Effects of Pulsed Electric Field on the Viscoelastic Properties of Potato Tissue

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ABSTRACT

We have investigated whether transient permeabilization would give rise to transient or permanent changes in the potato tissue texture. The changes on the viscoelastic properties of potato tissue exposed to PEF during small-amplitude oscillatory dynamic rheological measurements were monitored. The elastic (G′) and viscous moduli (G″) were measured every 30 s after the delivery of the pulse and the loss tangent (tan-δ) change calculated. Our results, supported by similar measurements on osmotically dehydrated control samples, clearly show that PEF causes a rapid change of the viscoelastic properties of the tissue that could be attributed to a partial loss in turgor pressure. This would be an expected consequence of electroporation. The recovery of tan-δ to values similar to those before pulsation, strongly suggests recovery of cell membrane properties and turgor.

1 Materials and Methods

1.1 Samples

Potato tubers (*Solanum tuberosum* L. cv. Bintje) grown in the south of Sweden, were manually washed and peeled. A rectangular cross-section sample, 15 mm long and 6.0 mm wide, was obtained from the phloem parenchyma tissue using a pair of parallel sharp blades.

1.2 PEF treatment

- Samples were treated at nominal field strength range of 30 to 500 V/cm, with single rectangular pulses of 1 ms, 10 μs and 100 μs, delivered axially to the tissue;
- Osmotically pre-treated samples were subjected to a single 1 ms pulse in the range of 30 to 500 V/cm. Additionally, a sequence of 9 rectangular pulses of 500 V/cm of 1 ms each was also tested;
- The pulsation for each PEF treatment was given 150 to 200 s after the start of the small-amplitude oscillatory dynamic rheological measurements;
- Five measurements were done for each condition.

1.3 Small-amplitude oscillatory dynamic rheological measurements

The potato sample was placed on the lower plate of the rheometer. The upper plate was brought into contact with the sample until a normal force of 1 N was reached. Measurements of the tan-d were recorded in the oscillatory mode as a function of time at intervals of 30 s The oscillation stress amplitude was 0.1 % and the frequency 1 Hz. Stress sweeps were applied to verify that data were acquired within the linear viscoelastic regime.

1.4 Experimental setup

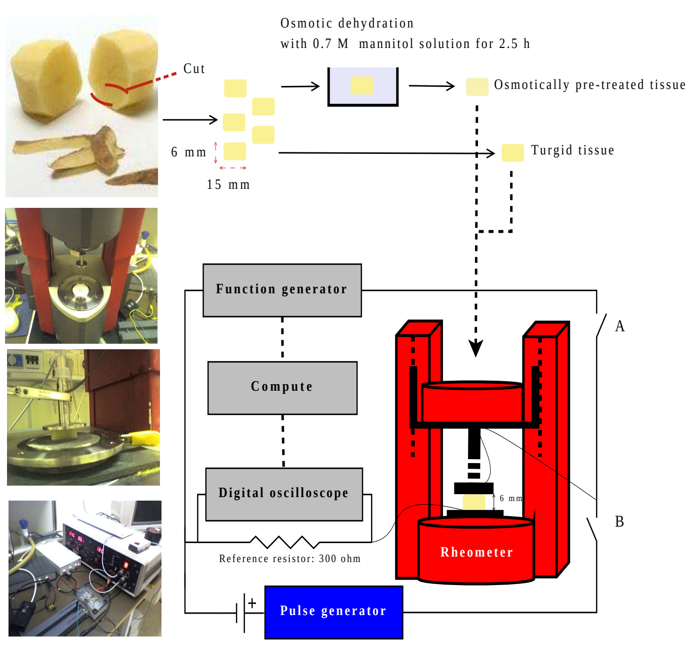


Figure 1. Application of PEF treatment during small-amplitude oscillatory dynamic rheological measurement

- Sample impedance was measured prior to pulsing and 30 s to 2 min after pulsing by using a low voltage sinusoidal wave form (V_s) at 5 V and 1 kHz, by turning on switch A;
- The resistance of the tissue samples during the pulsing period was done by turning on switch B, to deliver the high pulse voltage (V_t) from pulse generator;
- After PEF, response voltage (V_f) was monitored and recorded through the reference resistor (R_f) by using a digital oscilloscope. The impedance (I) of the tissue was calculated through Equation 1 [1];
- Electrical resistance of the tissue during pulsing period was calculated through Equation 1 by using V_t instead V_f.

