MEMBRANE PERMEABILITY CHANGES DURING MODERATE ELECTRIC FIELD PROCESSING OF VEGETABLE TISSUE

DISSERTATION

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ABSTRACT

Diffusion of beet dye from beet cubes was measured during a 3 min. moderate electric field (MEF) process using frequencies ranging from 0 (direct current) to 5000 Hz, and field strengths ranging from 0 (conventional heating) to 23.9 V/cm, while maintaining steady-state temperature at 45º C throughout the process. Diffusion increased with electric field strength and decreased with frequency. There was no enhanced diffusion from an agar cube or from previously frozen beet tissue. Electropermeabilization is suggested as the mechanism for enhanced diffusion.

The effect of ohmic heating on cell membranes of potato was investigated by measurement of dielectric spectra from 100 Hz to 20 kHz. Cylinders of potato were heated to various temperatures ranging from 30ºC to 70ºC either conventionally or ohmically. After cooling to 25ºC, the ohmically heated samples had significantly higher conductivity at all measurement frequencies for endpoint temperatures of 40ºC and 50ºC. At low frequencies, the apparent dielectric constant was also higher for these samples, but at high frequencies, the reverse pattern was shown. The ohmically heated samples apparently have greater membrane permeability than conventionally heated samples when heated to temperatures below 60ºC.

Microscopy shows that in beet tissue given a moderate electric field treatment, some cells lose their membrane selectivity while others remain intact. Raw, thawed, or
precooked potato cylinders were chilled, and then warmed to 25ºC by either allowing them to equilibrate or by MEF treatment. The conductivity from 100 Hz to 20 kHz and apparent dielectric constant from 100 Hz to 5 kHz was initially the same between raw, untreated samples and raw, MEF treated samples, but over 24 hours, that of the raw, MEF treated samples increased while that of the raw, untreated samples remained constant. No such distinct pattern emerged from the thawed or precooked samples. The apparent dielectric constant of raw, MEF treated potato above 5kHz was the same as raw, untreated potato and higher than thawed and precooked potato. None of the samples showed marked changes in dielectric constant at 5-20 kHz over the 24 hour period. Apparently, even mild electrical treatments permeabilize vegetable tissue, permitting enhanced diffusion.
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CHAPTER 1

1.1 INTRODUCTION

Ohmic heating is a thermal food process whereby the electrical resistance of the food itself generates heat as current is passed through it. Unlike conventional retort processes, ohmic heating allows high-temperature/short-time processing of particulates, thus avoiding excessive destruction of nutrients and sensory properties (Anonymous, 1990). In addition, a high-volume ohmic heater may be more cost-effective for low-acid foods than canning or freezing (Allen et al., 1996).

De Alwis and Fryer (1990) have reviewed technological developments in direct resistance heating up to 1989. Since then, researchers have developed and refined mathematical models for ohmic process design, which have been reviewed by Sastry and Palaniappan (1992). Other studies have focused on phenomena observed during ohmic heating, including changes in electrical conductivity (Halden et al., 1990), enhanced diffusion (Schreier et al., 1993), and microbial death kinetics (Palaniappan and Sastry, 1992).

Electrical treatments of vegetable and fruit tissue, such as ohmic heating and high voltage pulsed electric field (PEF) treatments, have been investigated for a variety of food processing applications. Such treatments have been investigated in attempts to
improve recovery of secondary metabolites (Hunter and Kilby, 1988; Dörnenburg and Knorr, 1993), dehydration (Rastogi et al., 1999; Sensoy, 2002; Lima and Sastry, 1999; Wang and Sastry, 2000), blanching (Sensoy, 2002; Mizrahi et al., 1975), and juicing (Lima and Sastry, 1999; Bazhal and Vorobiev, 2000) processes, with mixed results. The blanching studies have shown clearly that ohmic heating has the advantage of a rapid heating rate. However, effects on product quality have not been deeply investigated. Electric pretreatments have had positive effects on dehydration and juice extraction compared to raw tissue (Rastogi et al., 1999; Bazhal and Vorobiev, 2000; Lima and Sastry, 1999; Wang and Sastry, 2000), but results of comparisons of ohmically and conventionally heated tissue heated to temperatures above 60ºC have been inconsistent (Sensoy, 2002; Wang and Sastry, 2000). This is probably due to the increase in permeability that is only seen at temperatures below 60ºC. It is unlikely that yield increases over conventional heating will be substantial in processes that heat the tissue to over 60ºC, because thermal permeation is complete at such high temperatures. A deeper understanding of the permeation mechanisms should enable better design and prediction of these processes. With ohmic heating, greater electroporomeabilization is seen at low frequencies (Imai et al., 1995; Lima et al., 1999; Chapter 2), so they should be used where electroporomeabilization is desired. In addition, the chemical environment is likely to have a large effect on the electrical properties of cells (Osterhout, 1922), so it may be possible to further optimize processes by choice of chemical additives. Electric treatments for fruit and vegetable have great potential for improving product quality and
reducing costs, but much research remains to be done in order to develop applications for this technology.

The transport of molecules across living membranes at sub-lethal temperatures also has many other possible applications, including encapsulation, transformation, antibiotic infusion, and extraction. Encapsulation of enzymes (Zimmerman et al., 1976) and drugs (Zimmerman et al., 1980) by electroporation of blood cells has been used for extended release in vivo. The use of live cells as encapsulants could be an additional tool for food processors to control release of additives, which could be induced under any lethal condition. Membrane permeabilization of undesirable microorganisms could make them more vulnerable to preservatives. Many attempts to permeabilize Beta Vulgaris cell cultures while retaining viability have been made in order to optimize a process for continuous extraction of secondary products, which are normally not released from the plant vacuole. Successful attempts to extract betacyanin from live cell cultures include 400-750 V/cm DC pulses (Hunter and Kilby, 1988), a heat treatment (DiIorio et al., 1993), ammonium sulfate/EDTA (DiIorio et al., 1993), and ultrasound (Kilby and Hunter, 1990). Many drugs, coloring, flavoring, and other economically important chemicals are secondary products (Luckner, 1972), and their manufacture from cell culture could greatly reduce the cost and/or availability. The enhanced diffusion effect of ohmic heating could have many applications in both food processing and biotechnology.

Halden et al. (1990) observed that beet color seemed to diffuse more rapidly from beetroot during ohmic, as compared to conventional, heating. The electrical conductivity of beetroot was also higher for the ohmically heated sample at temperatures below 70°C.
The authors suggested four possible mechanisms for the electrical enhancement of conductivity:

1. Electroosmosis. This occurs when an immobile charged matrix, surrounded by mobile oppositely-charged ions, is placed in an electric field. The counterions move toward one electrode, causing a convection current which moves the solvent and other solutes with it. (Hunter, 1981).

2. Plasmolysis. This probably refers to irreversible dielectric breakdown of the cell membrane, also known as electroporation or punch-through (Zimmerman et al., 1974; Coster, 1965). Holes are formed in the cell membrane when it is exposed to an external electric field of sufficient intensity and duration. The membrane can reseal under mild conditions, but plasmolysis results under more extreme conditions.

3. Overcoming diffusion limitation in carrier mediated transport as per Athayde and Ivory (1985). If a chemical species is transported across a membrane by a charged carrier, its flux can be increased by application of an alternating current. The carrier moves from one interface to the other in response to the electric field, which is faster than by diffusion.

