REVIEW

REVERSIBLE ELECTROPORATION OF VEGETABLE TISSUES - METABOLIC CONSEQUENCES AND APPLICATIONS

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ABSTRACT

This review explores recent findings on gross metabolic responses of vegetable tissue induced by the application of pulsed electric field as well as discusses potential industrial applications of reversible electroporation./ *Este artículo explora recientes investigaciones sobre respuestas metabólicas de tejidos vegetales inducidas por la aplicación de pulsos eléctricos. También se discuten potenciales aplicaciones industriales de la electroporación reversible.*

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INTRODUCTION

The application of pulsed electric fields (PEF) to living cells causes a transient increase in the transmembrane potential difference, modifying the plasma membrane organization and bringing it locally to a permeable state (1). The application of PEF may cause lethal damage to cells, or induce sublethal stress by transient permeabilization of cell membranes and electrophoretic movement of charged species between cellular compartments (2).

The technique is, in its lethal version, at very high field strength, used for the inactivation of microorganisms in foods (3,4) and can, at lower field strength, be used for the improvement of extraction yield (5,6) or increased rate of drying (7,8). In its non-lethal version, the permeabilization may evade affecting cell viability. This is, under the name electrotransformation, routinely used for gene transfer with walled as well as wall-less systems (9).

When cell viabilily is kept after the application of the electric field, physiological responses are still largely unknown. It has been reported that PEF affects metabolism, including an oxidative burst with the consequent generation of reactive oxygen species (ROS) (1,10), and the stimulation of the production of secondary metabolites such as increased yields of a cytostatic compound in cell culture of *Taxus chinensis* (11) and of antioxidants and phytoesterols from oil seeds and fruits (12,13).

To our knowledge, little attention has been given to the application of PEF-induced stress to postharvest processing. Traditionally, postharvest processing is mainly concerned with slowing down or inactivating metabolic processes in the harvested product. Apart from the effects of ripening and senescence, reports on the active plant metabolism including stress-induced physiological events are not common in the literature. Recently, Gómez Galindo et al. (14) reviewed the importance and prospects of plant stress in postharvest treatments. Observed metabolic stress consequences include potato sweetening in cold storage, chilling damage of tomato and potato tissue suberization following industrial peeling. As most stresses include an oxidative burst and concomitant antioxidant defenses, the general utilization of stresses has been proposed as a means of increasing the plant tissue antioxidant levels in both preharvest (15) and postharvest (16).

Inducing stress by chemical means is not an attractive proposition for a healthy food product. Physical intervention, apart from ionizing radiation, is likely to be a more acceptable candidate. However, unlike chemical stress, temperature or pressure stress can not be easily targeted. The induction of sublethal stress by the application of PEF is likely to be a potential industrial alternative for metabolic stimulation, being unique as a stress regarding its primary effect on cell membranes and by causing a homogeneous effect over the whole treated tissue. Therefore,

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research is needed at first to find appropriate conditions (e.g. pulse shape and polarity, number, width, interpulse time) for PEF-induced metabolic stimulation. These conditions can then be refined upon an understanding of physiological responses.

This review explores recent findings on gross metabolic responses of potato tissue induced by the application of PEF as well as discusses potential industrial applications of reversible electroporation.

METABOLIC CONSEQUENCES

With the application of PEF, opening of pores in the plasma membrane will result in the efflux and influx of polar molecules. After the pulse application, the resealing process is accompanied by oxidative stress with the consequent production of ROS (2). H_2O_2 is a major constituent of ROS production upon stress (17,18). Recent studies, however, suggest that oxygen consuming pathways such as respiration and production of H_2O_2 are not the only metabolic responses to PEF. Gómez Galindo et al (19) demonstrated that PEF induces complex metabolic responses that may involve several aspects such as energy release from the movement of ionic species, ATP hydrolysis to rebuild gradients of charges across cell membranes and/or other physiological events taking place during electropermeabilization as well as long after resealing. Isothernal calorimetry studies on metabolic responses also showed evidence that this response is strongly influenced by the intensity and width of the pulse. It has been suggested (19) that the complex metabolic responses of plant tissues upon PEF-induced stress are strongly influenced by the size and the persistence of the created pores. The size and the distribution of the pores will depend on the pulsing conditions. Certain PEF conditions may create many small, transient pores (around 1.5-nm radius), that would relax and reseal in a time scale of ns (20, 21), with stronger PEF conditions, the distribution of pore sizes would include larger pores (around 50-nm radius) which tend to reseal more slowly (>1 s in some cases) or not at all (21). It is possible that the metabolic work that the cell needs to do when dealing with the formation of small or large pores is different and may depend on the extent of polar compounds migrating upon electroporation as well as the amount of energy required for resealing and further recovery.

