OHMIC HEATING AS AN ALTERNATIVE FOOD PROCESSING TECHNOLOGY

by

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Abstract

Ohmic heating for the food industry consists of using electrical energy to heat foods as a method of preservation, which can in turn be used for microbial inactivation or several other processes such as pasteurization, extraction, dehydration, blanching or thawing. Few studies have been conducted on the usefulness of this environmentally friendly processing technique. Due to the lack of sufficient information on research into ohmic heating for the food industry, a few of the published studies are discussed here in detail.

This report also focuses on self-conducted research using ohmic heating to determine its effect on *Lactobacillus acidophilus* inactivation versus conventional heating. *Lactobacillus acidophilus* was inoculated into MRS broth and incubated for 24 hours. The sample was then inoculated into sterile buffer at a dilution rate of 1:100. Samples of the diluted culture were subjected to either low voltage (18 V) or conventional heating (300°C) over a hotplate stirrer. Temperature was monitored on test and control samples to achieve an endpoint of 90°C. Samples were taken at regular intervals, plated onto MRS agar and incubated for 72 hours at 35°C to compare plate count expressed as colony forming units per milliliter (cfu/mL). Temperature was uniform throughout the ohmically heated sample and reached the endpoint more quickly than the conventionally heated sample, which also had cold spots. The total plate count at the end of the experiment was less for the ohmically heated sample versus the conventionally heated sample. Ohmic heating was more effective in inactivation of *Lactobacillus acidophilus* than conventional heating, most likely due to the more rapid and uniform heating of the sample, and possible electroporation of the cells.

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Preface

The demand for alternative food processing techniques has increased at a rapid rate as manufacturing moves further into the 21st century. As technology advances, more efficient equipment can be retrofitted to replace antiquated machinery and processing methods for the food industry. With growing concerns on the safety of food for consumption by animals and humans, a processing technique that is more easily controlled, more efficient, and just as effective as current techniques could revolutionize the food industry in the coming years.

Ohmic heating is one of the newest alternative processing techniques to emerge in the last 15 years. Using electric current, food can be pasteurized, fermented, or sterilized in a manner that is equally comparable, if not better, than the current methods of processing. The focus of this paper is the background of this technology, and the many applications it serves in the food industry.

Appendix A - Definitions

Electroporation- also known as electropermeabilization, is a significant increase in the electrical conductivity and permeability of the cell plasma membrane caused by an externally applied electrical field.

Electrical conductivity- also known as specific conductivity, is a measure of a material's ability to conduct an electric current.

Interstitial fluid- the extracellular fluid or tissue fluid, which is found in the spaces between the surrounding cells.

Electric field- the space surrounding an electric charge or in the presence of a time-varying magnetic field.

Electric arcing- an electrical breakdown of a gas, which produces an ongoing plasma discharge, resulting from a current flowing through normally nonconductive media such as air.

Voltage- also called electric or electrical tension, is the difference of electrical potential between two points of an electrical or electronic circuit, expressed in volts (V).

Electric current- the flow (movement) of electric charge, expressed in amperes (A), see direct current and alternating current below.

Electrical resistance- measure of the degree to which an object opposes an electric current through it, expressed in ohms (Ω).

Specific heat capacity- is the measure of the heat energy required to increase the temperature of a unit quantity of a substance by a certain temperature interval.

Fermentation- the process of releasing energy from a carbohydrate without oxygen by producing alcohol or lactic acid.

Optical density (OD)- the absorbance of an optical element for a given wavelength λ per unit distance.

Prokaryote- organism whose cells have no nucleus, generally bacteria

Eukaryote- organism whose cells have a defined nucleus, generally animals, plants and fungi.

Digital Multimeter- an instrument that measures the electrical potential difference between two points in a circuit, as well as current and resistance.

Joule (J)- measurement of heat, electricity and mechanical work, named after physicist James Prescott Joule. One Joule equals the force required to move one Coulomb through one volt.

Coulomb (C)- amount of electrical charge, measured in one ampere per second.

Direct Current (DC)- one directional flow of electric charge, sources include batteries and solar cells.

Alternating Current (AC)- electrical current whose magnitude and direction vary cyclically, sources include standard electricity.

Hertz (Hz)- unit of frequency, waves of energy expressed in cycles per second.

CHAPTER 1 - Technology and Design of Ohmic Heating Systems

Ohmic Heating

Ohmic heating is referred to as Joule heating, electrical resistance heating or electroconductive heating. For the purposes of this paper, the term of ohmic heating will be used exclusively. The food industry has shown a renewed interest in ohmic technology in recent years, with new systems being designed since the early 1990's. Ohmic heating is an advanced thermal process where food acts as an electrical resistor. The experimental design usually consists of electrodes that contact the food, whereby electricity is passed through the substance using a variety of voltage and current combinations. The substance is heated by the dissipation of electrical energy. When compared to conventional heating, where heat is conducted from the outside in using a hot surface, ohmic heating conducts heat throughout the entire mass of the food uniformly. The success of ohmic heating depends on the rate of heat generation in the system, the electrical conductivity of the food (see: Electrical Conductivity), and the method by which the food flows through the system (Leizerson and Shimoni, 2005a).

Electricity

All substances are comprised of atoms, which are in turn comprised of electrons (negatively charged), neutrons (neutral charge), and protons (positively charged). A substance is known as a conductor if the electrons move freely from one atom to another. An insulator is a substance in which the electrons are bound tightly together and do not move freely. This flow of electrons throughout a substance is known as electricity.

Electricity is comprised of electrical current, voltage and resistance. Electrical current is measured in amperes, where 1 ampere is the flow of ~$6x10^{18}$ electrons per second through a substance. Voltage is the electron pressure, or a measure of the ability to move an electrical charge through a resistance (opposition to flow of electricity) (Shugar and Ballinger, 1996). Voltage can be calculated by multiplying the current and the resistance. This principle is known as Ohm's Law, first published by physicist Georg Ohm in 1827.

Where voltage (V), current (I), and resistance (R)…

$V = I * R$

An electrical circuit that powers an ohmic heating system can be set up in two basic ways, either in series or parallel (*Fig. 1.1*). A series circuit has the resistors (for example, a standard light bulb) set in line with each other. The voltage from an electrical source (for example, a battery) is split equally across the resistors. The current and resistance remains the same. A parallel circuit has the resistors set next to each other, where the voltage is the same across each resistor, but the current and resistance are divided equally.

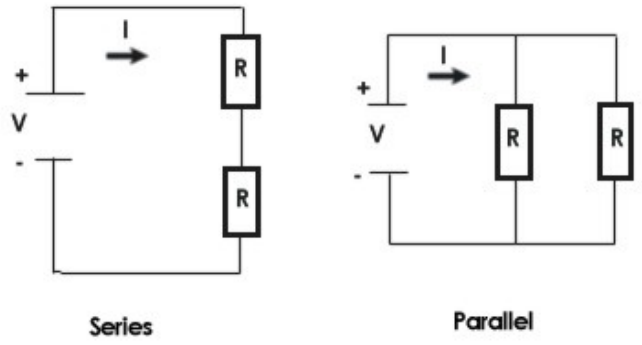

Figure 1.1 Series and Parallel Circuits

Specific Heat Capacity

Specific heat capacity is the amount of energy needed to increase the temperature of a substance by a certain interval. This is can be helpful for determining temperature distribution in a substance that is to be heated ohmically. An equation determines the specific heat of a substance by using the variables of heat added (expressed in joules), mass of the substance (expressed in grams), and the temperature change of the system (expressed in degrees Celsius). The joules added to a system can be calculated by measuring the Kilowatt Hours (the standard expression for electricity consumption) used to heat the substance to a particular temperature, and also the time it takes to reach that temperature.

1 Kilowatt Hour = 1000 Watt-Hours = 3,600,000 Joules

Using a form created by Georgia State University (http://hyperphysics.phy-astr.gsu.edu/hbase/thermo/spht.html), the specific heat of a substance can be determined by plugging in the variables. Otherwise, using the equation below, the same information can be calculated.

Where specific heat of substance (c), heat added (Q), mass (m), and change in temperature (ΔT)…

$$c = \frac{Q}{m * \Delta T}$$

For instance, 20,000 g (20 kg) of a substance heated from 20 to 90°C, using 1 Kilowatt Hour (3,600,000 J), would give a specific heat of 2.571 J/g°C.

Electrical Conductivity

Electrical conductivity is the measure of how well a substance transmits electric charge, expressed in Siemens per meter (S/m). Electrical conductivity is a ratio of the substance density to electric field strength and is affected by the chemical composition of a substance. In ohmic heating terminology, the conductivity is a measure of the mineral or ionic content. For food substances, the most common ionic ingredient is salt (NaCl). The higher the amount of dissolved salts in a substance, the higher the conductivity. Seawater has a conductivity measurement of ~5 S/m, whereas regular drinking water has a conductivity measurement of ~.0005 to .05 S/m (Janz and Singer, 1975). One method of measuring electrical conductivity is to use a TDS (Total Dissolved Solids) meter. TDS are the total amount of mobile charged ions in a substance (mg/L or ppm), calculated by measuring the number cations (positively charged) and anions (negatively charged) ions in the substance. Electrical conductivity is ~100 times the total number of cations and anions present (HM Digital, Inc., 2005).

Ohmic Heating System Design

There are endless possibilities for the design of an ohmic heating system, but there are several key elements that will be present in each one. A power supply (generator) is needed to produce the electricity. Electrodes connected to the power supply must be in physical contact

with the substance in order to pass the electric current through. The electrode gap (distance between the electrodes in the system) can fluctuate depending on the size of the system, but by changing this distance, the electric field strength, expressed in volts per centimeter [V/cm], can be varied. For a moving system, such as a conveyor or tube, the electrodes can be placed at various positions along the length of the product flow path for an in-line field (*Fig. 1.2*), or placed perpendicular to the flow path for a cross field (*Fig. 1.3*). In an in-line field system, the material upstream experiences higher field strength than the material downstream due to the drop in voltage throughout the system. In a cross-field system, the electric field strength is constant throughout (FDA-CFSAN 2000).

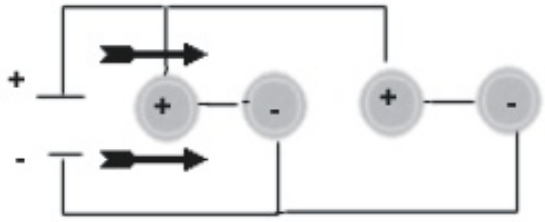

Figure 1.2 Inline Field Diagram

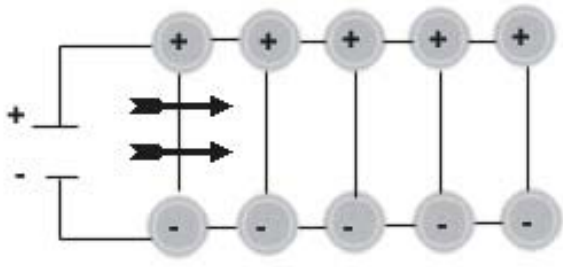

Figure 1.3 Cross Field Diagram

A device for measuring temperature in the system, such as an electrically isolated thermocouple, would be inserted at certain key positions. A data logger connected to the system would record essential information such as temperature, voltage, current and time. Additional

equipment may include a pH probe, a spectrophotometer for measuring optical density, a refractometer for measuring °Brix (soluble solids), and sampling ports (in a closed system).