4. Electrohydrodynamic mixing (Hoburg and Malihi, 1978; Plonski et al., 1979). Mass transfer of fluids of different conductivity separated by a non-selective barrier was enhanced by application of a static electric field (Hoburg and Malihi, 1978). The effect was attributed to an accumulation of free charge in the barrier due to the conductivity gradient. Plonski et al. (1979) also observed enhanced diffusion between two solutions of different conductivities across an octanol membrane when an alternating current was
applied. They describe electrohydrodynamic mixing as a type of convection driven by the electrical forces of accumulated charge at the membrane-solution interface. The effect is independent of the orientation of the electrodes.

Enhanced diffusion was further investigated by Schreier et al. (1993), who observed it from both beetroot and Visking semipermeable tubing. Electroporation is not a possible mechanism for enhanced efflux from tubing. Their data were consistent with equations for electroosmosis which predict a linear relationship between dye flux and electric field. However, the authors ruled out membrane effects because they found the diffusion enhancement to be the same regardless of tube orientation. Sims et al. (1991) have proven that electroosmotic effects occur across semipermeable tubing. They discuss the factors which affect molecular mobility across natural and artificial membranes, which include both electroosmotic and electrophoretic effects. Electrophoretic enhancement is a general increase in ion motion in response to the electric field. Diffusion of cations, anions, and neutral molecules across membranes are all affected by an electric field.

Schreier et al. (1993) discussed an electrophoretic mechanism, but the authors felt that more research was necessary to make a definite conclusion. It can not be the primary mechanism for enhanced flux from beetroot because living beet cell membranes are normally impermeable to betacyanins (Zhang et al., 1992). The cell membrane must be permeabilized in order for the dye to effuse from the cell.

A living cell has a thin, dielectric membrane with high resistance (about $10^4 \, \Omega$ cm$^2$) and a capacitance ($C$) of about 1 $\mu$F/cm$^2$ (Williams et al., 1964). When a cell of
radius R is exposed to an external electric field (E), charge accumulates at the membrane surface, establishing a potential difference (Δψ) which is maximal at the ends which are in line with the field (Tsong, 1992). The maximum Δψ is:

\[ \Delta \psi_{\text{max}} = 1.5 R E \]

For alternating currents, the charging time of the membrane is significant. The following equation should be used:

\[ \Delta \psi_{\text{max}} = 1.5 R E \{1+(2\pi f \tau)^2\}^{1/2} \]

where \( \tau = RC(\text{rint} + \text{rext}/2) \), \( R \) = radius of cell, \( C \) = capacitance/area, \( \text{rint} \) = electrical resistance of cell fluid, \( \text{rext} \) = electrical resistance of the external medium.

Living cells have a natural potential difference (Δψnat) of approximately -150 mV (Coster, 1965) which must be added to the induced field. Because the natural field is uniform, an externally applied field will be hyperpolarizing at the pole facing the positive electrode (anode) and depolarizing at the pole facing the negative electrode (cathode). Thus, the maximum potential difference will be only at one pole, and will be:

\[ \Delta \psi_{\text{max}} = \Delta \psi_{\text{nat}} -1.5 R E \]

Because of the effect of cell radius, larger cells will have a larger Δψ. Within dormant bacterial spores, electrolytes are immobile (Carstensen et al., 1971), so the Δψ should be much less than for normal cells. The membranes of dead cells lose their selectivity (Zhang et al., 1992; Osterhout, 1922) and will therefore not maintain any voltage gradient.
The maximum electric field within the cell membrane (Em) is simply the $\Delta \psi_{\text{max}}$ divided by the membrane thickness (d), which is about 10 nm for plants (Fensom, 1985). Thus,

$$Em = (\Delta \psi_{\text{nat}} -1.5 R E)/d$$

Beet cells have a $\Delta \psi_{\text{nat}}$ of -154 mV (Zhang et al., 1992) and an approximate diameter of 45 µm (Joersbo et al., 1990). The Em of a beet cell will increase from about $1.54 \times 10^5$ V/cm to $2.36 \times 10^5$ V/cm when placed in an electric field of 24 V/cm, which is typical of our laboratory ohmic heating conditions. This corresponds to a hyperpolarization of 83 mV, for a $\Delta \psi_{\text{max}}$ of 233 mV.

Coster (1965), while charging the membranes of giant algal cells with a microelectrode, observed that hyperpolarizing currents caused a sudden increase in permeability when the membrane potential reached about -300 mV. He called the phenomenon "punch-through", but it is now known as “electroporation” or “electropermeabilization”. Zimmerman et al. (1974) used an externally applied high-voltage field to cause "dielectric breakdown" of red blood cell membranes. Most research on electroporation uses a pulsed electric field method which is used to insert DNA into cells during transformation. Molecules, such as DNA, that are to be inserted are included in a suspension of cells which are electroporated. Under the right conditions, pores are formed in the membrane through which diffusion occurs. The pores close up, and the cell recovers (Tsong, 1989).

The mechanism of electroporation involves aqueous pores (Weaver, 1987), which are channels, about 0.4 nm in width, naturally present in biological membranes (Kotyk
and Janacek, 1975). The surface of a pore is continuous with the inner and outer surfaces of the cell membrane, which is composed of phospholipids (Stein and Danielli, 1956). Therefore, they have a negatively charged surface surrounded by hydrogen ions. They are normally permeable to water, but not to most ions or other molecules larger than water (Kotyk and Janacek, 1975). When a cell is exposed to an electric field, the ions accumulating at the membrane surface are preferentially drawn to the aqueous pores, which have a much higher capacitance than the lipid fraction of the membrane (Weaver, 1987). The ions pushing on the pore cause a pressure which expands it. When the hole has become large enough for ions to pass through, a decrease in resistance is measured.

Experiments which used single pulses of 10 nsec to 1000 µsec revealed that the threshold breakdown voltage is inversely proportional to pulse duration (Zimmerman and Benz, 1980; Joersbo et al., 1990). That is, a longer pulse can cause breakdown at a lower field strength. Because it takes several minutes for the cell membrane to recover completely (Zimmermann and Benz, 1980), pulses given in quick succession (every 0.5 sec) have a cumulative effect (Lindsey and Jones, 1987).

Ohmic heating, because it uses alternating current, is analogous to a sequence of pulses. The number of pulses per second is simply the frequency. The duration of an equivalent pulse would be the time spent above the threshold voltage and is therefore a function of the voltage amplitude, the threshold voltage, and the frequency. Pulsed radio-frequency (Chang, 1989) and 50 Hz alternating current (Joersbo and Brunstedt, 1990) pulses have been used for electroporation.
Both the pore formation (Zimmerman and Benz, 1980) and resealing events (Lindsey and Jones, 1987) are faster at higher temperatures. Diffusion through pores will continue after electroporation for minutes at 35°C to hours at 4°C (Lindsey and Jones, 1987). This is accompanied by a decrease in electrical resistance which is recovered when the pores reseal (Zimmerman and Benz, 1980).

The size of pores determines the permeability of the membrane to molecules of various sizes. Pore size can be controlled by the ionic strength of the suspension medium (Kinosita and Tsong, 1977). High ionic strength media cause formation of small pores, whereas large pores result from those of low ionic strength.

Another possible mechanism for enhanced mass transport during ohmic heating is electroosmosis, which occurs when an electric field is applied across a system of immobilized charge surrounded by mobile counterions (Hunter, 1981). In addition to colloidal suspensions, such systems include cell walls and cell membranes (Barry and Hope, 1969). Electroosmosis has been demonstrated by plant cells (Fensom and Dainty, 1963), but dye efflux from beet is only possible if the aqueous pores, which normally exclude betacyanins, are permeabilized by the electric field. Thus, electroosmosis induced across cell membranes could cause an increase in water mobility, but would not affect impermeant molecules such as betacyanin.