Other striking results on metabolic consequences of PEF-induced stress, suggests that PEF affects the cell wall organization. Gómez Galindo et al. (22) reported kinetic studies of the labeling of plasma membrane with the fluorescent dye FM1-43. This amphipathic styryl dye has a divalent cationic head group and a lipophilic tail, and it reversibly partitions into the outer leaflet of the cell membrane. FM1-43 fluoresces weakly in an aqueous environment, and its quantum yield increases by two orders of magnitude on interaction in the lipid membrane (23).

Kinetic studies on plant cells have shown that after application of FM1-43, staining of the plasma membrane is immediate and the cell wall does not act as a barrier that can significantly slow the staining process (24). However, it was shown through chemical treatment of the cell wall, that the diffusion of FM1-43 towards the cell membrane is slower if cell wall porosity decreases and, therefore, FM1-43 was tested as a marker for assessing cell wall permeability changes after the application of PEF.

In Figure 1a-d, typical microscopic observations of the fluorescent interaction of the dye with the cell membrane after PEF application are shown. Figures 1a and b show a dramatic decrease of the fluorescence intensity of the PEF-treated sample in comparison with that of the control. The fluorescence intensity is progressively restored when increasing the dipping time of the potato slice in FM1-43 from 2 to 5 min (Figures 1c, d), showing that PEF treatment has affected the rate of diffusion of the dye through the extracellular space, slowing the staining process.
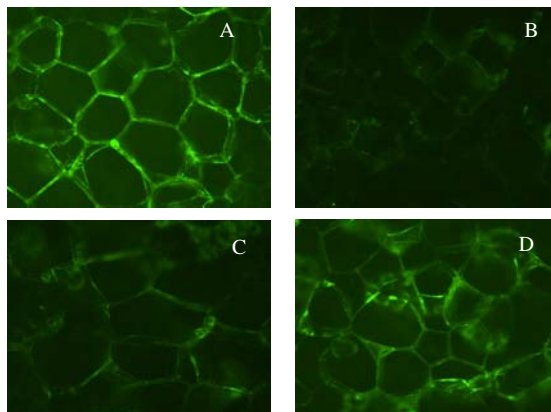
Figure 1. Typical experimental results from the effect of electropulsation on the fluorescence intensity of FM1-43. Potato tissue was treated with field strength of 400 V/cm (one 1 ms rectangular pulse) and FM1-43 was applied. (A) Potato tissue that was not PEF-treated (control) and dipped 2 min in FM1-43 (B, C, D) PEF-treated tissue dipped in FM1-43 for 2, 3 and 5 min respectively

Through further kinetic studies, Gómez et al (22) concluded that electropulsation, in the range of 100 to 500 V/cm (one 1 ms rectangular pulse), slows the diffusion of the dye FM1-43 through the cell wall of potato tissue, suggesting a significant decrease in cell wall permeability at nanometer scale. Experimental evidence linked those changes with the production of H_2O_2 by cell wall associated peroxidases.

These results suggest that, in vegetables with the metabolic capacity to induce cell wall structural changes upon stress, the application of PEF under the studied conditions would not only open pores in the cell membrane but also induce the reduction of cell wall porosity. The observed phenomena may affect the processing properties and composition of plant products extracted or otherwise processed using pulsed electric fields. Furthermore, the implications of these structural changes for industrial processes aiming at transferring substances inside cells with the aid of PEF should be further explored.

