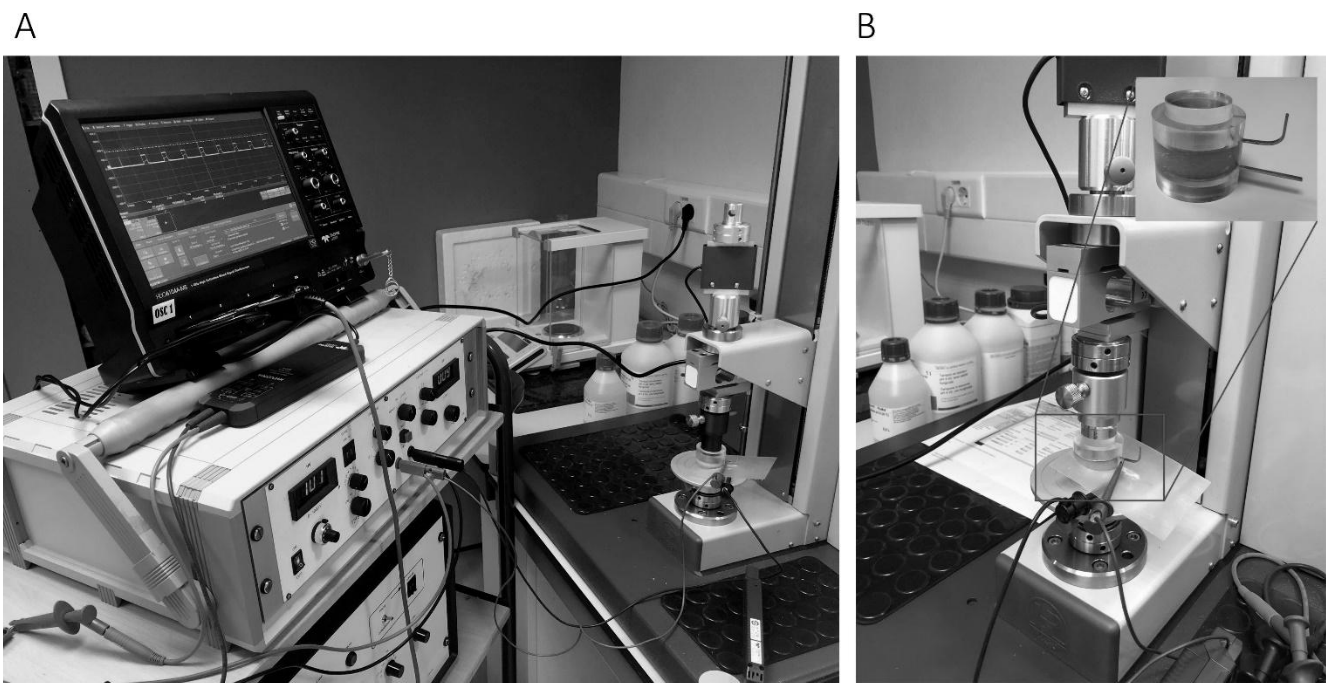

Figure 1: (A) Experimental setup showing the texture analyser, generator, and oscilloscope; and (B) A detailed view of the treatment chamber as set up under the texture analyser piston and of the treatment chamber setup on its own.

Experimental setup

Samples:

- Potato tuber (cultivar depending on availability);
- Each sample will be cut into a cylindrical shape: 6 mm thick, 25 mm in diameter.

Treatment chamber:

- Cylindrical chamber equipped with parallel plate electrodes at the top and bottom (see Figure 1B);
- Sample is inserted between the electrodes and placed under the piston of a texture analyser (Hegewald & Peschke Inspect solo 1 kN-M).

Instrumentation:

- A pulse generator (laboratory prototype device);
- An impedance analyser (LCR meter, Keysight E4980A);
- A switching circuit that allows for alternating between the generator and impedance analyser to prevent damage to the LCR meter during high-voltage pulses.