Ohmic heating has internal energy generation within its system. In theory, there is no upper limit to the temperature that can be produced. However, several other factors will influence the temperature achieved by the system: 1) the electrical conductivities of the food substance, 2) the design of the system, 3) the time that the substance is subjected to the heating, 4) the thermophysical properties of the food, 5) the electric field strength and constancy, 6) the temperature dependence of electrical conductivities, and 7) the extent of the interstitial motion in the food (FDA-CFSAN, 2000).

Commercial Applications

Ohmic heating systems made for commercial applications can vary greatly, but one example (*Fig. 1.4*) has 7 electrode columns, each comprised of polytetrafluoroethylene (PTFE) coverings and a single stainless steel cantilever electrode. These columns are structured vertically to allow for an upward flow of product, and are connected with insulated stainless steel tubes. Due to the increased electrical conductivity of products (see: Electrical Conductivity) as heat increases, the connecting tubes increase in length throughout the system to maintain the same electrical impedance. A temperature control system constantly monitors the temperature, flow rate, heat capacity and specific heat of a product to calculate the electrical power needed for the system. The processing capacities of such systems can range from 3 to 6 tons an hour. For validation of processing, a sterilized solution will be inoculated with a known organism, processed through the system at the known heat treatment for that organism, and then evaluated for survivor cell counts (Parrott 1992).

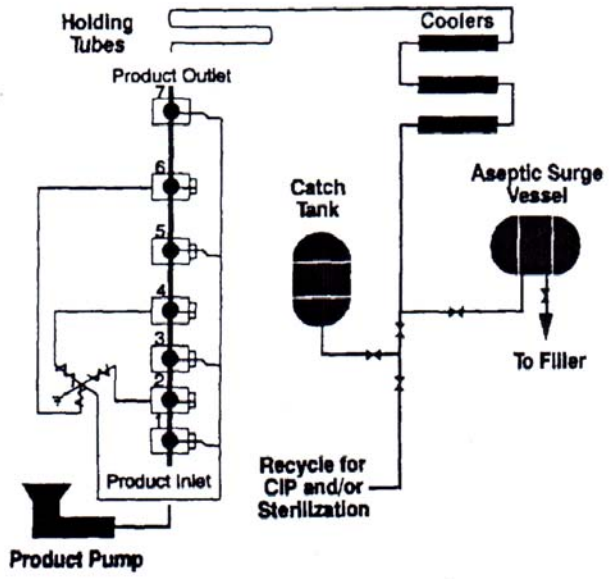

Figure 1.4 Schematic of Commercial Ohmic Design (Parrott 1992)

Thomas R. Parker of the United Kingdom patented an ohmic heating system to produce Japanese style breadcrumbs (Panko). For desirable Panko breadcrumbs, no crust or color must be formed. In comparison, American style breadcrumbs are produced with a golden brown color and crispy texture, which is formed when the bread dough is baked, ground, and then dried in an oven. Parker's device was designed to place the bread dough inside a container between two electrodes. The ohmic heating system bakes the dough without a crust, and the chamber constrains the dough, which forms the desired grain structure. The product is then cooled, ground and dried.

Yanagiya Machinery (http://www.ube-yanagiya.co.jp/Yanagiya/companyguide.htm) in Japan began manufacturing an ohmic heating system for the production of tofu in 1995 (Noguchi 2004). The "Big-J", named for joule heating, can process up to 4,800 pieces of tofu an hour. The estimated cost for this commercial sized machine is $40,000 to $1,500,000+ USD, depending on the desired capacity and installation. In 1999, Yanagiya developed the "Mini-J" (http://www.ube-yanagiya.co.jp/Yanagiya/mini-j-e.html), a tabletop version of their "Big J". The "Mini J" has titanium electrodes, with two models ranging from 100-200 V. The cost for

this machine is around $4,000 USD, although it is not available for export to all countries as of 2008.

Manufacturers of food products that use ohmic heating systems include Sous Chef Ltd. in England, where a 75-kilowatt unit processes low acid meats and vegetables in bags. Wildfruit Products, a division of Nissei Co. Ltd. of Japan, also uses a 75-kilowatt system to process whole fruits. Papetti's Hygrade Egg Products of Elizabeth, NJ uses a 200,000 Hz system manufactured by Raztek (http://www.raztek.com/home.html) to process between 10,000 and 20,000 pounds of liquid eggs per hour. This system heats the liquid eggs to 140-148°F and holds for 3.5 min. After processing, the eggs are given a 12-week shelf life for consumers, but cartons regularly surpass this shelf life at Papetti's plant (Mans & Swientek 1993). A shelf-life study was done by Raztek to compare conventional heating to ohmic heating using pasteurized liquid eggs for a measurement of microbial inactivation. Conventionally heated samples reached plate counts of 10,000 cfu/mL compared to ohmically heated samples with plate counts of <10 cfu/mL after 12 weeks (Reznik, 1999). The initial plate counts at the start of the study were similar, but as time progressed, the survivor bacteria in the ohmically heated samples decreased. The study concluded that the injury effects to the cells were from electroporation (see: Electroporation), obtained from the ohmic heat treatment.

Emmepiemme SRL (http://www.fiereparma.it/spazi/200720/03011026.html) in Piacenza, Italy uses ohmic heating to process many varieties of foods including: baby food, artichokes, carrots, mushrooms, ketchup, fruit nectars, fruit juice, peppers, cauliflower, tomato paste, sausages, pâté, and fruit purée.

Several other applications for ohmic heating in the food manufacturing industry include: heating liquid foods such as soups, stews, and fruits in syrup; heat sensitive liquids processing; juices treated to inactivate proteins (such as pineapple or papaya); blanching; thawing; starch gelatinization; sterilization; peeling of fruits (eliminating the need for lye-a harmful corrosive chemical); dehydration; extraction; fermentation (see: Fermentation); and processing protein-rich foods which tend to denature and coagulate when thermally processed (Ramaswamy et al., 2005).

Fermentation

Fermentation is the process of releasing energy from a carbohydrate without oxygen by producing alcohol or lactic acid. An ohmic heating system may be particularly useful in this important process of food manufacturing, particularly the dairy industry, where fermentation by *Lactobacillus acidophilus* is necessary for the production of cheese and yogurt. Cho et al. (1996) showed that ohmic heating of a fermentation vessel containing *L. acidophilus* reduced the lag period of the bacteria in the early stages of growth. With this knowledge, a dairy manufacturer utilizing the ohmic heating process in the early stages of fermentation may shorten the total processing time of a dairy product. This speedier process would save untold amounts of time in overhead and labor costs.

Advantages of Ohmic Heating

In general, ohmic heating systems are advantageous due to an optimization of investment (increased efficiency), instant shutdown of the system, and reduced maintenance costs because of the lack of moving parts. In order to comply with governmental regulations concerning microbial lethality in food products, heating methods are applied at the coldest point of a system, which is generally the center of the largest particle. In conventional heating, the time it takes to increase the temperature at this cold point may overprocess the remaining particles and the surrounding liquid. This overprocessing leads to a destruction of nutrients and decreased flavor. Ohmic heating processes the particles and surrounding liquid simultaneously, preventing overcooking (Parrott 1992).

Microbial Inactivation

Microbial inactivation in relation to ohmic heating is primarily thermal in nature, much like conventional heating. Cho et al. (1996) demonstrated that electrical pretreatment by ohmic heating can reduce the intensity of additional thermal applications for subsequent microbial inactivation. Microbial inactivation curves of ohmic heating are similar to conventional heating curves except for a difference in the slope, which can most likely be explained by the presence of the electric field (FDA-CFSAN, 2000). No particular pathogens with a unique resistance to ohmic heating have been discovered; therefore the thermal death kinetic studies available for pathogens and spoilage bacteria may be followed when designing experiments and systems that utilize ohmic heating.

An experiment using *Bacillus subtilis* revealed that a two stage ohmic treatment (ohmic heating, holding period, ohmic heating) resulted in accelerated death rates (Cho et al., 1999). Another experiment involving *Saccharomyces cerevisae* showed that ohmic heating enhanced the leakage of intracellular components as compared with the conventional method of boiling in water (Lee and Yoon, 1999).

Electroporation

All living cells, whether a prokaryote or eukaryote, contain cell membranes. These membranes are comprised of lipids (fatty molecules) and proteins (Alberts et al., 2002). Prokaryotes have an additional layer outside the membrane known as the cell wall. At low frequencies (50-60 Hz) and high field strengths (>100V/cm) most commonly associated with ohmic heating, the naturally porous cell walls (*Fig. 1.5)* can allow the cell membrane to build up charges, forming disruptive pores (Cho et al., 1996) (*Fig. 1.6*). Electroporation occurs because the cell membrane has a specific dielectric strength, which can be exceeded by the electric field. The dielectric strength of a cell membrane is related to the amount of lipids (acting as an insulator) present in the membrane itself. The pores formed can vary in size depending on the strength of the electric field, and can reseal after a short period of time. Excessive exposure causes cell death due to the leakage of intracellular components through the pores (Lee and Yoon, 1999). Therefore, electroporation is highly damaging to a cell and would enhance the lethal effects of thermal abuse already present from the ohmic heating.

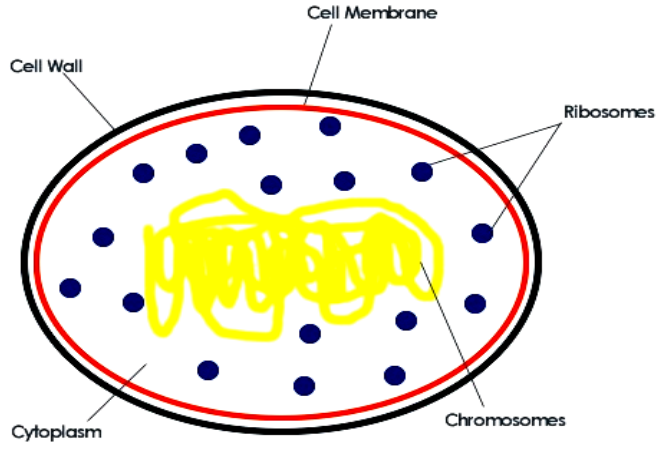

Figure 1.5 Diagram of Bacterial (Prokaryote) Cell

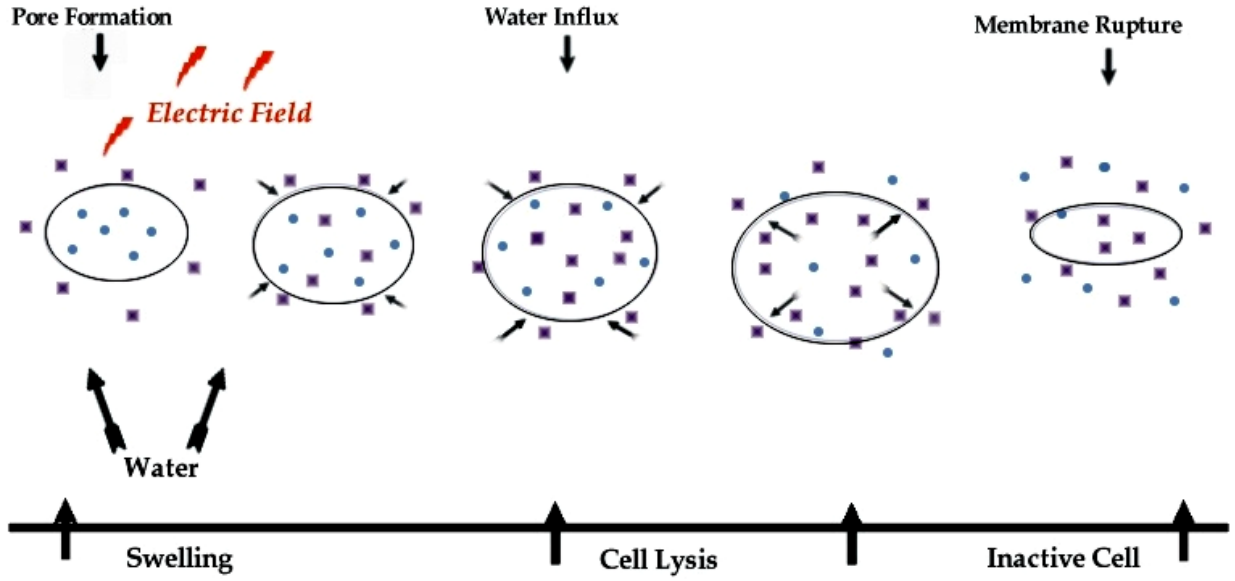

Figure 1.6 Electroporation Process of a Cell

Disadvantages to Ohmic Systems

The costs of commercial ohmic heating systems, including installation, can be in excess of $9,000,000 USD, which is a costly investment for a manufacturing facility. A Nestlé food service plant in Missouri uses an ohmic heating system to process beef stew, where the cost to process a pound of stew at the initial startup ranged from $0.25 to $0.49 USD. However, after 5 years, the cost per pound of processed stew decreased to approximately $0.21 to $0.36 USD (Rahman 1999). In comparison, commercial conventional pasteurizers cost upwards of $1,000,000 USD, including installation. As an example, a commercial pasteurizer used to produce pasteurized juice products (100 gallon per min flow rate) will cost a company anywhere from $0.11 to $0.17 per gallon to generate a finished product (Stark 2008).