INDUSTRIAL APPLICATIONS

Attempts to transfer substances inside fruit and vegetables tissues through electroporation are not common in the literature. Recently, however, Yeu Phoon et al. (25) reported the successful insertion of trehalose as cryoprotectant in spinach leaves.

Attempts have been made to improve the resistance of horticultural products to freezing damage by treating the tissues with cryoprotectants. Industrial techniques, such a vacuum impregnation (VI), have been used to promote compositional changes on porous tissues (e.g. apples), by filling porous fractions of the product with an external solution of the cryoprotectant. Resistance to freezing damage has then been improved through the reduction of freezable water (26). However, when applying VI, the solution containing the cryoprotectant is located in the apoplast, whereas in nature, cryoprotectants accumulate inside the cell conferring their protective effects to the plant tissues.

Sugars could be forced inside the cells through osmotic dehydration, by immersing the tissue in a hypertonic solution. To accelerate the mass transfer during this operation, pulsed electric fields (PEF) have been used to affect plasma membrane permeability properties (27, 28). However, these operations will induce permanent damage on cell membrane properties and cell death (29), consequences that will not provide cryoprotection to the tissue.

The impregnation of a cryoprotectant solution inside the cells of spinach leaves, reported by Yeu Phoon et al. (25) through a combination of VI and PEF, showed a substantial improvement of their freezing tolerance. Figure 2 shows the processing steps that were tested. The success of the operation was assessed by vital staining with fluorescein diacetate (FDA) and a wilting test consisting in simply holding the centre of the sample with a small pincer and

observing whether the leaves would bend or not. Figure 3 shows the results where it is demonstrated that the tested procedure (Process 1 in Figure 2) resulted in viable, turgid spinach leaves.

A comparison between process 1 and 2 in Figure 2 reveals that improving the freezing tolerance of spinach leaves could be achieved only when the VI process was used in combination with PEF.

FUTURE PERSPECTIVES

Recent investigations on reversible electropermeabilization of vegetable tissues have resulted in striking results as well as in interesting questions. Much remains to be done on:

Process 1
PEF ⟶ VI ⟶ Resting ⟶ Freezing
⟶ Thawing

Process 2
VI ⟶ Resting ⟶ Freezing ⟶ Thawing

Figure 2. A combination of treatments for improving the freezing tolerance of spinach leaves. PEF was performed by treating the spinach leaves with ten trains of bi-polar, rectangular electric field pulses with a nominal electric field strength of 580 V/cm. Each train lasted 20 ms and consisted in 25 µs pulses. The resting period between the trains was 10 s to avoid heating of the samples. The VI process was carried out at 20ºC in a chamber connected to a vacuum pump (86 kPa (man)). The spinach samples were immersed in a 40 % (w/w) solution of trehalose for 25 min. The resting step was performed by submerging the samples in deionised water overnight at 4ºC to regain turgor. Freezing was done by submerging the individual samples in liquid nitrogen for 7 s. The samples were then immediately thawed in water at room temperature.
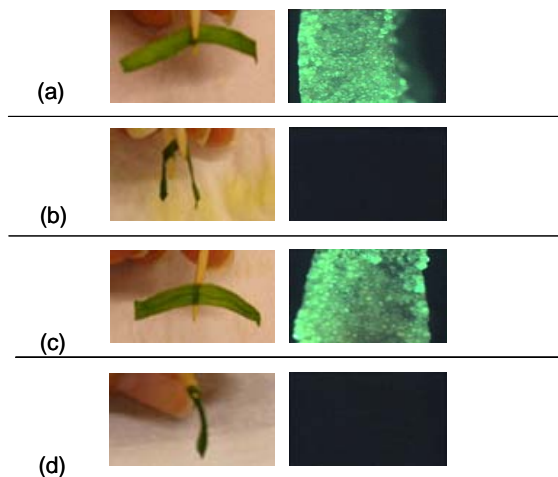

Figure 3. The freezing tolerance of spinach leaves after different treatments. **Left panel:** Typical results (10 measurements) from the wilting test, showing the turgidity of the samples. **Right panel:** Typical results (10 measurements) from microscopic observations using fluorescein diacetate to identify viable cells. Viable cells are distinguished by a bright fluorescence. **(a)** A fresh spinach leaf. **(b)** A leaf frozen in liquid nitrogen for 7 s and immediately thawed in water at room temperature. **(c)** Spinach leaves subjected to "Process 1", as schematised in Fig.2. **(d)** Spinach leaves subjected to "Process 2", as schematised in Fig. 2.