Electroosmosis could be important in the transport of solutes through phloem, a transport tissue in vascular plants such as beets. The main conducting tubes, or sieve tubes, originate from sieve elements- cells which have formed connections to the surrounding cells (Parthasarathy, 1975). The sieve elements are lined up end to end and
are separated by porous walls, the sieve plates. Many of the pores in the sieve plates contain P-protein filaments, which extend through the pore in parallel like wires in a cable. The negatively charged P-protein filaments form a good matrix for electroosmotic flow between adjacent sieve elements (Spanner, 1975). In cut beet tissue, betacyanin could come out from the phloem because sieve plate pores are not covered by cell membranes. Thus, during ohmic heating, beet dyes may be extracted from the phloem by electroosmosis.

In addition to electroporation and electroosmosis, electric fields affect the conformation of proteins in the membrane (Tsong, 1989; Miller et al., 1994). This affects the transport of carrier-mediated permeants such as ions. The effect can be reversible (McLeod, 1995; Liu et al., 1990) or irreversible (Teissie and Tsong, 1980) depending on the field strength. Recovery of normal protein activity takes longer than shrinkage of pores in the lipid phase of the membrane (Tsong, 1989). While there is no known carrier for betacyanins, ions and other chemicals can affect permeability, and thus may indirectly influence the effusion of beet dye (Dilorio et al., 1993; Cooke et al., 1986; Siegal and Daly, 1966; Pooviah and Leopold, 1976; Osterhout, 1922).

This dissertation focuses on plant cell membrane permeability changes during ohmic heating of tissue. In the second chapter, frequency and voltage effects are described as quantified by the effusion of betacyanin from beet cells. In the third chapter, the effect of heating temperature endpoint is described as quantified by dielectric spectra. In the fourth chapter, long-term changes in permeability are studied by measuring the dielectric spectra over a 24 hour period after the treatment has ended. These results have
provided insight into the mechanism for enhanced diffusion and can provide direction in future attempts to improve electrical heating processes and to develop new applications for the technology.
1.2 BIBLIOGRAPHY


CHAPTER 2

FREQUENCY AND VOLTAGE EFFECTS ON ENHANCED DIFFUSION
DURING MODERATE ELECTRIC FIELD (MEF) TREATMENT

2.1 ABSTRACT

Diffusion of beet dye from beet cubes was measured during a 3 minute moderate electric field (MEF) process using frequencies ranging from 0 (direct current) to 5000 Hz, and field strengths ranging from 0 (conventional heating) to 23.9 V/cm, while maintaining steady-state temperature at 45º C throughout the process. Diffusion increased with electric field strength and decreased with frequency. There was no enhanced diffusion from an agar cube or from previously frozen beet tissue. Electroporation is suggested as the mechanism for enhanced diffusion. Mass transfer enhancement appears to be significant when the product initially possesses an intact cell structure. There appears to be a threshold potential above which significant increases in permeabilization occur. Except for DC, this potential is found to depend on frequency – the higher the frequency, the higher the threshold potential for permeabilization.
2.2 INTRODUCTION

Halden et al. (1990) observed that beet color seemed to diffuse more rapidly from beetroot during ohmic, as compared to conventional, heating. The electrical conductivity of beetroot was also higher for the ohmically heated sample at temperatures below 70º C. Schreier et al. (1993) observed enhanced diffusion both from beetroot and Visking semi-permeable tubing. Further work (e.g. Wang and Sastry, 1998) has shown that the presence of a moderate electric field (arbitrarily identified here as being roughly in the range of 1 to 1000 V/cm, lower than that for pulsed electric fields) has interesting effects on biological materials. Studies on cell breakdown under electric fields have shown that permeabilization can be monitored by frequency versus conductivity relationships (Angersbach et al., 1999); and that moderate electric field pulses can enhance diffusion (Jemai and Vorobiev, 2002). In moderate electric field (MEF) processing of foods, an understanding of diffusion effects will be important for the prediction of nutrient losses and of increases in conductivity during processing. Such knowledge will also be valuable in optimizing novel applications of MEF treatments, including pretreatment of vegetable tissue for drying (Wang and Sastry, 1998).

In studying such treatments, it is important to assess the effects of electrode impedances on field strength determinations. At low frequencies, errors in electrical measurements may result from electrode impedances (Schwann, 1963). A discussion of
potential drops at the surface of electrodes during ohmic heating can be found in Amatore et al. (1998). Ohmic heating and MEF processing employ alternating currents and voltages substantially higher than those typically required for electrochemical (faradaic) reactions. The electrodes have a capacitive reactance, which causes a voltage drop inversely proportional to the frequency of the alternating electric field. In addition, electrochemical reaction products may form at the electrode surface, forming a physical barrier to current flow. Therefore, the impedance at the electrodes has both capacitive and resistive components, which are dependent on frequency, applied voltage, and other cell conditions. The details of these processes were outside the scope of this experiment. However, because frequency and applied voltage were varied in this experiment, it was necessary to measure the electric field within the bulk solution in order to eliminate possible electrode effects. The principles of using probes to measure the electric field in bulk solutions are discussed by Schwan (1963) and Misakian et al. (1993).

The objective of this study was to determine how variations in frequency and voltage affect enhanced diffusion from beetroot.
2.3 MATERIALS AND METHODS

Sample preparation. Beets were purchased from a local grocer and stored no longer than 1 week in a refrigerator (4º-10º C) before use. Cubes (1.17 cm³) were cut from the beet roots and soaked in 0.05% NaCl to leach out betacyanins from the extracellular spaces within the beet tissue. A serial soaking procedure was used to leach as much pigment as possible while maintaining the viability of the cells. They were first soaked at room temperature for 15 min. to remove the majority of the pigment, then drained, then soaked in fresh solution overnight (10-18 hours) in the refrigerator (4º-10º C) to remove the remaining pigment, then drained again, and then soaked at room temperature in fresh solution until testing (1-12 hours). The solution from the final soaking remained virtually colorless, indicating that extracellular betacyanins had been thoroughly removed and that the cell membranes had retained their impermeability to betacyanin.

In a separate experiment, beet cubes prepared as above were frozen in order to rupture the cell membranes without destroying the betacyanin, then thawed and brought to room temperature for testing. These samples will be referred to as “previously frozen”.
A firm gel was made with 10% Bacto-Agar (Difco Laboratories, Detroit, MI) and 2% beet root powder (Weinstein Nutritional Products, Irvine, CA) and cut into 1.17 cm³ cubes.

**Experimental procedure.** The static MEF treatment chamber was a 250 mL beaker with titanium electrodes held in place with a plastic cap (Fig. 1). The heater was partially immersed in a water bath and swirled to control the temperature. For each combination of frequency and applied voltage, the water bath temperature was adjusted so that the solution (50 mL of 0.05% NaCl) would maintain a steady-state temperature of 45°C ± 1°C during MEF processing. For each observation, a beet cube was dropped into the preheated solution and was MEF processed for 3 min at the specified frequency and voltage. The absorbance of the decanted solution was measured at 527 nm, the wavelength of maximum absorbance of betacyanin (Vincent and Scholz, 1978), with a spectrophotometer (Beckman DU-50, Fullerton, CA) after it was cooled to room temperature. The concentration of betacyanin was estimated from the extinction coefficient of betanin, 1120%⁻¹cm⁻¹ (Vincent and Scholz, 1978). Measurements were made in pentiplicate at each frequency and applied voltage combination. Samples were tested at various levels of frequency (0 (DC), 10, 50, 250, or 5000 Hz) over a range of field strengths from 0 (conventional extraction) to 23.9 V/cm. Previously frozen samples were tested at field strengths of 0 (conventional extraction) and 23.9 V/cm at 250 Hz. Agar samples were tested at electric fields of 0 (conventional extraction) and 23.9 V/cm at 50 Hz.