Treatment protocol

A loading force of 10 N will be applied via the texture analyser piston to potato samples.

Sequence of events (total duration: 2 minutes):

1. Apply mechanical load (constant force) to the sample.
2. At 10 seconds, measure the pre-pulse impedance.
3. At 30 seconds, deliver 8 pulses (100 µs duration, 1 s^{-1} repetition rate).
4. Immediately (i.e., within seconds) after pulse delivery, measure the post-pulse impedance.
5. Continue mechanical loading until a loading time of 120 seconds total is reached.

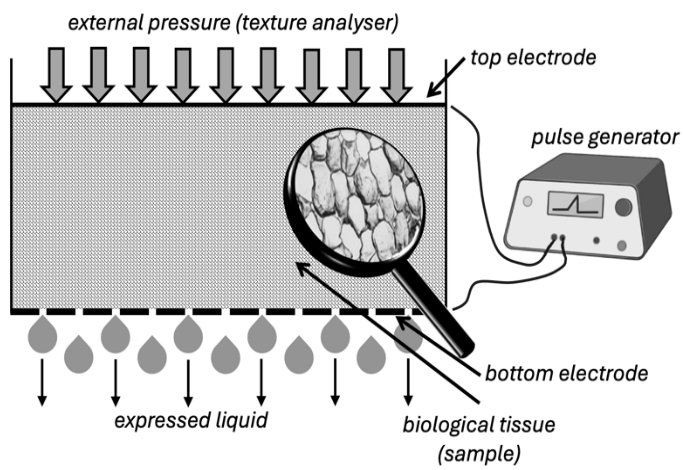

Figure 2: A schematical illustration of a sample placed into a cylindrical treatment chamber with plate electrodes at the top and bottom.

Data analysis and interpretation

After completing the measurements, you will analyse the impedance and deformation data using pre-prepared MATLAB scripts. The analysis will focus on quantifying electroporation effects and comparing the mechanical and electrical responses of the two plant tissues.

Step 1: Electrical impedance analysis

- Calculate the ratio of post-pulse to pre-pulse impedance (take the absolute value first and disregard the angle) at 50 kHz for each treatment condition.
- Plot this impedance ratio against the applied voltage to observe how electrical properties change with increasing electroporation intensity.

Step 2: Texture (deformation) analysis

- Determine the total deformation of each sample from the moment of pulse delivery until the end of the 2-minute constant loading period.
- Plot the deformation against the applied voltage to assess how mechanical properties are affected.

You will perform the analysis for all samples of potato tissue in a voltage escalation study. In your own measurement set, you will work with six voltage conditions (five voltage levels plus one control), resulting in a total of twelve paired datasets (impedance and deformation). Apple fruit data is already available ahead of time to make this lab work feasible within the available two hours.

Interpretation and discussion

- Compare the impedance and deformation trends across both tissue types and treatment intensities.
- Discuss the correlation between membrane permeabilization and tissue electrical homogenisation (from electrical data) and mechanical softening (from texture data).
- Comment on the relative sensitivity and practicality of each method for detecting electroporation effects in different plant matrices.
- Consider how tissue structure, water content, and solute composition influence the results.

The lab session concludes with a brief review of your findings. You will print the generated graphs and paste them into your lab workbook under the "NOTES & RESULTS" section for submission and documentation.

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.

[3] Grimi N., Lebovka N., Vorobiev E., Vaxelaire J. Compressing behavior and texture evaluation for potatoes pretreated by pulsed electric field. *J Texture Stud,* 40:208-224, 2009.

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

[5] Pavlin M., Miklavčič D. Theoretical and experimental analysis of conductivity, ion diffusion and molecular transport during cell electroporation — Relation between short-lived and long-lived pores. *Bioelectrochemistry,* 74:38-46, 2008.

[6] Mahnič-Kalamiza S., Vorobiev E. Dual-porosity model of liquid extraction by pressing from biological tissue modified by electroporation. *J Food Eng,* 137:76-87, 2014.

NOTES & RESULS