(1)   Effect of transient loss of internal compartmentalisation on cellular composition, structure and metabolic activity.

(2)   Defense-related consequences of PEF-induced stress (e.g. activation of metabolic pathways).

(3)   Cell wall structural changes and their effects on physical (e.g. texture) and processing properties of vegetable tissues.

(4)   Transport phenomena of exogenous substances (e.g. trehalose) at the cellular level upon PEF application.

The use of new analytical techniques such as metabolic profiling and novel microscopic techniques such as digital holography would contribute to expand our knowledge in the field.

ACKNOWLEDGEMENTS

REFERENCES

1.   SABRI, N., PELISSIER, B., & TEISSIÉ, J. Electropermeabilization of intact maize cells induces an oxidative stress. European Journal of Biochem istry, 1996, 238, 737-743.
2.   TEISSIÉ, J., GOLZIO, M., & ROLS, M.P. Mechanisms of cell membrane electropermeabilization: A minireview of our present (lack of ?) knowledge. Biochimica and. Biophysica Acta – General Subjects, 2005, 1724, 270-280.
3.   GÓNGORA-NIETO, M.M., PEDROW, P.D., SWANSON, B.G., & BARBOSA-CÁNOVAS, G.B. Energy analysis of liquid whole egg pasteurized by pulsed electric fields. Journal of Food Engineering, 2003, 57, 209-216.

4.   TOEPFL, S. HEINZ, V., & KNORR, D. High intensity pulsed electric fields applied for food preservation. Chemical Engineering Process, 2007, 46, 537-546.
5.   FINCAN, M., DE VITO, F., & DEJMEK, P. Pulsed electric field treatment for solid-liquid extraction of red beetroot pigment. Journal of Food Engineerring, 2004, 64, 381-388.
6.   CHALERMCHAT, Y., & DEJMEK, P. Effect of pulsed electric field pretreatment on solid-liquid expression from potato tissue. Journal of Food Engineering, 2005, 71, 164-169.
7.   ADE-OMOWAYE, B.I.O., RASTOGI, N.K., ANGERSBACH, A., & KNORR, D. Combined effects of pulsed electric field pre-treatment and partial osmotic dehydration on air drying behaviour of red bell pepper. Journal of Food Engineering, 2003, 60, 89-98.
8.   LEBOVKA, N.I., SHYNKARYK, N.V., & VOROBIEV, E. Pulsed electric field enhanced drying of potato tissue. Journal of Food Engineering, 2007, 78, 606-613.
9.   GANEVA, V., GALUTZOV, B., & TEISSIE, J. Electric field mediated loading of macromolecules in intact yeast cells is critically controlled at the wall level. Biochimica and Biophysica Acta, 1995, 1240, 229-236.
10.   GABRIEL, B., & TEISSIE, J. Generation of reactive oxygen species induced by electropermeabilization of Chinese hamster ovary cells and their consequence on cell viability. European Journal of Biochemistry, 1994, 223, 25-33.
11.   YE, H., HUANG, L.L., CHEN, S.D., & ZHONG, J.J. Pulsed electric field stimulates plant secondary metabolism in suspension cultures of *Taxus chinensis*. Biotechnology and Bioengineering, 2004, 88, 788-795.
12.   GUDERJAN, M., ELEZ-MARTÍNEZ, P., & KNORR, D. Application of pulsed electric fields at oil yield and content of functional food ingredients at the production of rapeseed oil. Innovative Food Science and Emerging Technologies, 2007, 8, 55-62.
13.   GUNDERJAN, M., TOPFL, S., ANGERSBACH, A., & KNORR, D. Impact of pulsed electric field treatment on the recovery and quality of plant oils. Journal of Food Engineering, 2005, 67, 281-287.
14.   GÓMEZ GALINDO, F., SJÖHOLM, I., RASMUSSON, A.G., WIDELL, S., & KAACK, K. Plant stress physiology: opportunities and challenges for the food industry. Critical Reviews in Food Science and Nutrition, 2007, 47, 729-763.
15.   ATKINSON, C.J., NESTBY, R., FORD, Y.Y., & Dodds, P.A.A. Enhancing beneficial antioxidants in fruits: A plant physiological perspective. Biofactors, 2005, 23, 229-234.
16.   SCHREINER, M., & HUYSKENS-KEIL, S. Phytochemicals in fruit and vegetables: Health promotion and postharvest elicitors. Critical Reviews in Plant Science, 2006, 25, 267-278.