To ensure that electrode impedance effects were eliminated from the measurement of the electric field strength, insulated wires were attached with waterproof
silicone sealant to the center of the face of each electrode (Fig. 1). The wires were bare at the tips, which were 0.7 mm from the surface of the electrode. That was the minimum distance the wires could be from the electrodes without touching, as limited by the thickness of the insulation. The wires were connected to a voltage transducer, and the measured potential difference was recorded. The electric field was estimated by dividing the measured potential difference by the distance between the wires (5.03 cm). This was considered a realistic estimate of the minimum field strength experienced by the sample. To verify this assumption, a model was developed for the electric field by solving Laplace’s equation within the vessel domain. Nondimensionalized results (Fig. 2) show that the electric field within most of the chamber is reasonably uniform, except for the electrode edges. Since the beet cube moved around the interior of the vessel due to the swirling agitation of the shaker, staying near the center, without approaching the electrode edges during the treatment, the assumed field strength was considered realistic.
2.4 RESULTS AND DISCUSSION

For agar and previously frozen samples, diffusion of betacyanin from the MEF cubes and conventionally treated cubes were not significantly different (Table 1). This suggests that electrical treatment results in no diffusion enhancement when samples have either no cell structure, or one that is previously denatured by freezing. The results in Table 1 show that diffusion from the agar gel matrix was considerably lower than that from the previously frozen sample. We note that this experiment was intended for comparison between treatments only, and not between gel and previously frozen samples (the concentration of betacyanin within the gel was not the same as that of native tissue). Still, slow diffusion within a gel is to be expected, since the diffusion coefficient in a liquid medium is typically inversely proportional to viscosity, as evidenced by a number of existing models (e.g. Stokes-Einstein or Wilke-Chang; Bird et al. 1960) for diffusion coefficient in the literature. The previously frozen samples would be expected to consist of an aqueous medium in a ruptured cell structure matrix, while the gel would approximate a cell-free, high-viscosity medium.

The diffusion of betacyanin from fresh beets was a function of frequency and voltage (Fig. 3). The diffusion was enhanced by smaller frequencies and larger voltages. An exception to this pattern was direct current, which we would expect to have the greatest enhanced diffusion, but which was comparable to 50 Hz. The differences among
frequencies became apparent at applied voltages above 20 V. Logistic, exponential, and Gompertz growth models were tested because they are applicable to many diffusion phenomena (Banks, 1994). The logistic model provided the smallest residual standard error. The fitted model (p<0.05) is as follows:

\[
C = \frac{3.33}{1+210\exp\{-(0.000721F+0.0821V)\}}
\]

where:

\(C\) = concentration of betacyanin (ppm)

\(F\) = frequency (Hz)

\(V\) = measured potential difference (V)

Several possible mechanisms have been proposed for enhanced diffusion during electric treatment via ohmic heating (Halden et al, 1990; Schreier et al, 1993). Electrophoresis is the movement of ions in response to an electric field. Electroporation is the formation of holes in a cell membrane resulting from local pressure of ions, which cannot initially permeate the cell membrane, but are forced against it by the electric field (Weaver, 1987).

The results of this study support the electroporation hypothesis. Once betacyanin has been purged from the extracellular tissue of the fresh beet, the remaining betacyanin is trapped inside the vacuole until the selectivity of the membrane has been altered (Zhang et al, 1992). Thus, the cell membrane must be damaged in order for the betacyanin to effuse from the beet tissue. There is no such restriction for agar or frozen beet, so we would expect enhanced diffusion from these materials if electrophoresis had
been the mechanism. Sample temperatures taken shortly after each MEF pretreatment were found to be close to or slightly less than that of the surrounding solution; thus we may eliminate the possibility of thermal denaturation from ohmic heating effects.

The effects of frequency and voltage on enhanced diffusion are consistent with electroporation. Like a capacitor, the charge that accumulates on a cell membrane is proportional to the strength of the electric field and the duration of exposure (Tsong, 1992), which are both important factors for electroporation (Lindsey and Jones, 1987). At lower frequencies, there is more time for charging of the cell membrane, as has been shown by the modeling study of Bruhn et al. (1997).

The electroporation hypothesis alone does not explain why DC did not cause the greatest enhanced diffusion. Living cells have a natural potential difference across their cell membranes (Coster, 1965), which must be added to the induced field. Thus, a cell in an electric field will have a larger membrane potential at one end, or pole, than the other. If the field is alternating, it is possible that both poles will alternately experience the maximum membrane voltage.

The increased efficacy of an alternating field has been previously discussed in the literature in the context of microbial inactivation using pulsed electric field (PEF) treatment (Barbosa-Canovas et al., 2000) where it is noted that bipolar pulses are more effective at microbial inactivation than monopolar pulses, since the polarity reversal results in alternating charge movement, and consequently, increased stress on the cell membrane. We expect that a similar mechanism may be in effect in the present instance.

In addition to the above, we may note the following points from Fig. 3. In all cases, increasing field strength enhances diffusion. All treatments require a minimum
threshold potential above which diffusion is significantly increased. With the exception of the DC treatment, this threshold potential appears to increase with frequency. This observation is entirely consistent with the theory that low frequency allows greater charge buildup around the cell membrane. At high frequencies, higher potentials are necessary to overcome the reduced charge buildup time per cycle. The efficacy of DC appears to be limited. The lowest effective frequency would be a worthwhile subject for further research, since improved mass transfer has been reported for frequencies as low as 4 Hz (Lima and Sastry, 1999).
2.5 CONCLUSIONS

Except for direct current, diffusion enhancement by Moderate Electric Field (MEF) processing increases with increasing field strength and decreasing frequency. The enhancement appears to be significant when the product initially possesses an intact cell structure. No enhancement is observed when a cell structure is either absent or previously completely permeabilized. Consequently, the mechanism of diffusion enhancement may be attributed to pore formation in cell membranes. There appears to be a threshold potential above which significant increases in permeabilization occur. Except for DC, this potential is found to depend on frequency – the higher the frequency, the higher the threshold potential for permeabilization.
2.6 BIBLIOGRAPHY


<table>
<thead>
<tr>
<th>Sample</th>
<th>Betacyanin concentration (ppm) (mean ± standard deviation)</th>
<th>Conventional extraction</th>
<th>MEF treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thawed beet cube</td>
<td>2.87&lt;sup&gt;a&lt;/sup&gt; ± 0.82</td>
<td>3.04&lt;sup&gt;a&lt;/sup&gt; ± 0.63</td>
<td></td>
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<tr>
<td>Agar gel</td>
<td>0.345&lt;sup&gt;b&lt;/sup&gt; ± 0.041</td>
<td>0.325&lt;sup&gt;b&lt;/sup&gt; ± 0.039</td>
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Table 2.1. Betacyanin concentrations in fluid medium following a 3-minute conventional and MEF (23.9 V/cm, 50 Hz) extraction treatment at 45 C. a,b – Within the same row, mean values followed by the same letter are not significantly different (p>0.05).
Figure 2.1. Experimental Apparatus
Figure 2.2. Nondimensionalized electric field distribution within treatment chamber.
Figure 2.3. Diffusion of betacyanin from fresh tissue MEF processed for 3 min. at 45°C: Effect of electric field strength and frequency. Error bars represent 95% confidence intervals.
CHAPTER 3