$$I = R_f\left(\frac{V_f}{V_s} - 1\right) \quad (1)$$

2 Results

2.1 Tan-δ measurement during PEF treatment

- Tan-δ change was calculated by subtracting the tan-δ values measured at time intervals of 30 s after pulsation by the initial value of tan-δ, immediately measured before pulsation (Figure 2a). The values corresponding to the initial stabilization of the oscillatory measurements were then subtracted (Figure 2b and Figure 3).

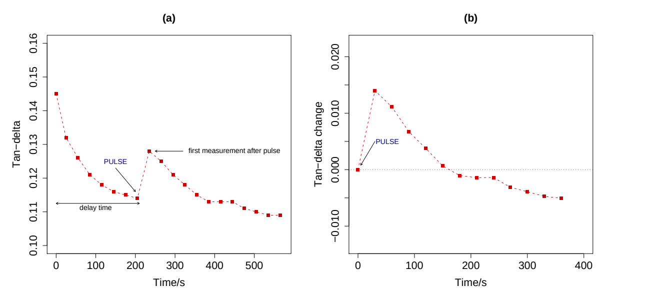
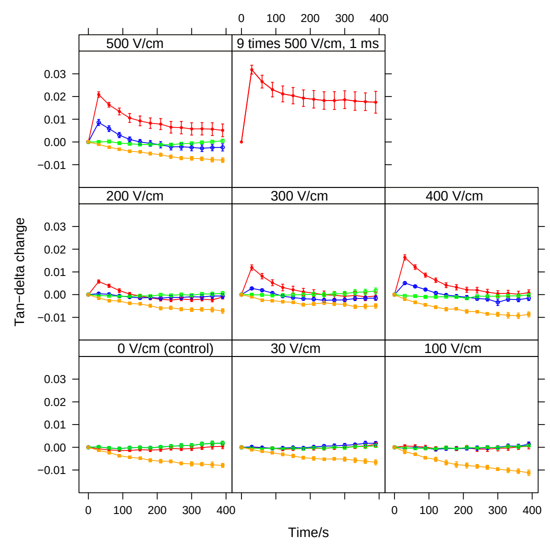


Figure 2. Example of tan-δ measurement (a) and tan-δ change (b) before and after a pulse of 1 ms at 400 V/cm being applied

- For the PEF treatments at pulse widths of 100 μs and 1 ms, above the 200 V/cm the tendency observed was the increase of tan-δ (decrease of stiffness) within 30 s after pulsation and its recovery at a time scale of minutes;
- Increase of tan-δ in turgid samples followed the increase of the intensity and pulse width of PEF treatments applied;
- Application of PEF on the osmotically pre-treated tissues did not produce any effect in tan-δ.



Pulse widths of 10 μs (—), 100 μs (—) and 1 ms on turgid tissue (—) and on osmotically treated tissue (—).

Figure 3. Effects of PEF treatment on tan-δ of potato tissues, when one single pulse of 10 μs, 100 μs, and 1 ms, on turgid and osmotically pre-treated tissue, were applied at different field strengths. The error bars represent the standard error of the mean of 5 experiments.