A disadvantage related to the type of food that can be processed lies in the presence of fat globules. A food that has fat globules can be troublesome to effectively heat ohmically, as it is non-conductive due to lack of water and salt (Rahman 1999). If these globules are present in a highly electrical conductive region where currents can bypass them, they may heat slower due to lack of electrical conductivity. Any pathogenic bacteria that may be present in these globules may receive less heat treatment than the rest of the substance (Sastry 1992).

Another slight disadvantage relates to the electrical conductivity of a substance. As the temperature of a system rises, the electrical conductivity also increases due to the faster movement of electrons. Thus, this creates the possibility of 'runaway' heating (FDA-CFSAN, 2000). An ohmic heating system that has not been cleaned thoroughly enough may result in electrical arcing due to protein deposits on the electrodes. But, by utilizing the knowledge of the aforementioned issues in designing an ohmic heating system, these disadvantages may be more easily controlled.

CHAPTER 2 - Industry Studies

Ohmic Heating Treatments of *Lactobacillus Acidophilus*

H.-Y. Cho, A.E. Yousef, and S.K. Sastry performed an experiment, **Growth Kinetics of *Lactobacillus acidophilus* Under Ohmic Heating (1996)**, to examine the effects of ohmic heating during fermentation of the dairy industry's most important organism, *Lactobacillus acidophilus.* An inoculated solution of *L. acidophilus* was subjected to fermentation temperatures (30, 35 or 40°C) using ohmic heating or conventional heating. Both low (15V) and high (40V) voltage was used for the ohmically heated sample. The growth parameters, which included lag period, minimum generation time, and maximum growth, and pH of the solution were measured during the experiment. The metabolic activities of the bacteria were also determined by measuring the consumption of glucose and the production of lactic acid and bacteriocin (an antimicrobial protein byproduct unique to *Lactobacillus acidophilus*). The lag period for the bacteria was affected significantly by the ohmic heating in the early stages of fermentation, but declined in effectiveness in the later stages. At temperatures of 30°C, the lag period was decreased by 94% compared with the conventional heating method. The final product of the ohmically heated sample had a higher final pH and lower bacteriocin levels, but had little effect on glucose consumption and lactic acid production by *L. acidophilus*. The conclusion drawn from this study was that ohmic heating enhances the fermentation in early stages of growth, but tends to hinder the later stages.

For the experiment, *L. acidophilus* culture was inoculated into MRS broth at a rate of 1% and then incubated for 24 hr at 37°C. After the initial incubation, the treatment was repeated a second time, again transferring the culture at a rate of 1% into MRS broth. A fermentation vessel (*Fig. 2.1*) was built for the design of the experiment, utilizing a 5-L glass container with a 2-L working volume. The lid for the vessel had ports for the thermocouple, pH probe, inoculation, water circulation coil, and two stainless steel plate electrodes with a surface area of 73.5 cm^2 that were placed 14 cm apart inside the vessel. Any metal surfaces present in the experimental vessel were coated with epoxylite for electrical insulation. To keep temperatures constant for both treatments, a water bath fitted with a controller, manufactured by The Partlow Company in New York, was used to operate the flow of water in the circulation coil. A

thermocouple coated with Teflon was used to measure the temperature in the system, which was connected to the water bath controller. A pH probe measured the pH of the medium, while a double-beam spectrophotometer was set up inside the system to measure the optical density at 600 nm (OD_{600}). The reference sample for the spectrophotometer was non-inoculated MRS broth. The voltage and current in the system were recorded using transducers. All of the data produced by this equipment was connected to a data logger that was in turn linked to a microcomputer. The conventional heating design mirrored that of the ohmic heating design, save for the insertion of electrodes (Cho et al., 1996).

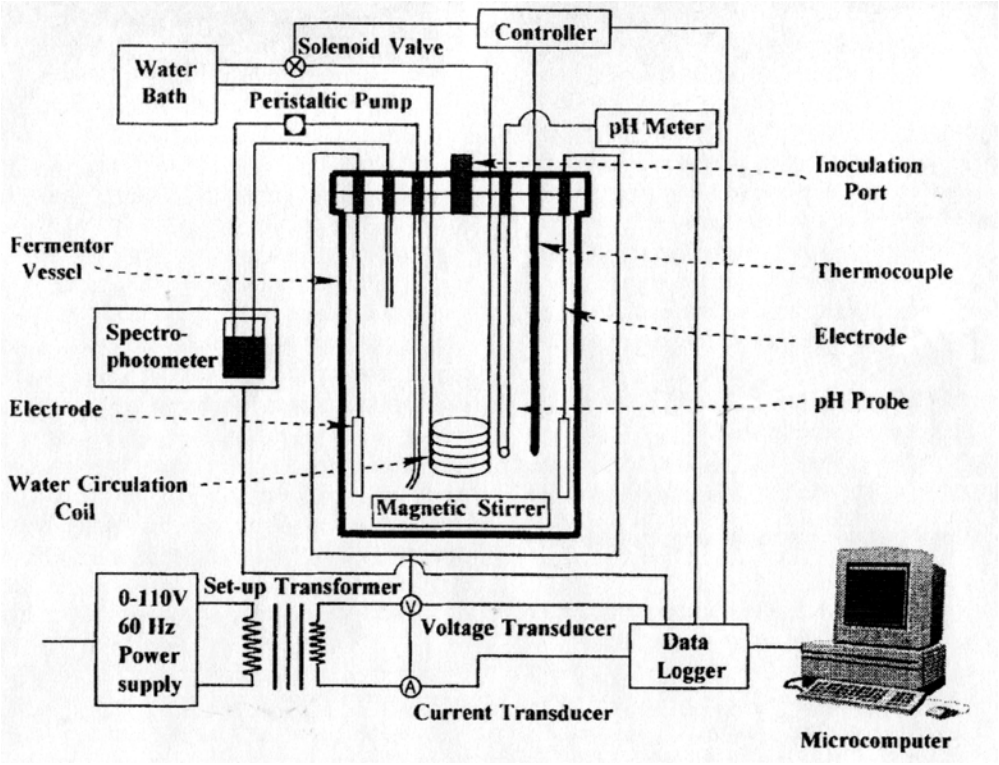

Figure 2.1 Ohmic Heating Fermentation Vessel diagram (Cho et al., 1996)

Fermentation began in the vessel when the broth was heated either ohmically or conventionally. The ohmically heated vessel was supplied with an alternating current of 60 Hz, at a constant voltage of either 15V (low) or 40V (high). The aforementioned cooling water circulation coil maintained the temperature in this system, as well as the conventionally heated vessel. The three temperatures studied for fermentation were 30, 35, and 40°C. The vessel was then filled with 2 L of MRS broth and autoclaved at 121°C for 20 min. Prior to inoculation, the sterile MRS broth was agitated for 30 min, and then the solution of *L. acidophilus* was added at a

rate of 0.01% by volume. As fermentation progressed, all the data was analyzed by the attached microcomputer in 30-min intervals. The metabolic activity was measured by collecting samples in sterile culture tubes at 2 to 5 hr intervals, at a temperature of 35°C.

The experiment was repeated in triplicate for each temperature. The bacterial growth was measured from the OD_{600} of the cultures, by comparing the results to a special calibration curve. This curve was obtained by gathering cells of the *L. acidophilus* from a 250 mL sample of the fermentation broth through centrifugation. The subsequent obtained cells were added to 10 mL of distilled water. Several different dilutions of the 10 mL solution were made and then either measured using OD_{600} or plated onto MRS agar, which was incubated at 37°C for 48 hr and the colonies were visually counted. A linear relationship was obtained between OD_{600} and $\log_{10}$ cfu/mL, which was then used to estimate cell counts in ensuing experiments using a Gompertz model (Gibson et al., 1987, Zwietering et al., 1990, Zwietering et al., 1992).

> Where Y_8 is $\log_{10}$ cfu/mL, X is time in hours, and A, B, C, and M are the model parameters (calculated by the "Nonlin" module of the Systat statistical program, created by Systat Inc. of Illinois)…
>
> $$Y_8 = A + C \exp\{ -\exp[-B(X - M)]\}$$

The data from the growth parameters (lag period, minimum generation time, and maximum growth) and pH changes during the experiment were fitted to similar models and equations. All data results were analyzed statistically using the General Linear Model of the SAS statistical program of the SAS Institute Inc. located in Cary, NC (Cho et al., 1996).

Results from the study are as follows: The lag period of the *L. acidophilus* was significantly affected ($p<0.01$) by the ohmic heating treatment. At 30°C, the lag period for fermentation was 18-fold less under low voltage (15V) ohmic heating, compared to conventional heating. However, the lag period for the conventional heating treatments decreased as the fermentation temperature rose, whereas the ohmic heating treatment had an opposite effect. The minimum generation time was not affected by the ohmic heating treatment, but the overall generation time decreased as the temperature increased. Maximum growth decreased slightly using ohmic heating (*Table 2.1*). The statistical analysis of the final pH of both systems showed that the method of heating notably affected the decline of the pH during fermentation. Although

both the glucose and lactic acid concentrations were less using the ohmic heating methods, there was not a significant difference compared to conventional heating. The bacteriocin activity was measured at a lower activity for the ohmic treatment. The electrical current in the ohmic heating system decreased with the progress of the experiment, even though the voltage remained constant. The variability in the current can be explained by the high ionic content of the MRS broth, accuracy of the measuring device, and noise from the electric source (Cho et al., 1996). The data from the current changes mirrors that of the changes in *L. acidophilus* growth during the fermentation.

Table 2.1 Growth parameters for *L. acidophilus* during fermentations under ohmic and conventional heating.