LOW-FREQUENCY DIELECTRIC CHANGES IN POTATO FROM OHMIC HEATING: EFFECT OF END POINT TEMPERATURE

3.1 ABSTRACT

The effect of ohmic heating on cell membranes of potato was investigated by measurement of dielectric spectra from 100 Hz to 20 kHz. Cylinders of potato were placed in a glass static ohmic heater and heated to various temperatures ranging from 30°C to 70°C either conventionally or ohmically. After cooling to 25°C, the ohmically heated samples had significantly higher conductivity at all measurement frequencies for endpoint temperatures of 40°C and 50°C. At low frequencies, the apparent dielectric constant was also higher for these samples, but at high frequencies, the reverse pattern was shown. The ohmically heated samples apparently have greater membrane permeability than conventionally heated samples when heated to temperatures below 60°C. This is reflected in the diffusion of KCl solution into the tissues, which is faster at higher endpoint temperatures.
3.2 INTRODUCTION

During ohmic heating, the electric field may cause changes in the permeability of cell membranes of plant cells below the temperature at which membranes are permeabilized due to thermal effects (Personius and Sharp, 1938; Chapter 2). Diffusion is enhanced, electrical conductivity changes are more linear during heating, and moisture migrates more easily out of the tissue (Halden et al., 1990; Schreier et al., 1993; Lima et al., 2001; Imai et al., 1995). Thus, ohmic pretreatment has been found useful in drying and juice extraction processes (Wang and Sastry, 1998). Electropermeabilization is a mechanism that can account for these effects. According to this hypothesis, pores formed in the cell membranes upon electric field exposure cause a drop in resistance as ions are allowed to pass through the membrane (Coster, 1965). If electropermeabilization occurs in a vegetable tissue, the apparent dielectric constant and conductance should be affected due to the changes in membrane permeability. Thus, low-frequency dielectric measurements may be used for examining the effects of ohmic heating and other processes on cell membranes.

The permeability of cell membranes has long been measured by the electrical conductivity of the tissue at sub-MHz frequencies (Osterhout, 1922; Personius and Sharp, 1938; Knorr and Angersbach, 1998; Lebovka et al., 2000). Living tissues, such as plant
cells, have dielectric dispersions in the low-frequency region due to the effects of cell membranes (Schwan, 1957) from ionic conduction and membrane charging relaxation mechanisms that do not occur at high frequencies. Thus, low-frequency measurements may be used for examining the effects of ohmic heating and other processes on cell membranes. Kuang and Nelson (1998) have published a review of the low-frequency dielectric properties of biological tissues, with explanations of the underlying mechanisms.

Dielectric constant data have great potential for revealing underlying mechanisms. Biological tissues show three major dispersions, or drops in capacitance, with increasing frequency (Schwan, 1957): the $\alpha$ dispersion in the kHz range or below, the $\beta$ dispersion in the tens of kHz to tens of MHz range, and the $\gamma$ dispersion in the microwave region. The mechanism for the $\alpha$ dispersion remains poorly understood despite decades of research (Kuang and Nelson, 1998), although many theories have been proposed (Schwan, 1981). Just higher than the $\alpha$ dispersion range, cell membranes are the dominant capacitive charging mechanism (Kuang and Nelson, 1998). The decline in membrane capacitance up to the MHz range is known as the $\beta$ dispersion, above which water rotation becomes the dominant mechanism and the state of the cell membrane is relatively unimportant. Thus, the best frequencies for measuring membrane effects should be higher than the $\alpha$ dispersion range and lower than the $\beta$ dispersion range. This study used frequencies within and just higher than the $\alpha$ dispersion range.

In Chapter 1, the increase in permeation of beet cell membranes at frequencies as low as 4 Hz suggests that the membrane is not saturated with charge even at very low
frequencies, implying a much longer time constant than the dielectric literature suggests. It is possible that the capacitance of beet tissue is higher than that of erythrocytes and other animal tissues that are commonly cited, or that the resistance of the intact beet tissue, which was soaked in a relatively dilute salt solution, is higher than the samples commonly cited.

The objective of this study was to examine the effect of ohmic heating on dielectric spectra of potato from 100 Hz to 20 kHz after heat treatments ranging from final temperatures of 25°C -70°C.
3.3 METHODS

Cylinders of potato, 5 cm long, 2.6 cm diameter, were placed coaxially in a cylindrical glass static ohmic heater (Fig. 3.1), with the sample completely blocking the path of the current. Although the samples contacted the stainless steel electrodes, an isoconductive KCl solution (described below) was poured over the sample to fill microscopic gaps between the electrodes and the potato cylinder. A thermocouple was inserted to the geometric center of the sample. Conventional samples were heated in a water bath, which was kept at 10°C above the endpoint temperature so that the maximum temperature in the sample would be no more than 10°C above the center temperature. The ohmic samples were heated at 18 V/cm, 60 Hz. The voltage was chosen so that the heating rate of the ohmic sample would be the same as that of conventional when heated to 40°C. Matching other heating rates exactly proved to be too difficult. Samples were heated to final temperatures of 30°C, 40°C, 50°C, 60°C, or 70°C, then allowed to cool in air at 25°C for 1.5 hours, which was the maximum time required to equilibrate the sample to 25°C. Control samples were simply held at 25°C for 1.5 hours. The thermocouple was removed, and the dielectric spectra measured. In order to measure the low-frequency dielectric spectra, power leads were connected to an LCZ meter (HP 4276A, medium measuring speed, high (1V) test signal level). The impedance and phase angle of the
samples were measured at frequencies ranging from 100 Hz to 20 kHz. Electrical conductivity (S/m) and apparent dielectric constant were calculated from the sample geometry and dielectric measurements.

Calculation of dielectric properties at low frequencies is subject to errors at low frequencies due to electrode polarization (Schwan, 1963, 1992). We have attempted to estimate the effect of electrode capacitance by comparing potato samples to isoconductive KCl solution, which has a static dielectric constant of 79 (Craig, 1995). Although solutions of salts are supposed to have a constant dielectric constant at the static value in the frequency range used in this study, in practice, the literature values are measured in the MHz to GHz range to eliminate conductivity effects (Craig, 1995). Salt solutions form diffuse double layers at the electrodes, which act as capacitors in series with the sample and cause very high apparent dielectric constants (Ferry, 1948). Electrode capacitance can be influenced by conductance because of the formation of a depletion layer at the electrode surface upon passage of faradaic current (Hill and Pickup, 1985). It was not possible to calculate this value with enough accuracy for a rigorous correction of the data, but our principal purpose was in comparative behavior rather than actual property measurements. Thus, we have chosen to present data as apparent, rather than true, dielectric constants.

**Preparation of isoconductive KCl solution.** In order to minimize artifacts from diffusion gradients, a KCl solution with the same conductivity as potato juice was prepared for contact between sample and electrodes. Resistivity of tissues is due to both the salt concentration and the presence of physical barriers, such as cell membranes and
cell walls. Since our intention was to study the physical barriers, an attempt was made to minimize the conductivity effects of the diffusion of KCl solution into the potato tissue.

Raw potato was juiced, and then centrifuged to remove solids. The mean conductivity of the clarified potato juice from three trials was 1.009 S/m, as measured by a conductivity meter (Cole-Parmer Instrument Co., EC meter 19101-00). The KCl solution was then made by titration to 1.009 S/m, and had a concentration of 5.65 mg/ml or 0.076 M. A total volume of 16 ml was poured over the sample through the sampling ports.
3.4 RESULTS AND DISCUSSION

The conductivity of conventionally heated samples increased sharply when samples were heated above 50°C (Fig. 3.2). However, the conductivity of ohmically heated samples increased more gradually and became significantly higher than the control at a 40°C endpoint temperature (Fig. 3.3). Samples heated to 50°C conventionally were no greater than the control (Fig. 3.2), whereas samples heated to 50°C ohmically were as conductive as those heated to higher temperatures (Fig. 3.3). Fig. 3.4 shows that ohmically heated samples are significantly more conductive at 100 Hz than conventionally heated ones at 40°C and 50°C, but are the same at lower and higher temperatures. Conductivity changed very little over the frequency range used in this study, so a virtually identical pattern is observed at all other frequencies used here, up to 20 kHz (Fig. 3.5). Previous reports of comparisons of ohmically and conventionally heated vegetable tissues have shown a similar increase in conductivity at lower temperatures with ohmic, rather than conventional, heating (Personius and Sharp, 1938; Palaniappan and Sastry, 1991).