2.2 Tan-δ recovery

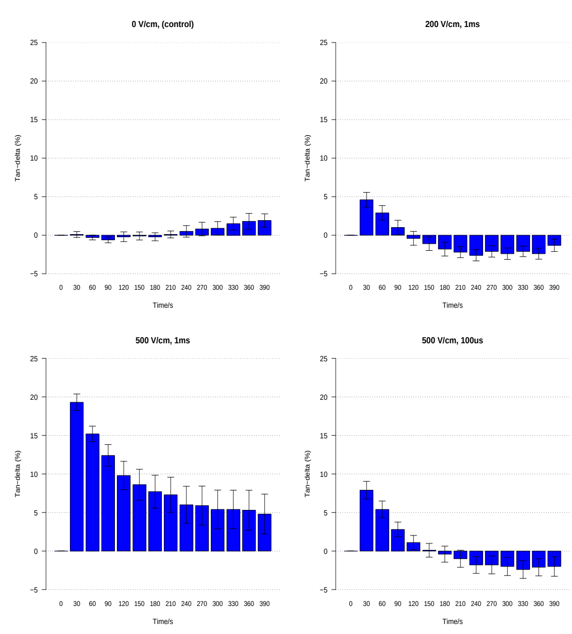
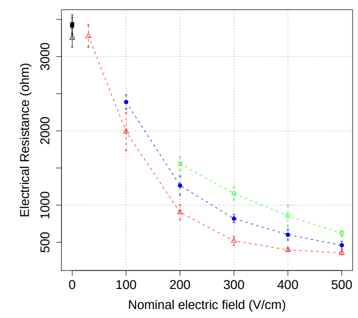


Figure 4 Examples of tan-δ percentage recovery when pulses of 1 ms (at 200 V/cm and 500 V/cm) and 100 μs (at 500 V/cm were applied. The error bars represent the standard error of the mean of 5 experiments.

2.3 Electrical response upon PEF treatment

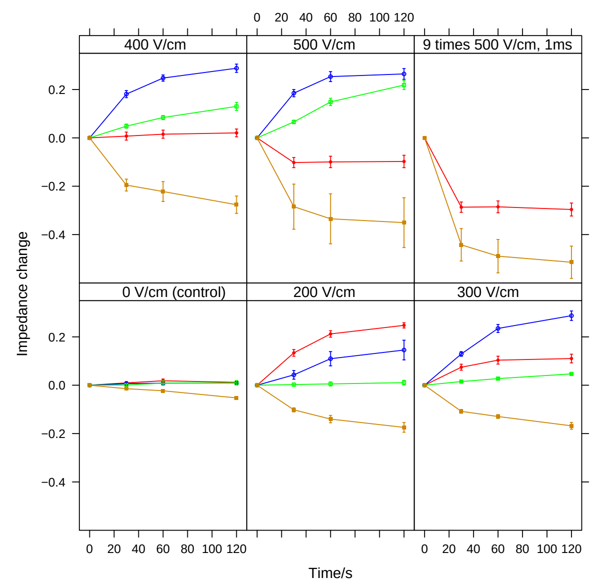
- Total permeabilization of the tissue reached similar values when widths of 1 ms, 100 μs and 10 μs were applied during pulsation, particularly for 500 V/cm;
- At pulse widhts of 10 μs a drastic decrease of the electrical resistance of the tissue was not accompanied by a measurable viscoelastic response.



Pulse widths of 10 μs (—), 100 μs (—) and 1 ms on turgid tissue (—).

Figure 5. Electrical resistance during pulse delivery at different field strengths. The error bars represent the standard error of the mean of 5 experiments.

- The electrical impedance is presented as a change compared to the value of the first measurement before pulsing.



Pulse widths of 10 μs (—), 100 μs (—) and 1 ms on turgid tissue (—) and on osmotically treated tissue (—).

Figure 6. Change of the impedance after PEF treatment at different field strengths. The error bars represent the standard error of the mean of 5 experiments.

3 Conclusions

I. Small-amplitude oscillatory dynamic rheological measurements for potato tissue show evidently that PEF treatments give rise to dynamic changes of its viscoelastic properties;

II. The initial increase of the tan-δ values could be attributed to a partial loss in turgor pressure;

III. This increase, an expected consequence of electroporation, was strongly dependent on the pulse conditions;

IV. At certain PEF conditions, the recovery of the tan-δ to values similar to those before pulsation was observed, suggesting the recovery of cell membrane properties and turgor;

V. This study has also resulted in interesting questions regarding different events taking place in the cells upon reversible electroporation. The complex cell stress physiology involving both cell membrane functional properties and cell wall structure would influence the physical properties of the tissue in a pattern that needs to be further explored at the micro or nanoscale level.

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References

[1] Y. Chalermchat. *Effects of Pulsed Electric Fields on Plant Tissue.* PhD thesis, Lund University, 2005.