Heating Method	Fermentation Temperature (°C)	Lag Period (h)	Minimum Generation Time (h)	Maximum Growth (log_{10}cfu/mL)
Conventional	30	6.09	1.03	9.80
Low Ohmic (15V)	30	0.34	1.26	9.69
High Ohmic (40V)	30	1.44	1.41	9.68
Conventional	35	1.54	0.56	9.81
Low Ohmic (15V)	35	1.56	0.47	9.70
High Ohmic (40V)	35	0.92	0.64	9.74
Conventional	40	1.24	0.37	9.89
Low Ohmic (15V)	40	1.85	0.33	9.82
High Ohmic (40V)	40	1.28	0.38	9.71

Although this study was exhaustive, there still remained the difficulty of concluding whether there exists an effect on the growth of the bacteria due to the presence of the electric field. Cho et al. (1996) hypothesized that the lag period in the early stages of the fermentation of *L. acidophilus* was appreciably affected by the ohmic heating treatment due to the fluctuating electric field displacing the polar antimicrobials (found in MRS broth) and the macromolecules (produced by the *L. acidophilus*) that are attached to the cell walls and membranes. This activity may improve the absorption of nutrients and minimize the inhibitory action of fresh MRS broth,

which in turn can shorten the lag period. The low bacteriocin activity created a higher final pH in the medium due to the variability of the different heating methods.

This particular study showed that treating foods fermented with *Lactobacillus acidophilus* with electric current might be potentially useful for the dairy industry. Ohmic heating can be used to shorten the lag period in the early stages of fermentation of *Lactobacillus acidophilus*, which would considerably shorten the processing time needed for products, thereby increasing efficiency and lowering cost. The study was important to the concept of ohmic heating by showing that a wide variety of uses exist for the technology in the food manufacturing industry.

In a similar industry study, **Effect of Moderate Electric Field on the Metabolic Activity and Growth Kinetics of *Lactobacillus acidophilus* (2006)**, L. Loghavi, S.K. Sastry, and A.E. Yousef studied the effect of applying moderate electric fields (MEF) to an inoculated solution containing *L. acidophilus* to determine if the permeability of the cell membranes would be affected during fermentation. The MEF application was also studied to record its influence on bacteriocin production. This antimicrobial protein unique to *L. acidophilus* is gaining interest in the food industry as a defense against foodborne pathogens. It was shown that the bacteriocin activity increased during fermentation without a significant change to the final biomass. However, the lag time, maximum growth rate, biomass production and pH were not significantly affected by the application of MEF (Loghavi et al., 2006).

Applications of MEF have been shown to permeabilize and increase dispersion across cell membranes in eukaryotes (Halden et al., 1990; Imai et al., 1995; Jemai and Vorobiev, 2002; Kulshrestha and Sastry, 2003; Schreier et al., 1993; Sensoy and Sastry, 2004) and possibly prokaryotes (Cho et al., 1996). MEF has also been shown to accelerate growth in early stages of fermentation of *L. acidophilus* and inhibit at later stages (Cho et al., 1996).

For the experiment, four different treatments were performed:

1) Conventional heating -no electric field was present.

2) A MEF with field strength of 1 V/cm -applied for 40 hr.

3) A MEF (Early) with field strength of 1 V/cm -applied for the first 5 hr and then consequently turned off for the remaining 35 hr.

4) A MEF (Discrete) with field strength of 1 V/cm -repeatedly turned off and on for 2-min intervals throughout the fermentation.

All treatments were performed at a constant temperature of 30°C for 40 hours, except for the comparison of the conventional heating method and the discrete MEF treatment, which was additionally performed at 37°C for 24 hours.

An experimental fermentation vessel (*Fig. 2.2*) was designed similarly to the design of the vessel created by Cho et al. (1996). The electrodes consisted of two platinized titanium plates, which had a 14 cm electrode gap and 73.5 cm^2 surface area. A thermocouple, two pH probe tubes and a fraction collector tube were also present in the system. The power supply was a transformer that delivered 0-110 V and 60 Hz. A heat exchange jacket was connected to a water bath to maintain the constant temperature in the vessel. A computerized controller was connected to the jacket to control the flow of the coolant from the water bath. The thermocouple used to monitor the temperature was Teflon coated.

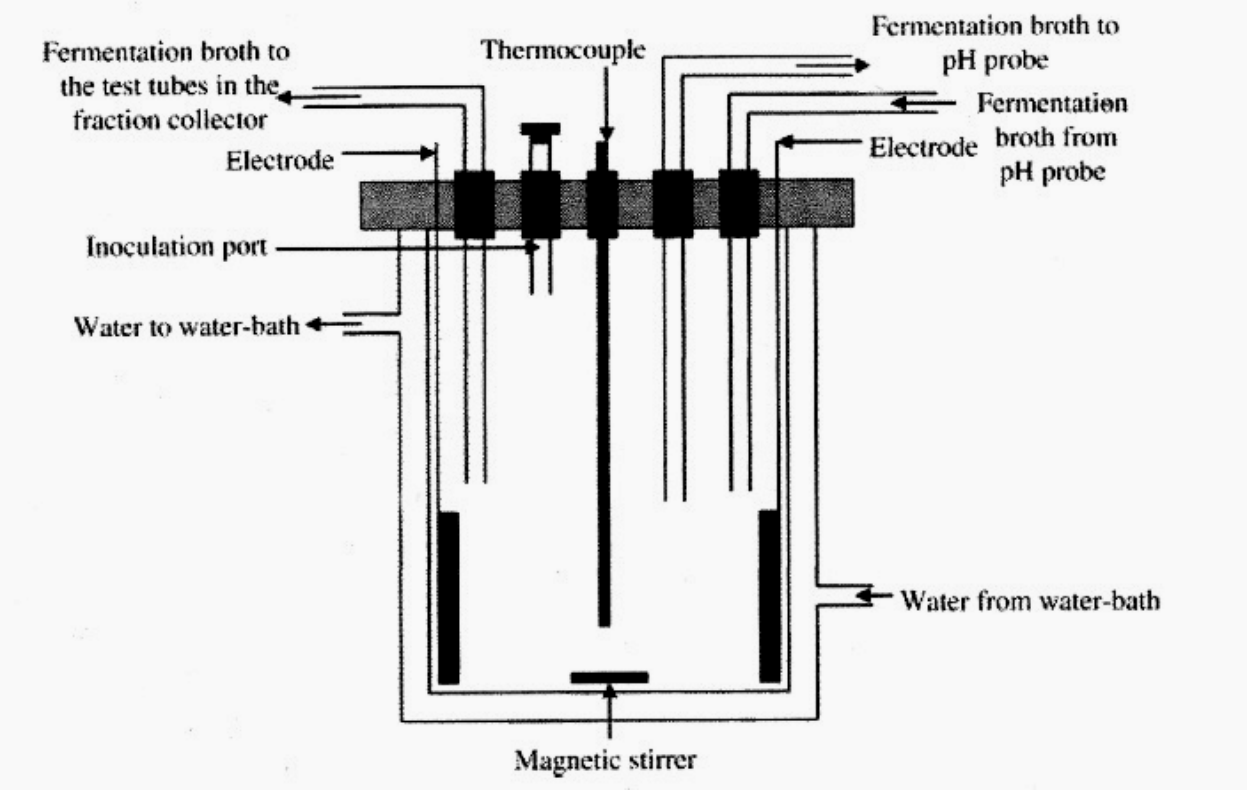

Figure 2.2 Fermentation Vessel diagram for MEF experiment (Loghavi et al., 2006)

The bacterial strains of *L. acidophilus* and *L. leichmannii* (used as an indicator strain for bacteriocin activity) were each inoculated into separate MRS broths and incubated at 37°C for 24

hr. The *L. acidophilus* inoculation was performed twice, both at a rate of 0.1%, divided in half by volume, and either incubated at 30°C for 16 hr or 37°C for 24 hr. The *L. acidophilus* solution was inoculated into 2.5-L of sterile MRS broth at a rate of 0.1%. The solution was continuously agitated during fermentation to ensure homogeneity using a magnetic stir bar, and a constant temperature was maintained using the cooling water circulation in the heat exchange jacket (Loghavi et al., 2006). To measure the pH, a sample of the broth was circulated through a flow-through pH probe, which was manufactured by Cole-Parmer in Illinois, and then reintroduced to the system. This design avoided interfering with the electric field. Samples were collected with a fraction collector every 30 min to measure bacteriocin activity. Data was collected every 5 min using a data logger.

Bacteriocin activity was calculated using a bioassay method (Yousef and Carlstrom 2003) that indirectly measured the bacteriocin amount released during fermentation by determining the antimicrobial activity of the fermentation medium against *L. leichmannii*, which is sensitive to bacteriocin (Loghavi et al., 2006).

Bacterial count estimation was obtained by measuring OD_{600} versus a cfu/mL curve in the procedure described by Cho et al. (1996), but instead using a 0.085% saline solution as both a reference blank in the spectrophotometer and the dilution liquid. The data was fitted to the Gompertz model that was also described by Cho et al. (1996) and Zwietering et al. (1990, 1992).

> Where $y(t) = \log_{10}$count at time t, A is the log number at $t = -\infty$, B is the maximum specific growth rate, M is the time at which the bacterial culture achieves its maximum growth rate, and C is the final natural log increase in the population…
>
> $$y(t) = A + C \exp\{ -\exp[-B(t - M)]\}$$

Results from the study are as follows: The temperature of the fermentation and the heating methods significantly affected the effect of MEF on bacteriocin activity (*Fig. 2.3*). Lower activity was observed under the conventional heating treatment at 37°C compared to the discrete MEF treatment at the same temperature. Maximum bacteriocin activity was observed with the early MEF treatment at 30°C. Bacteriocin activity increased with the application of MEF in combination with conventional heating at each specific temperature. However, the bacteriocin activity decreased when the MEF was applied continuously throughout the

fermentation at 30°C compared to the other treatments at the same temperature. The growth data for *Lactobacillus acidophilus* showed significant differences in the lag period using early MEF compared to other treatments at 30°C. The bacterial population changes, maximum specific growth rates, and pH changes were lower at 30°C than at 37°C. Minimum generation times were higher at 30°C. The fermentation temperature rather than the application of heating treatments seemingly affected these growth parameters more.