17.   BRADLEY, D.J., KJELBOM, P., & LAMB, C.J. Elicitor and wound-induced oxidative cross-linking of a proline-rich plant cell wall protein: a novel, rapid defense response. Cell, 1992, 70, 21-30.
18.   WOJTASZEK, P. Oxidative burst: an early plant response to pathogen infection. Biochemica, 1997, 322, 681-692.
19.   GÓMEZ GALINDO, F., WADSÖ, L., VICENTE, A., & DEJMEK, P. Exploring metabolic responses of potato tissue induced by electric pulses. 2008, Submitted for publication.
20.   JOSHI, R.P., HU, Q., & SCHOENBACH, K.H. Simulation of electroporation dynamics and shape deformations in biological cells subjected to high voltage pulses. IEEE Transactions of Plasma Science, 2002, 30, 1536-1546.
21.   JI, Z., KENNEDY, S., BOOSKE, J.H., & HAGNESS, S.C. Experimental studies of persistent poration dynamics of cell membranes induced by electric pulses. IEEE Transactions of Plasma Science, 2006, 34, 1416-1424.
22. GÓMEZ GALINDO, F., VERNIER, P.T., DEJMEK, P., VICENTE, A., & GUNDERSEN, M. Pulsed electric field reduces the permeability of potato cell wall. Bioelectromagnetics, 2008, in press.
23.   SCHOTE, U, & SEELING, J. Interaction of the neuronal marker dye FM1-43 with lipid membranes. Thermodynamics and lipid ordering. Biochimica and Biophysica Acta, 1998, 1415, 135-146.
24.   BOLTE, S., TALBOT, C., BOUTTE, Y., CATRICE, O., READ, N.D., & SATIAT-JEUNEMAITRE, B. FM-dyes as experimental probes for dissecting vesicle trafficking in living plant cells. Journal of Microscopy, 2004, 214, 159-173.
25.   YEU PHOON, P., GÓMEZ GALINDO, F., VICENTE, A., & DEJMEK, P. Pulsed electric field in combination with vacuum impregnation with trehalose improves the freezing tolerance of spinach leaves. Journal of Food Engineering, 2008, in press.
26.   MARTÍNEZ-MONZÓ, J., MARTÍNEZ-NAVARRETE, N., CHIRALT, A., & FITO, P. Mechanical and structural changes in apple (var. Granny smith) due to vacuum impregnation with cryoprotectants. Journal of Food Science, 1998, 63, 499-503.
27.   ADE-OMOWAYE, B.I.O., RASTOGI, N:K:, ANGERSBACH, A., & KNORR, D. Osmotic dehydration of bell peppers: influence of high intensity electric field pulses and elevated temperature treatment. Journal of Food Engineering, 2002, 54, 35-43.
28.   TAIWO, K., ESHTIAGHI, M.N., ADE-OMOWAYE, B.I.O., & KNORR, D. Osmotic dehydration of strawberry halves: influence of osmotic agents and pre-treatment methods on mass transfer and product characteristics. International Journal of Food Science and Technology, 2003, 38, 693-707.
29.   MAVROUDIS, N. Mass transport in apple tissue – effects on tissue structure and osmotic processing conditions. PhD Thesis, Food Engineering Department, Lund University, Sweden. 2003