For both conventionally (Fig. 3.6) and ohmically (Fig. 3.7) heated samples, those that were heated to a higher endpoint temperature had a greater dispersion of dielectric
constant across the low-frequency range than those heated to lower temperatures. At 100 Hz, samples heated ohmically to 50ºC had significantly higher apparent dielectric constants than those heated conventionally, while at 70ºC, the reverse was true (Fig. 3.8). This pattern is similar to that of conductivity. The lower apparent dielectric constant for ohmically heated samples at 70ºC could be due to the reduced time required to heat to the endpoint temperature, thus allowing less time for denaturation of macromolecules and other chemical reactions. Another possibility is that enzymes released by electropermeabilized cells at lower temperatures change the conductance or polarizability of the tissue before they are denatured by catalyzing reactions involving ions. For instance, pectin methylesterase, which causes firming of cell walls during vegetable blanching, is active upon cell lysis, but is denatured at temperatures above 70ºC (Bartolome and Hoff, 1972).

Usually, dispersions in the sub-kHz region are interpreted according to hypothesized α dispersion mechanisms. One proposed α dispersion mechanism hypothesizes counterion polarization effects due to an ionic double layer formed at the surfaces of cells or macromolecules with fixed charges (Schwan, 1981, Craig, 1995). Such a mechanism would be inconsistent with our results from Chapter 2 because cell membranes are known to become freely permeable at high temperatures, with the effect being particularly pronounced at frequencies associated with the α dispersion. This suggests that membrane structure is an important component of the mechanism, since increased capacitance suggests and increase in surfaces capable of being charged. It is possible that permeation opens many possible channels for ion movement, and exposes
many previously inaccessible surfaces of membranes and macromolecules. The probability of a cell rupturing in an electric field is proportional to cell size and temperature (Tsong, 1989) and whether or not a cell has ruptured nearby (Lebovka et al., 2001). Thus, in a tissue with a random distribution of cell size, larger cells will be vulnerable to electropermeabilization at a lower temperature than others, and will be ruptured first. As the temperature increases, more and more cells will be vulnerable to electropermeabilization. Finally, the whole tissue will be above the lethal temperature for the cells, resulting in the maximum change in dielectric properties. Kuang and Nelson (1997), in a study of artificial membranes, observed higher capacitance and stronger dispersions in those membranes with the greatest degree of permeation, which is in agreement with this work.

At frequencies above 5 kHz, a different pattern emerges. This range is normally associated with the β dispersion region, and is sometimes attributed to membrane charging. However, as our work in Chapter 2 shows, permeability is relatively less affected at high frequencies. When cell membranes lose their selectivity, the ionic double layers on membrane surfaces, caused by negative charge accumulation within the cell, are not available to provide a capacitive reactance within the time scales associated with these higher frequencies, so the apparent dielectric constant at frequencies higher than the α dispersion range decreases (Schwan, 1957). In this study, samples heated to higher temperatures have lower apparent dielectric constants at higher frequencies (Fig. 3.6, 3.7). At 20 kHz, samples heated to 50ºC have a lower apparent dielectric constant if heated ohmically, rather than conventionally (Fig. 3.9).
The dielectric properties of potato heated to various endpoint temperatures indicate that permeabilization of cells occurs at lower temperatures during ohmic heating than conventional heating. We have shown that the dielectric properties of potato change abruptly in the 50ºC-60ºC range when conventionally heated. The sharp increase in conductivity found upon conventional heating to 60ºC corresponds to cell death caused by cell membrane dysfunction (Gonzalez-Martinez, 2002). Thus, most of the cells will rupture in a narrow range of temperature, above which they cannot survive. Although potato starch gelatinization also occurs over the temperature range 58ºC-70ºC (Briant et al., 1945), its effect is a small increase in electrical resistance (Personius and Sharp, 1938).

The gradual change in dielectric properties that is seen over the same temperature range for ohmically heated samples suggests that a nonthermal mechanism causes membrane changes in an electric field. Electropermeabilization is a mechanism that can explain this gradual change. When cells are permeabilized, channels are opened for diffusion of KCl into the tissue. As the tissue becomes infused with the KCl solution, the dielectric properties of the tissue become more like those of the KCl solution. This is evident from Figs. 3.3-3.7, where the more severe the treatment, the greater the permeabilization, and the more closely the properties approach that of the KCl solution.

Since the KCl had the same electrical conductivity as the potato juice, the results for electrical conductivity are likely not influenced by the presence of the KCl solution. Thus, the results generally resemble those of Palaniappan and Sastry (1991). However, the apparent dielectric constant of the KCl solution differs markedly from potato tissue,
thus the progression of treatments show different extents of permeabilization as shown by the increasing approach of the dielectric constant towards that of KCl with increasing treatment severity. These results also show that the dielectric spectra of ohmically heated tissues have a different signature than conventionally heated tissue.

Vegetable blanching often involves a preheating step between 50°C -70°C to lyse the cells and to allow pectin methylesterase to firm the cell walls (Bartolome and Hoff, 1972; Andersson et al., 1994). It is possible that electrical treatment can be used for firming of plant tissues at lower temperatures. For example, PEF treatments have been successful in recovery of secondary metabolites, although cell viability was affected (Hunter and Kilby, 1988; Dörnenburg and Knorr, 1993). Theoretically, reversible electropermeabilization is possible (Weaver and Chizmadzhev, 1996), thus allowing cell survival, but the distribution of vulnerability within a population of cells should make it challenging to have a significant effect on most of the cells without some lethal effects.
3.5 CONCLUSIONS

Ohmic heating of raw potato tissue causes an increase in conductivity at a lower temperature than conventional heating. The apparent dielectric constant of the tissue samples is a function of the frequency. The higher the final temperature of heating up to 70°C, the greater the permeabilization. For the same final temperature of heating, ohmic heating yields greater permeabilization than conventional heating. Although the effects of both methods are similar at temperatures around 70°C, the dielectric spectra of ohmic heating show a different signature than conventional heating.
3.6 BIBLIOGRAPHY


Figure 3.1. Apparatus for dielectric measurements.
Figure 3.2. Conductivity spectrum of potato cylinders heated conventionally to various endpoint temperatures. Error bars represent 95% confidence intervals.
Figure 3.3. Conductivity spectrum of potato cylinders heated ohmically to various endpoint temperatures. Error bars represent 95% confidence intervals.
Figure 3.4. Conductivity at 100 Hz of conventionally and ohmically heated potato cylinders heated to various endpoint temperatures. Error bars represent 95% confidence intervals.
Figure 3.5. Conductivity at 20 kHz of conventionally and ohmically heated potato cylinders heated to various endpoint temperatures. Error bars represent 95% confidence intervals.
Figure 3.6. Apparent dielectric constant spectrum of potato cylinders heated conventionally to various endpoint temperatures. Error bars represent 95% confidence intervals.
Figure 3.7. Apparent dielectric constant spectrum of potato cylinders heated ohmically to various endpoint temperatures. Error bars represent 95% confidence intervals.
Figure 3.8. Apparent dielectric constant at 100 Hz of conventionally and ohmically heated potato cylinders heated to various endpoint temperatures. Error bars represent 95% confidence intervals.
Figure 3.9. Apparent dielectric constant at 20 kHz of conventionally and ohmically heated potato cylinders heated to various endpoint temperatures. Error bars represent 95% confidence intervals.
CHAPTER 4