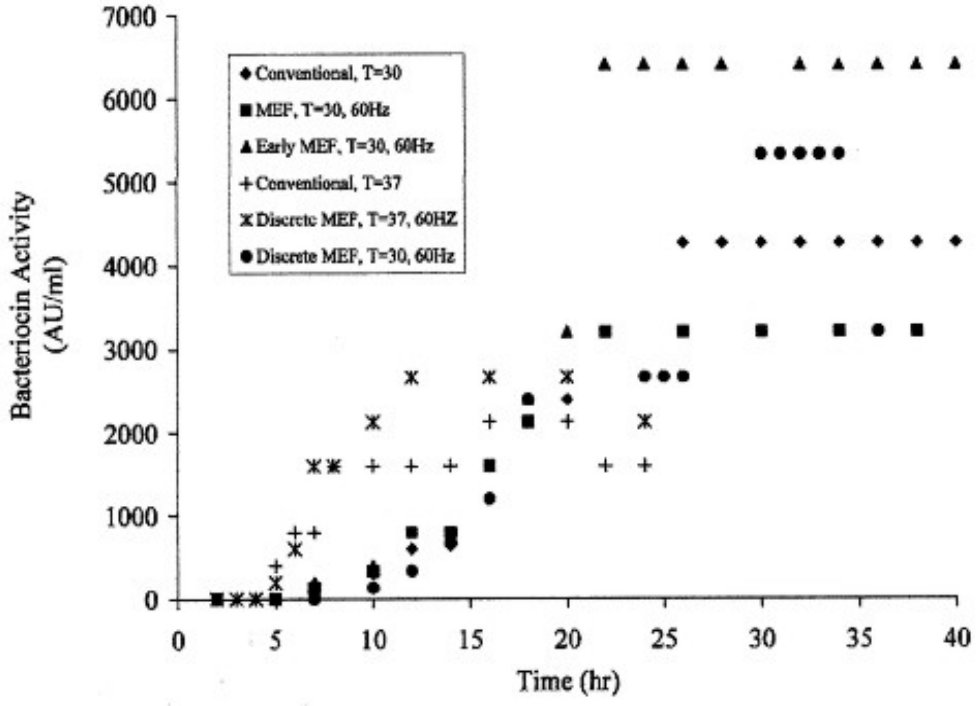

Figure 2.3 Bacteriocin production measured in arbitrary units (AU/ml) during fermentation (Loghavi et al., 2006)

The results of this study on MEF at 60 Hz, excluding the continuous treatment, resulted in increased bacteriocin activity. The early MEF treatment affected the bacteriocin activity the greatest, even though this activity is associated with the growth of bacteria. As stated before, the growth of the bacteria was not significantly affected by using different treatments. The differences in these results may be explained by the extra stress placed on the bacteria. This may induce an increase in production of defensive molecules such as bacteriocin. Another explanation is that the exposure of the cell membranes to electric fields may cause temporary

pores (electroporation) and an increase in transmembrane conductivity and diffusive permeability of nutrients, surfactants, autoinducers, and bacteriocin (Loghavi et al., 2006).

This particular study showed that applying MEF during fermentation of *Lactobacillus acidophilus* is useful for increased production of bacteriocin, which acts as food and feed preservative due to its antimicrobial effects. This knowledge would be invaluable as a natural way to preserve food and feed, increasing its shelf life and attractiveness to consumers who are interested in using organic products or products without the addition of chemical preservatives.

Sensory Aspects of Ohmically Heated Orange Juice

S. Leizerson and E. Shimoni studied the effects of ohmic heating on the stability of orange juice compared with conventional pasteurization in their study, **Stability and Sensory Shelf Life of Orange Juice Pasteurized by Continuous Ohmic Heating (2005)**. The attributes assigned to measure the quality aspects of orange juice were ascorbic acid (vitamin C) concentration, pectin esterase (PE) activity (cloudiness), color, and five representative flavor compounds: decanal, octanal, limonene, pinene, and myrcene. There was no significant difference between heating methods for the degradation of vitamin C concentrations or residual PE activity, although the particle size of the cloud was noticeably less in the ohmically heated sample. Both the ohmic heating and conventional heating treatments had the same effect on microbial load for 105 days, but the sensory shelf life as determined by the flavor compounds was twice as long for the ohmically heated orange juice as the conventionally heated juice at >100 days (Leizerson and Shimoni, 2005a).

Several industry studies (Nagy et al., 1977; Roig et al., 1999; Kaanane et al., 1988 and Kennedy et al., 1990) have been performed to examine the influence of different processing techniques on the quality and shelf life stability of orange juice. These studies include the kinetics of vitamin C degradation during storage as a marker for the end of shelf life. High pressure processing [500-900 MegaPascals (MPa)] has been utilized to preserve the cloud that consumers associate with fresh orange juice (Goodner et al., 1999).

For the preparation of the orange juice samples, the juice of fresh Shamuti oranges was extracted using a citrus juice extractor and then filtered through a sieve with 1 mm holes. The fresh orange juice was processed in a 50-kilowatt ohmic heating system designed by Raztek of

Sunnyvale, California. The design for this system includes two feeding tanks: one for a salt solution and the other for the untreated product, which continuously enters the system through a mono pump. The first part of the system is the rapid cooler, where the untreated product is first pumped. This cooler contains two sets of coiled tubes, the upper being for the untreated product and the lower for the heated product flow. The upper part of the tank is filled with steam, and the lower part is filled with water. The entire system is subjected to a vacuum to maximize the heat transfer. As the hot product passes through the tube, the water is quickly brought to boiling and the cold product passes through the tube above the boiling liquid, which heats the cold product through steam condensation. After this, the product enters the ohmic heating chamber. Inside the chamber, there are two pairs of adjacent graphite electrodes with a 20 cm gap between each pair. The product flows along the axis between the electrodes and is subjected to alternating current at 50 Hz and a maximum voltage of 8 kilovolts. A sophisticated controller determines the necessary current and voltage, using information from a thermocouple situated at the exit of the treatment chamber for the set treatment temperature. Following the ohmic heating treatment, the product enters a 120 cm holding tube where the thermal treatment takes place. The product then enters the rapid cooler and is then cooled to room temperature by a tubular heat exchanger. The samples of the orange juice were collected in sterilized jars and stored at 4°C (Leizerson and Shimoni, 2005a).

For determination of microbial counts, the orange juice was plated on orange serum agar (OSA) for total plate counts and oxytetracycline glucose yeast extract agar (OGYE) for yeast and mold counts. 1 mL samples of the orange juice (fresh and treated) were diluted 1:10 using 0.1% peptone water. From each of these dilutions, two samples were plated and incubated at 30°C for 48 hr.

Residual PE activity determination was based on the formation of galacturonic acid, as described by Rouse and Atkins (1955). A 3 mL sample of orange juice was added to 50 mL solution of 1.0% citrus pectin and 0.2M NaCl, and hydrolyzed at 30°C. The pH remained constant due to the addition of 0.1N NaOH with an auto-titrator. Using the measured NaOH usage over a 10 min period, PE activity percent and PE activity unit (PEU) were calculated using the equation below:

$$PEU = \frac{(mL\ of\ NaOH) \times (0.1N\ NaOH)}{(3\ mL\ sample) \times (10\ min)} \times 10^4$$

$$PE\ \% = \frac{PEU\ of\ thermally\ treated\ OJ}{PEU\ of\ fresh\ OJ} \times 100$$

Concentration of ascorbic acid in the orange juice samples was determined using a reverse phase high performance liquid chromatograph (RP-HPLC) that eluted the samples at a flow rate of 1 mL/min, with a filtered mobile phase of 10% methanol in water solution that had a pH of 2.9 due to the addition of citrate. 20 µL of an orange juice solution that had been centrifuged at 12500 x *g* for 10 min to remove pulp and large particles was injected manually into the system. The elution time was 3.4 min. A standard calibration curve of *L*-ascorbic acid in concentrations ranging from 10-80 mg/100 mL was used to determine vitamin C (Leizerson and Shimoni, 2005a).

Measurements of flavor compounds were performed by headspace solid-phase microextraction gas chromatography (SPME-GC). A SPME fiber coated with 65µm polydimethylsiloxane-divinylbenzene was used for the adsorption of the flavor compounds inside a vial containing orange juice, and then incubated at 60°C for 20 min. The SPME fiber was injected into the GC at 220°C and held for 2 min. The flavor compounds were separated using another GC with a capillary column and a flame ionization detector. The GC chromatograph peaks were analyzed using the ChemStation software package developed by Hewlett-Packard. Comparing the retention times with five standard compounds performed the identification of α-pinene, myrcene, limonene, octanal, and decanal (Leizerson and Shimoni 2005a).

Browning of the orange juice was determined by centrifuging the orange juice at 12500 x *g* for 10 min and then filtering through a 0.45 µm Millipore filter. The samples were then analyzed at room temperature in a spectrophotometer at 420 nm.

The orange juice samples were also subjected to a particle size analyzer from the Beckman Coulter Company of Miami, Florida. This analyzer was "equipped with a polarization intensity differential scattering system. This method is based on laser diffraction analysis. When a parallel beam of a laser passes through the suspension, the diffracted light is focused on a

detector, which senses the distribution of scattered light intensity." (Leizerson and Shimoni, 2005a)

The Weibull-Hazard Shelf Life determination method directly measures the probability of unacceptable sensory evaluation of a product, and is consequently used to determine the end of shelf life. Shelf life can be defined as the time when 50% of untrained tasters find a product unacceptable (Labuza and Schmidl, 1998). Testing for this experiment was carried out using five panelists throughout the study at an interval of 7 days. After 50% of the panelists found the orange juice to be unpalatable, the testing intervals increased to 4 days.

Results are as follows: Vitamin C dissipation curves followed a linear decrease (r^2 = 0.923) in both ohmically and conventionally heated samples at 4°C. There appeared to be no significant difference in the final concentration of ascorbic acid between the two methods. The ohmically heated samples showed a significant increase in browning compared to conventionally heated samples (Leizerson and Shimoni, 2005a). However, this browning effect never became noticeable to the human eye. Color deterioration affects the consumer's perception of the quality of orange juice. Browning occurs in orange juice during storage and can be accelerated due to abusive storage conditions, presence of oxygen or metal ions, or degradation of ascorbic acid. Ascorbic acid provides reactive carbonyl groups that can be precursors to non-enzymatic browning.

PE activity was neither slowed nor increased with ohmic heating compared to conventional heating, as both treatments showed a 90-98% decrease in activity compared to fresh orange juice. Ordinarily, particles in orange juice contribute to its sensory characteristics for consumers. The visual turbidity stems from a suspension of pectin particles. PE causes cloud instability by deesterification of pectin; therefore a thermal process is applied to inactivate the enzyme.

For the five flavor compounds, higher concentrations of all were measured in the ohmically treated samples compared to conventionally treated samples (*Fig. 2.4*). These results indicate better flavor retention, which most likely originates from lower residence times in the ohmic heating system. These flavor compounds in orange juice give it its unique aroma. Usually, these compounds are very susceptible to high temperatures and the presence of microorganisms.

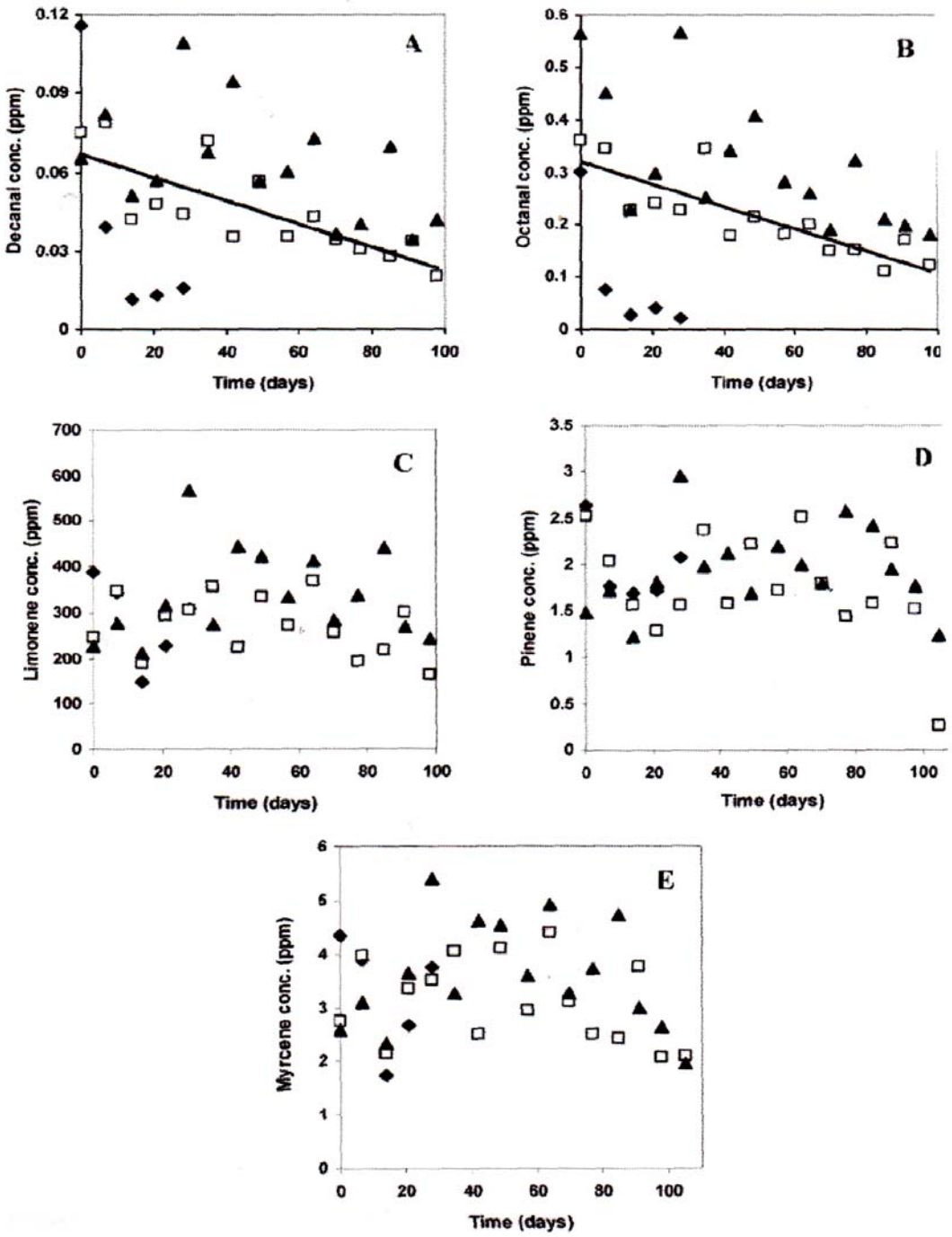

Figure 2.4 Flavor Compounds Concentration in Fresh and Thermally Treated OJ (4°C) (Leizerson and Shimoni, 2005a)

Where (A) decanal; (B) octanal; (C) limonene; (D) pinene; (E) myrcene and (♦) fresh OJ; (▲) ohmically heated OJ; (□) conventionally pasteurized OJ.

The shelf life of pasteurized orange juice may expire due to a number of reasons, including microbial load, vitamin C content, and sensory characteristics. The upper level of acceptable microbial load was set as 10^3 cfu/mL. After 105 days, both ohmically heated and conventionally heated samples reached this limit. For vitamin C content, the U.S. Recommended Daily Allowance (U.S. RDA) set forth by the United States Department of Agriculture (USDA) is at least 25 mg per 100 mL at the time of expiration date for 100% vitamin C supply. Both heat treatments reached this level in 79 days. As the microbial load reached the set limit at 105 days, the sensory study was terminated for safety considerations. The sensory shelf life of the ohmically heated samples was >105 days, compared to the conventionally heated juice, which had a sensory shelf life of 50 days.

The results of the study indicated that a thermal treatment such as ohmic heating could be used to extend the sensorial shelf life of pasteurized fresh squeezed orange juice. Ohmic heating can be performed without overheating, due the heat transfer aspects of electrical current through the juice, which results in a high retention of the sensorial attributes of the fresh juice. Unfortunately, this report did not specify at what temperatures the orange juice was subjected to for either ohmic or conventional treatments, which makes replication of the experiment difficult.

In a similar experiment, **Effect of Ultrahigh-Temperature Continuous Ohmic Heating Treatment on Fresh Orange Juice (2005)**, S. Leizerson and E. Shimoni studied the effects of ultrahigh-temperature ohmic heating on the quality of orange juice as compared with conventionally pasteurized orange juice. The orange juice was treated at temperatures of 90°C for 1.13 s, 120°C for 0.85 s, and 150°C for 0.68 s in an ohmic heating system or 90°C for 50 s in a conventional pasteurizer. The ohmic heating treatment resulted in a higher quality product with minimal sensory deterioration. This study also focused on the inactivation of microbes, heat-sensitive compounds and physical characteristics. The orange juice was inoculated with *Bacillus subtilis* spores to examine the inactivation effect of the ohmic system.

For preparation of samples, the orange juice was extracted and thermally treated as described in the previous Leizerson and Shimoni study, **Stability and Sensory Shelf Life of Orange Juice Pasteurized by Continuous Ohmic Heating (2005)**. A matrix of three set point temperatures and three flow rates was tested. Orange juice was treated by the ohmic-heating system at set point temperatures of 90, 120, and 150°C and in flow rates of 3, 4, and 5 L/min.

Conventional pasteurization of orange juice was conducted at 90°C for 50 s using a plate heat exchanger with a 1-L holding tube. Residual PE activity, microbial counts, identification of the concentration of ascorbic acid, flavor compound analysis, and browning measurement were also performed as previously described (Leizerson and Shimoni, 2005a). Additional tests included testing of °Brix on a refractometer, and color determination using a chroma meter.

Sensory tests were given to a series of panelists to evaluate sensory attributes of ohmically heated orange juice. A triangle test was given to compare fresh, pasteurized, and ohmically heated orange juices, where each panelist received three coded samples, of which two were identical and one was different. Each panelist was then asked to distinguish the different sample. Each set of three samples was replicated 25 times.

Citrus juices are distinguished by their highly acidic conditions, which lead to the growth of yeast and mold, and some types of bacteria tolerant of low pH. Both ohmic and conventional heat treatments reduced initial microbial loads by at least a 2-3 log reduction compared to fresh orange juice (initial count of approximately $10^{2.5}$ cfu/mL). There was no significant difference between the two, since both methods rely on thermal treatment for inactivation (Leizerson and Shimoni, 2005b).

For ohmic heating treatment, PE activity was reduced to 2-10% compared to 5% with conventional heating. These results do not show a significant difference between the two thermal treatments on inactivation of PE.

Vitamin C degradation of fresh orange juice is due to nonenzymatic reactions, which are accelerated by high temperatures during thermal processing. In ohmic heating, the Vitamin C degradation compared to fresh orange juice was 7-25%, and 16% for conventional heating. Again, no significant difference could be seen in the two types of heat treatments (Leizerson and Shimoni, 2005b).

Nonenzymatic browning may produce off-flavors, a decrease in nutrient content, loss of color, and the appearance of brown pigments. Vitamin C degradation is thought to be the major source of this browning, which is initiated by thermal processing. The initial browning index for ohmic heating was 0.25 to 0.43 depending on the temperature. The index for conventional heating was 0.27 at the same temperatures.

Flavor compounds are 0.02% of the total weight in a sample of orange juice. 75-98% of the compounds are hydrocarbons, 0.6-1.7% aldehydes, 1% esters, 1% ketones, and 1-5%

alcohols (Leizerson and Shimoni, 2005b). The flavor of the juice can be damaged through thermal heating due to the chemical reactions that are accelerated by the heating process. In the ohmic heating treatment, higher concentrations of all flavor compounds were retained after heating. This may be explained by the short residence time in the ohmic system, which does not as quickly degrade them as in conventional heating, and also because the thermal treatment releases bonded components from the medium.

The results of the sensory tests showed that panelists could distinguish between fresh and conventionally pasteurized samples and between conventionally and ohmically heated samples. However, panelists could not distinguish between fresh and ohmically heated samples, since they exhibited similar levels of flavor compounds.

The results of this study suggest that continuous ohmic heating can be used to effectively pasteurize orange juice, and yet retain the flavor and sensorial appeal of freshly squeezed juice. The ohmic heating technique is just as efficient in the required microbial and enzymatic inactivation as conventional pasteurization, but retains the flavor compounds associated with fresh orange juice.

Increased Juice Yield from Plant Tissue Using Ohmic Heating

I. Praporscic, N.I. Lebovka, S. Ghnimi, and E. Vorobiev studied the effects of ohmic heating on juice yield from potato and apple tissues in the study, **Ohmically Heated, Enhanced Expression of Juice from Apple and Potato Tissues (2006)**. Two different experiments were carried out involving different treatment chambers: textural and conductivity study of cylindrical samples and juice yield tests of tissue slices. The results showed that degree of tissue disintegration and yield was dependent on the field intensity, temperature, treatment duration, and type of plant tissue. The best juice extraction was obtained when the plant tissue was treated electrically at a moderate temperature of 50°C. The combined effect of possible electroporation and thermal softening of the tissues may explain the increased yield.

Pressure extraction technologies are widely used in the food industry for production of juices, wines, sugar and vegetable oils. Different treatments have been proposed for the improvement of juice extraction efficiency and yield. A pulsed electric field (PEF) treatment at moderate field strength of 0.5-1 kV/cm and treatment time of 10^{-4} to 10^{-2} s can create a higher

efficiency of plant tissue pressing (Praporscic et al., 2006). Electroporation appears to be the reason for the PEF-induced damage of plant tissues (Weaver and Chizmadzhev, 1996). Higher field strengths have shown to be promising as a method of microbial inactivation. The aim of this study was to examine how temperature and moderate electric field treatment under 100 V/cm for 1-100 s affect the juice yield from plant tissues.

For the experiment, Granny Smith apples and Milva potatoes were stored at refrigeration temperatures, and the moisture content measured. Apples ranged between 85-88% and 83-85% for the potatoes. Electric fields were supplied using either an alternating current (AC) generator or a PEF generator. The AC generator can supply 5-250 V at 50 Hz. "The PEF generator provides monopolar pulses of rectangular shape and allows for variation of the pulse duration within the range of 10-1,000 μs, pulse repetition of 1-100 ms, and the number of pulses within the range of 1-100,000" (Praporscic et al., 2006). The temperature inside the system was measured using a Teflon coated thermocouple and the electrical conductivity of the samples was recorded using an LCR meter, manufactured by Hewlett-Packard, at a frequency of 1,000 Hz.

Two different types of tests were carried out in different treatment chambers: textural and conductivity tests with cylindrical samples and juice yield tests with tissue slices (*Fig. 2.5*). The experimental design (*Fig. 2.6*) shows that the treatment chambers were both connected to the AC and PEF generators.