CHANGES IN PERMEABILITY OF MODERATE ELECTRIC FIELD (MEF) TREATED VEGETABLE TISSUE OVER TIME

4.1 ABSTRACT

Microscopy shows that in beet tissue given a moderate electric field treatment, some cells lose their membrane selectivity while others remain intact. Raw, thawed, or precooked potato cylinders were chilled, and then warmed to 25°C by either allowing them to equilibrate or by MEF treatment. The conductivity from 100 Hz to 20 kHz and apparent dielectric constant from 100 Hz to 5 kHz was initially the same between raw, untreated samples and raw, MEF treated samples, but over 24 hours, that of the raw, MEF treated samples increased while that of the raw, untreated samples remained constant. No such distinct pattern emerged from the thawed or precooked samples. The apparent dielectric constant of raw, MEF treated potato above 5 kHz was the same as raw, untreated potato and higher than thawed and precooked potato. None of the samples showed marked changes in dielectric constant at 5-20 kHz over the 24 hour period. Apparently, even mild electrical treatments permeabilize vegetable tissue, permitting
enhanced diffusion.
4.2 INTRODUCTION

The permeability of cell membranes has long been measured by the electrical conductivity of the tissue at sub-MHz frequencies (Osterhout, 1922; Personius and Sharp, 1938; Knorr and Angersbach, 1998; Lebovka et al., 2000). Living tissues, such as plant cells, have dielectric dispersions in the low-frequency region from the effects of cell membranes (Schwan, 1957) due to ionic conduction and membrane charging relaxation mechanisms that do not occur at high frequencies. Thus, low-frequency measurements may be used for examining the effects of ohmic heating and other processes on cell membranes. Kuang and Nelson (1998) have published a review of the low-frequency dielectric properties of biological tissues, with explanations of the underlying mechanisms.

During MEF treatment, the electric field may cause changes in the permeability of cell membranes of plant cells below the temperature at which membranes are permeabilized due to thermal effects (Personius and Sharp, 1938; Chapters 2 and 3). Diffusion is enhanced, electrical conductivity changes are more linear during heating, and moisture migrates more easily out of the tissue. (Halden et al., 1990; Schreier et al., 1993; Lima et al., 2001; Imai et al., 1995; Chapter 2). Thus, MEF pretreatment has been found useful in drying and juice extraction processes (Wang and Sastry, 1998). Electropermeabilization is a mechanism that can account for these effects. According to
this hypothesis, pores formed in the cell membranes upon electric field exposure cause a
drop in resistance as ions are allowed to pass through the membrane (Coster, 1965).
Visual observation of treatment-induced changes may provide some insight. If
electropermeabilization occurs in a vegetable tissue, the permittivity and conductance
should be affected due to the changes in membrane permeability. Thus, low-frequency
measurements may be used for examining the effects of MEF treatment and other
processes on cell membranes. The objective of this study was to examine the structural
and low-frequency dielectric spectral response of vegetable tissue subjected to MEF
processing for 24 hours after exposure.
4.3 METHODS

**Microscopy.** In order to visualize changes in the tissue structure, a slice of beet tissue approximately 1 cell layer in thickness was immersed in 0.5% agar between two electrodes 22 mm apart on a petri dish. The sample was photographed under a microscope at 10X magnification before MEF treatment and one hour after a 10 second exposure to a 120V, 60 Hz electric field. The time after exposure was required to allow the betacyanin to effuse from permeabilized cells. The temperature increase was less than 2ºC.

**Dielectric changes over time.** Cylinders of potato, 5 cm long and 2.6 cm in diameter, were placed coaxially in a cylindrical glass static MEF treatment device (Fig. 4.1), with the sample completely blocking the path of the current. Cooked and thawed samples were prepared as described below. Although the samples contacted the stainless steel electrodes, an isoconductive KCl solution (preparation described below) was poured over all samples to fill microscopic gaps between the electrode and potato surfaces. For all samples, the treatment device, containing the sample, was placed in a refrigerator (3ºC) overnight (12-24 hours). A thermocouple was inserted to the geometric center of the sample. Conventional samples were allowed to equilibrate to 25ºC for 2.5 hours. The MEF samples were heated to 25ºC at 24 V/cm, 60 Hz. The thermocouple was removed, and the dielectric spectra measured every 30 minutes for 24 hours. In order to
measure the low-frequency dielectric spectra, power leads were connected to an LCZ meter (HP 4276A, medium measuring speed, high (1V) test signal level). The impedance and phase angle of the samples were measured at frequencies ranging from 100 Hz to 20 kHz. Conductivity (S/m) and relative permittivity were calculated from the sample geometry and dielectric measurements.

**Cooked and thawed sample preparation.** Cooked samples were heated at 30 V/cm at 60 Hz to an internal temperature of 100ºC. Before being placed in the treatment device, thawed samples were wrapped in parafilm, placed in a freezer (-10ºC, 12 hours-3 days), and allowed to thaw at room temperature for 2 hours, when it was soft enough to be placed in the treatment device.

**Preparation of isoconductive KCl solution.** In order to minimize artifacts from diffusion gradients, a KCl solution with the same conductivity as potato juice was prepared for contact between sample and electrodes. Resistivity of tissues is due to both the salt concentration and the presence of physical barriers, such as cell membranes and cell walls. Since our intention was to study the physical barriers, an attempt was made to minimize the conductivity effects of the diffusion of KCl solution into the potato tissue.

Raw potato was juiced, and then centrifuged to remove solids. The mean conductivity of the clarified potato juice from three trials was 1.009 S/m, as measured by a conductivity meter (Cole-Parmer Instrument Co., EC meter 19101-00). The KCl solution was then made by titration to 1.009 S/m, and had a concentration of 5.65 mg/ml or 0.076 M.
4.4 RESULTS AND DISCUSSION

The photomicrographs shown in Figs. 4.2 and 4.3 are of the same tissue before and 1 hour after MEF treatment. After treatment, many of the beet cells have completely lost their pigmentation, indicating that those individual cells have lost their viability and the membranes have lost their selectivity (Zhang et al., 1992). The remaining pigment is entirely within individual cells. Apparently, partial permeability changes in a tissue reflect complete damage to a fraction of the cells, rather than partial damage to all of the cells. Also, it is not obvious from Figs. 2 and 3 what significant structural changes may have occurred within individual cells. It appears as though any permeabilization has been temporary, and just long enough to permit movement of intracellular pigments.

The conductivity of the raw, MEF treated samples was initially the same as raw, untreated samples, but increased over time to approach that of thawed and cooked samples (Figs. 4.4-4.7). This increase was not observed in any other treatment. Cooked and thawed samples had constant conductivity over the 24 hour period, and there were no differences between MEF treated and untreated samples. A similar pattern was observed for conductivity at all frequencies.

As with conductivity, the relative permittivity below 5 kHz of raw, MEF treated samples increased over the 24 hour period, whereas the raw, untreated samples did not
(Figs. 4.8-4.10). At higher frequencies, the apparent dielectric constant did not significantly decrease due to MEF treatment, and did not change over time (Fig. 4.11).