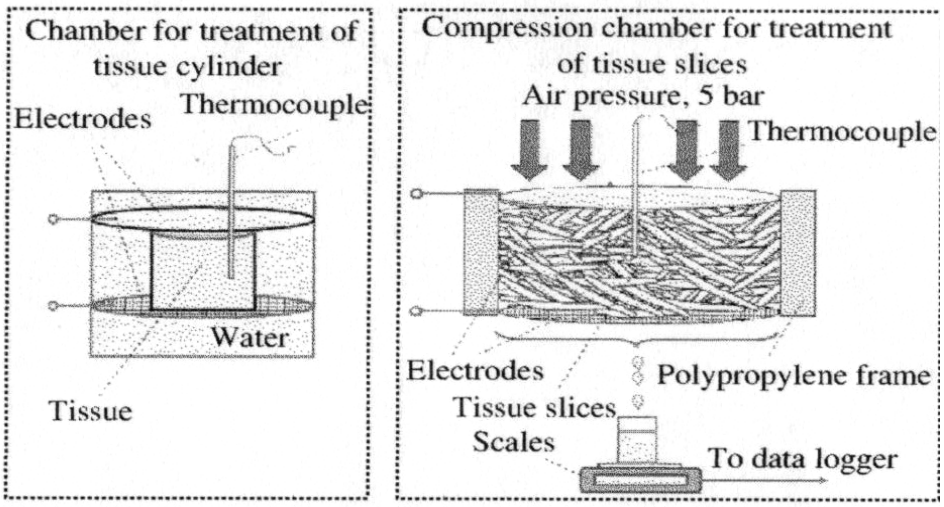

Figure 2.5 Textural and Conductivity Test Chambers (Praporscic et al., 2006)

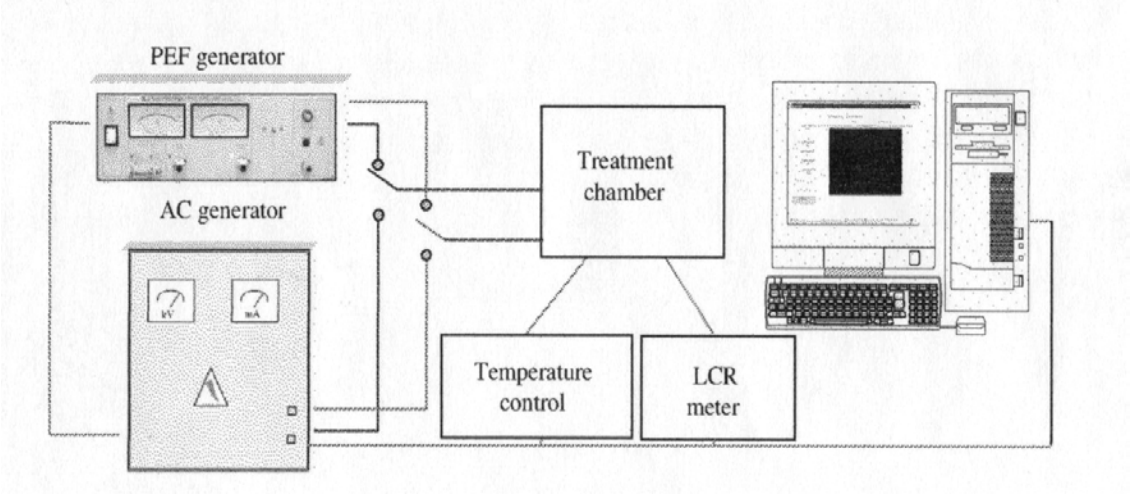

Figure 2.6 PEF and AC schematics for experiment (Praporscic et al., 2006)

For the testing, the apples and potatoes were cut into cylindrical samples 26 mm in diameter and 10 mm in height. The AC generator treated these samples and the temperature was monitored throughout using the thermocouple. A texture analyzer from Stable Microsystems of England measured the textural properties of the samples after they had cooled to room temperature. Then, 10 mm by 10 mm cylindrical samples were cut from the treated samples and subjected to a stress relaxation test by the same texture analyzer. The highest force recorded to compress the samples was 20 Newtons (N), with a speed of piston displacement at 1 mm s^{-1}. The degree of damage to the tissue was calculated using the electrical conductivity disintegration index Z (Lebovka et al., 2002):

> Where σ is the measured electrical conductivity in Siemens (S)/m; σ_i is the electrical conductivity of intact tissue in S/m; and σ_d is the electrical conductivity of totally destroyed tissue in S/m…

$$Z = \frac{\sigma - \sigma_i}{\sigma_d - \sigma_i}$$

The experiments for juice yield were performed with tissue slices using a compression chamber connected to the AC or PEF generators. The treatment chamber was designed with a cylindrical polypropylene frame with an inside compartment that was 25 mm thick by 56 mm in diameter. The compartment was filled with tissue slices (1.5 mm wide by 1 mm tall by 30-40 mm long for potatoes and 6 mm wide by 1.5 mm tall by 30-40 mm long for apples) and then

tightly sealed with steel plates. One of the plates, covered with a filter cloth, acted as a stationary electrode. The other plate was attached to an elastic rubber diaphragm. Installed between the diaphragm and the tissue slices was a wire gauze electrode. These samples were treated electrically before being subjected to the pressing at 5 bar for 3600 s after they had cooled to room temperature. The mass of the expressed juice was measuring using a scale situated beneath the cylinder. The mass of the expressed juice was subtracted from the initial mass of 40 g (for all samples) and multiplied by 100 for a percentage of juice yield.

The results are as follows: The electrical treatment was more damaging to the potato tissue than the apple tissue, as well as the rise of the sample temperature was more pronounced for the potato tissue. This can be attributed to the different cell structure and electrophysical properties of potato and apple tissues. The high content of trapped air in apples results in a lower electrical conductivity when compared with potato tissue (Praporscic et al., 2006). Apple tissue is spongy, while potato tissue is texturally stronger due to a high starch content which preserves its cell structure.

The conclusion drawn from this study is that treating of plant tissue with electric fields less than 100 V/cm provides a dramatic increase in membrane destruction and mechanical softening. Previous studies (Lebovka et al., 2004a, 2004b) showed that thermal damage is not significant for treatment times of 20 s and temperatures less than 60°C, so the changes in the mechanical properties of the tissues can be explained by electroporation. In the range of 50-60 V/cm and treatment times of 20 s, the threshold of electroporation is exceeded, and the majority of the cells are destroyed, releasing the juice. The final temperature of the tissues can greatly affect the juice yield also (*Fig. 2.7*). For this phase of the experiment, different end point temperatures and electric fields strengths were used in conjunction to determine the greatest yield possible.

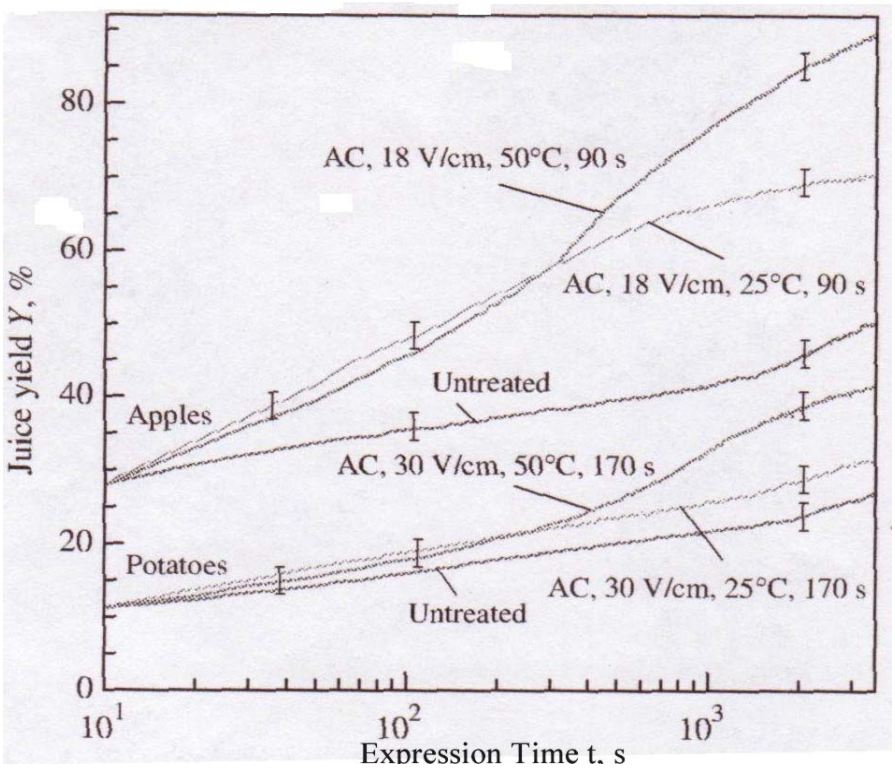

Figure 2.7 Juice Yield Percent using Varying Electric Fields and End Point Temperatures (Praporscic et al., 2006)

Based on this study, the tissue disintegration depends on several treatment parameters including: field intensity, temperature and time duration, and type of plant tissue. The best efficiency of juice extraction from pressing was obtained when either tissue was treated electrically at 50°C. This information could be invaluable for the aforementioned industries using pressing and extraction technologies for the production of various juices and wines. By treating these products with electrical current before processing, the juice yield would be increased, also increasing efficiency and profit.

In another experiment, **Does Electroporation Occur During the Ohmic Heating of Food? (2005)**, N.I. Lebovka, I. Praporscic, S. Ghnimi, and E. Vorobiev studied the effect of temperature on electroporation of plant tissues during pulsed electric field (PEF) or alternating current (AC) treatment. "The characteristic damage time, which determines effectiveness of the

electroporation mechanism in plant tissue damage, was estimated for potato tissue in the range of electric field strengths of 40 to 500 V/cm and temperatures of 22°C and 49°C in PEF treatments" (Lebovka et al., 2005). Moderate electric field (MEF) treatments allow extraction and expressing processes to be enhanced considerably in different food substances.

Effective plant tissue disintegration under a PEF treatment can be achieved at room temperature when the field strength is within 500 to 1000 V/cm and treatment time ranges from 10^{-4} to 10^{-2} seconds. The objective of this experiment was to study the relationship between MEF induced damage of plant tissues, temperature, electric field intensity, and time of treatment (Lebovka et al., 2005).

For the experiment, Granny Smith apples and Milva potatoes were obtained and analyzed as described in the study done by Praporscic et al. (2006). The tissue samples were cylindrical in shape, with a diameter of 26 mm and height of 10 mm for PEF treatment and 10 mm in diameter and 10 mm in height for textural analysis.

The treatment chamber was designed around a polypropylene tube with an inner diameter of 26 mm and an electrode at the bottom. The sample was placed into the chamber and then covered by another electrode. Temperature was monitored using a Teflon coated thermocouple. Electric field treatment was applied as previously described in the Praporscic et al. (2006) experiment, using the same PEF and AC generators. The PEF generator provided up to 1500 V, number of pulses between 1 and 30,000, and duration of 10^{-5} to 10^{-3} s at intervals of 10^{-2} s. To avoid ohmic heating effects, a longer inter-train pause time was used between PEF pulses compared to the other PEF study done by Praporscic et al. (2006). In AC treatment experiments, tissues were ohmically heated started from room temperature of 22°C to temperatures not exceeding 50°C, with a voltage of 10-100 V at 50 Hz. The electrical conductivity was measured using an LCR meter at a frequency of 1000 Hz and the stress relaxation measurements were performed in a textural analyzer, with a maximum force of 20 N.

In a typical PEF treatment at electric fields less than 100 V/cm and treatment times of 10^{-4} to 10^{-2} seconds, the cell damage from electroporation increased and the electrical conductivity of the plant tissue decreased. The mild treatment conditions of the study exclude nearly any thermal damage and softening of tissue. These effects are mainly related to the PEF induced damage (Lebovka et al., 2005).

The thermal damage and softening of the tissue are not essential at mild thermal conditions (Lebovka et al., 2004a, 2004b). The fact that the electrical conductivity of the tissues decreased considerably at electric field strengths above 20 V/cm showed the significance of electrical current on damage to plant tissue. The force relaxation curves (*Fig. 2.8*) of the tissues evidently show softening as a result of ohmic treatment. The underlying changes to the observed structure of the tissue can be explained by the electroporation mechanism. If fields are less than 40 V/cm, "the disintegration index of apples exceeds that of potatoes and the textural changes in apples are more constitutive than in potatoes" (Lebovka et al., 2005). This can be explained by the variation in tissue structure, different size of cells, and dissimilar content of air cavities.