Previous studies have generally shown that ohmically heated vegetable samples show greater conductivity over untreated or conventionally heated (Palaniappan and Sastry, 1991, Lima et al., 1999). Initially, this was not seen in this study, possibly due to the low temperature range. In Chapter 2, no increase in conductivity was observed upon MEF treatment to 30ºC, but an increase was found at higher temperatures. However, Personius and Sharp (1938) observed a conductivity increase up to 30ºC, and were able to increase the conductivity of MEF treated potato to that of cooked potato by repeated cycles of MEF treatment to 30ºC and cooling to room temperature. This may be have been due to the difference in electric field strength, which was 18 V/cm in Chapter 2 and 44 V/cm in the Personius and Sharp study. Most previous studies of conductivity changes in MEF treatment have involved temperature ranges of 25ºC -100ºC, usually measuring a conductivity change during the heating itself.

The gradual increase in conductivity and apparent dielectric constant below 5 kHz over time indicates that some process has been initiated. If the process was an irreversible permeation, we would have expected the apparent dielectric constant above 5 kHz to decrease as the cell membranes lost their selectivity. A chemical process may have been initiated that increases the conductivity without affecting the capacitance of the cell membranes. In the case of reversible electropermeabilization, or if only a small fraction of the cells have been irreversibly damaged from the electric field, we may see effects of enzyme catalyzed reactions without greatly altering the apparent dielectric constant.
constant at frequencies above 5 kHz. In the present set of experiments, the KCl solution had the same electrical conductivity as the potato juice, although the intact tissue would be expected to have a lower electrical conductivity. However, the apparent dielectric constants were not matched. The change in electrical conductivity over time (Figs. 4.6 and 4.7) suggests an equilibration process between the potato and the KCl solution. Likewise, the apparent dielectric constant of the raw MEF treated samples (Figs. 4.8-4.11) suggests an approach towards the values for KCl. These results suggest that permeabilization has occurred in the raw MEF treated tissue. Thus, even mild electrical treatments cause permeabilization.

The one result that is more difficult to explain is in Fig. 4.8, Fig. 4.9, where it appears that the apparent dielectric constant of the thawed, MEF treated sample exceeds that of the KCl solution. By contrast, none of the other samples, either cooked, MEF treated, cooked, untreated, or thawed, untreated, show such a trend. Results may be influenced by the exudate from thawed MEF samples, which may have different properties than the others. Cooked samples would be expected to have gelatinized starch, and consequently, little exudate that might mix with KCl. By contrast, thawed samples would contain sufficient exudate. However, the thawed, untreated samples do not show the behavior of the thawed MEF samples. This suggests that the MEF process, combined with thawing, may have resulted in some chemical changes that affected the apparent dielectric constant. A freezing and thawing cycle is likely to release numerous potential reactants into the extracellular fluid. At the electrodes, reactive species are often formed that may interact with these constituents. Many species may be oxidized or reduced.
directly at the electrodes. Many enzymes may remain active after a freezing and thawing cycle, so a biochemical mechanism is also possible.

Lebovka et al. (2001) have shown that pulsed electric fields initiate changes in the cell tissue structure that evolve on the order of seconds after the pulse. The authors attributed the effects to moisture migration within the tissue and membrane pore resealing. In this study, it is unlikely that pore resealing has a major effect. Pore resealing occurs on the order of 1 s (Knorr et al., 2000), and would result in a decrease in conductivity, rather than an increase. Moisture migration and diffusion processes are a possible factor. In the microscopy study, the treated tissue immediately after MEF treatment appeared identical to that before treatment. It took some time for the dye to diffuse out of the cells. Likewise, it should take any permeated cell some time to come to equilibrium with its surroundings. In this study, an attempt was made to minimize the effects of ion migration and diffusion by using the isoconductive KCl. However, the gradual changes in conductivity and low-frequency dielectric constants only below 5 kHz are consistent with an enhanced diffusion process. Further investigation into these processes has potential to yield valuable information about the details of the interaction of electric fields with cellular tissue.
4.5 CONCLUSIONS

Visualization studies on beet tissue indicate that enhanced diffusion occurs even after mild MEF treatment, without obviously visible permanent cell damage. Mild MEF treatment of raw potato did not initially alter its dielectric properties. It does result in permeabilization of the tissue as reflected in the properties changing over time to equilibrate with a KCl solution. These effects appear to be entirely nonthermal in character, since the temperature range of our study was well below that associated with irreversible membrane denaturation. This suggests that very low energy treatments may be used effectively to enhance diffusion in cellular materials. By contrast, tissue treated with more severe treatments, such as cooking or thawing, exhibit properties that are closer in value to this KCl solution and do not subsequently change in a significant manner.
4.6 BIBLIOGRAPHY


Figure 4.1. Apparatus for dielectric measurements.
Figure 4.2. Beet before ohmic treatment.
Figure 4.3. Beet after ohmic treatment.
Figure 4.4. Conductivity of potato over 24 hours at 100 Hz. Error bars represent 95% confidence intervals.
Figure 4.5. Conductivity of potato over 24 hours at 20 kHz. Error bars represent 95% confidence intervals.
Figure 4.6. Initial conductivity spectrum of potato. Error bars represent 95% confidence intervals.
Figure 4.7. Conductivity spectrum of potato after 24 hours.
Figure 4.8. Initial apparent dielectric constant low-frequency spectra. Error bars represent 95% confidence intervals.
Figure 4.9. Low-frequency apparent dielectric constant spectra after 24 hours. Error bars represent 95% confidence intervals.
Figure 4.10. Apparent dielectric constant of potato over 24 hours at 100 Hz. Error bars represent 95% confidence intervals.
Figure 4.11. Apparent dielectric constant of potato over 24 hours at 20 kHz. Error bars represent 95% confidence intervals.
CHAPTER 5

CONCLUSIONS

This dissertation focuses on plant cell membrane permeability changes during MEF processing of vegetable tissue. Except for direct current, diffusion enhancement by Moderate Electric Field (MEF) processing increases with increasing field strength and decreasing frequency. The enhancement appears to be significant when the product initially possesses an intact cell structure. No enhancement is observed when a cell structure is either absent or previously completely permeabilized. Consequently, the mechanism of diffusion enhancement may be attributed to pore formation in cell membranes. There appears to be a threshold potential above which significant increases in permeabilization occur. Except for DC, this potential is found to depend on frequency – the higher the frequency, the higher the threshold potential for permeabilization.

Ohmic heating of raw potato tissue causes an increase in conductivity at a lower temperature than conventional heating. The apparent dielectric constant of the tissue samples is a function of the frequency. The higher the final temperature of heating up to 70°C, the greater the permeabilization. For the same final temperature of heating, ohmic heating yields greater permeabilization than conventional heating. Although the effects
of both methods are similar at temperatures around 70°C, the dielectric spectra of ohmic heating show a different signature than conventional heating.

Visualization studies on beet tissue indicate that enhanced diffusion occurs even after mild MEF treatment, without obviously visible permanent cell damage. Mild MEF treatment of raw potato did not initially alter its dielectric properties. It does result in permeabilization of the tissue as reflected in the properties changing over time to equilibrate with a KCl solution. These effects appear to be entirely nonthermal in character, since the temperature range of our study was well below that associated with irreversible membrane denaturation. This suggests that very low energy treatments may be used effectively to enhance diffusion in cellular materials. By contrast, tissue treated with more severe treatments, such as cooking or thawing, exhibit properties that are closer in value to this KCl solution and do not subsequently change in a significant manner.

MEF processing has already been found useful in dehydration and juice extraction processes, and has great potential for blanching processes. Reversible electropermeabilization may also be useful for encapsulation and secondary product extraction processes, but no studies have been done investigating these possibilities. The results presented in this dissertation have provided insight into the mechanism for enhanced diffusion and can provide direction in future attempts to improve electrical heating processes and develop new applications for the technology.