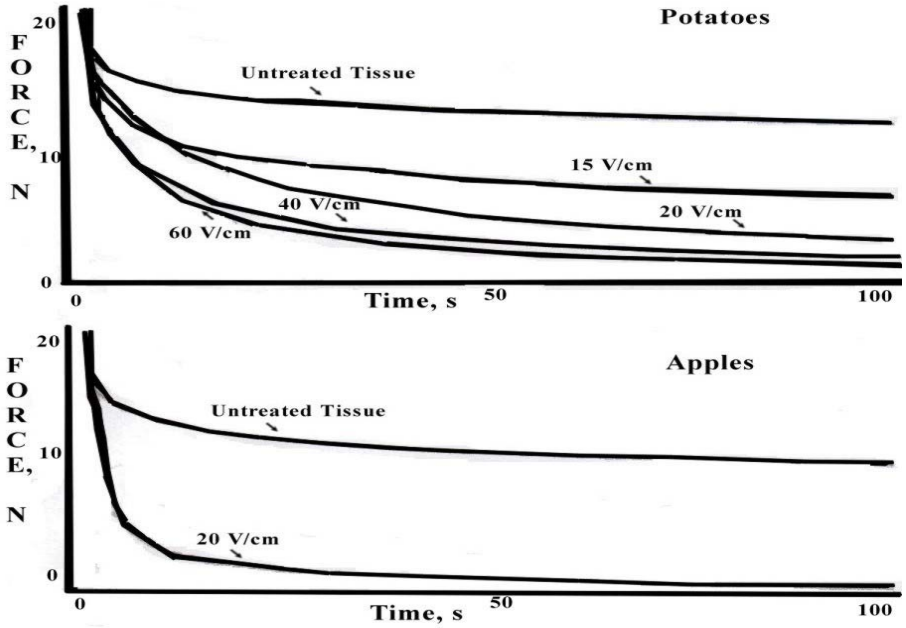

Figure 2.8 Textural relaxation curves for potato and apple tissues ohmically heated to 50°C (Lebovka et al. 2005)

This study shows a noticeable effect of temperature on the degree of damage using PEF or AC treatment of a plant tissue. At temperatures less than 50°C and a MEF under 100 V/cm, an ohmic heating treatment results in a marked amount of damage to tissues. The observed effects of ohmic heating show the important role that electroporation plays in plant tissue damage. This knowledge can be useful in increasing the juice yield of fruit and vegetable products that are generally processed by compression. Electrically pretreating those foods will yield higher profits due to the increased amount of juice from the same product.

CHAPTER 3 - Self-Conducted Research

Effect of Ohmic Heating on *Lactobacillus acidophilus* inactivation

In recent years, the food industry has showed interest in alternative processing technologies for different types of products. One of these technologies, ohmic heating, relies on utilizing electrical energy to thermally process foods. This provides a food processor more opportunities to produce high quality, shelf-stable products for an emerging market.

The ohmic heating of *Lactobacillus acidophilus* for the purpose of microbial inactivation was compared with the technology of conventional heating microbial inactivation. This study was meant to observe if the ohmic heating technique would be considered effective enough for commercial use. As no pathogenic or spoilage bacteria with a unique resistance to ohmic heating have been found, a probiotic bacteria such as *Lactobacillus acidophilus* would be an appropriate substitute for process determination and validation of microbial inactivation.

Organism

Lactobacillus acidophilus (*Fig. 3.1*) is an organism normally found in the gastrointestinal tract of humans and animals. It is identified as a probiotic bacterium due to its non-pathogenic and beneficial nature to humans. It is a homofermentative organism, producing lactic acid as its sole product of fermentative metabolism. It grows with or without the presence of oxygen and can survive in highly acidic environments of pH 4-5 or lower (EBI 2006), allowing for survival in the harsh environment that is present in the gastrointestinal tract. *L. acidophilus* is most important in the fermentation of foods, particularly in dairy foods such as yogurt and cheese. For this experiment, *L. acidophilus* was chosen for its ease of use and low potential for contamination and harm to humans.

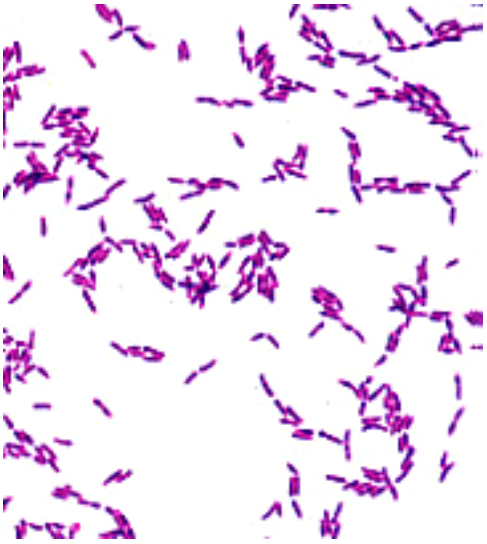

Figure 3.1 ***Lactobacillus Acidophilus***

Experimental Treatments

The following treatments were investigated:

a. Conventional Heating Treatment: Experimental design vessel situated on top of hotplate stirrer (Fisher Scientific, Isotemp Ceramic [0-540°C]), continuously agitated at 300°C until 90°C was reached (~7 min).
b. Ohmic Heating Treatment: Experimental design vessel situated on top of magnetic stirrer (Fisher Scientific, Isotemp Analog [60-1200 rpm]), continuously agitated while being subjected to 18V of direct current by a generator (Tenma Laboratory DC Power Supply model 72-2005) until 90°C was reached (~9 min).

Experimental Design

The design for the experiment (*Fig. 3.2*) was closely modeled after the fermentation vessel schematic of Cho et al. (1996). A 250 mL Pyrex® glass beaker was topped with a stainless steel lid equipped with a small opening for the addition of the inoculated solution and sample taking. A glass beaker was most suitable due to the fact that glass is an insulator and would not interfere with the electric currents. A Teflon-coated magnetic stir bar was placed at the bottom of the beaker. The lid for the ohmic treatment was also equipped with two small holes on either side for insertion of two electrodes, made by soldering 20 cm^2 copper plates onto the ends of each wire, placed 5 cm apart inside the beaker. The entire assembly was autoclaved

at 121°C for 10 min. The electrodes were supplied with 18V of DC from the generator, measured using a digital multimeter (PHC Enterprise, Inc., Torrance, CA model DT-830D). A magnetic stirrer was situated under the beaker to aid in the equalization of the temperature. The conventional heating assembly and preparation mirrored that of the ohmic heating assembly, but without the addition of the electrodes and the addition of a hotplate stirrer underneath as the mode of conventional heating. The temperature was monitored using an infrared temperature gun (Raytek® Raynger ST). Infrared thermometers measure temperature using blackbody radiation (thermal) emitted from objects. The infrared thermometer would not significantly interfere with the electrical field in the system due to its ability to measure temperature without contacting the system.

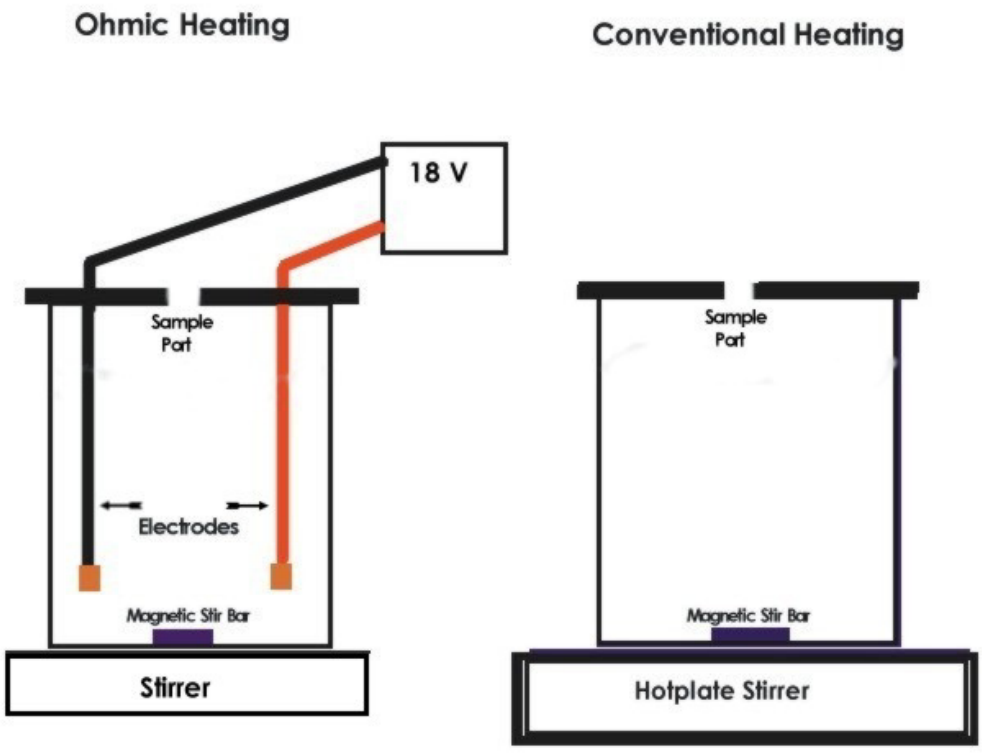

Figure 3.2 Experimental Design for Ohmic and Conventional Systems

Bacteria Preparation

L. acidophilus was obtained from Fisher Scientific as a culture suspended in agar. Upon receipt, the culture was stored at 0°C for the duration of the experiment. The surface of the

culture was scraped with a sterilized inoculating loop, and the cells were then inoculated into de Man Rogosa Sharp (MRS) broth (for optimum growth of *Lactobacillus* strains). This solution was incubated at 37°C for 24 hr in a sterile container. The subsequent inoculated solution was diluted into Butterfield 99 sterile buffer (Weber Scientific) at a rate of 1:100.

Experimental Procedure

For determination of sample taking times, the beakers were first filled with 100 mL of Butterfield 99 sterile buffer and heated either ohmically or conventionally. In order to obtain uniformity in sample collection, 6 temperature points approximating 37°C, 48°C, 59°C, 70°C, 79°C and 90°C were chosen for a broader range of testing. Since the ohmically heated solution reached 90°C more rapidly than the conventionally heated treatment, the time during the run of the experiment at which the samples were collected was not equivalent for both systems. After sample-taking times had been calculated for each system, both beakers were filled with 100 mL of the inoculated solution. Both assemblies were continuously agitated at 300 rpm with the magnetic stir bar to equalize temperature. Observation at this point showed that the ohmically heated sample had variances in temperature of only 0.1-0.5°C throughout the system. The conventionally heated sample had variances in temperature of 1.2-2.0°C throughout the system.

At the pre-determined time intervals, 1mL samples were taken from the approximate middle of each system, placed into glass test tubes, promptly capped and stored at refrigeration temperature (4°C) for the duration of the experiment. The test tube assemblies and pipette tips were autoclaved at 121°C for 10 min prior to the experiment. Both treatments were performed in a laboratory hood with positive airflow. The voltage and current were monitored at 2-min intervals throughout the experiment using the digital multimeter (*Table 3.1*). The entire experiment was performed in triplicate and all results are reported as the averages of all trials. Due to the relatively short time of the experiment, the voltage and current (expressed in milliamps [mA]) remained almost constant throughout.

Table 3.1 Voltage and Current Changes Throughout Ohmic Treatment

Time (min)	Voltage (V)	Current (mA)	Temperature (°C)
0	18.04	72	25
2	18.01	72	51
4	18.08	73	77
6	18.12	74	90

As previously mentioned, the ohmically heated assembly reached the end point temperature of 90°C more quickly than the conventionally heated assembly. The conventional heating rate showed a linear increase ($r^2 = 0.995$), while the ohmic heating rate showed a larger jump in temperature halfway through, most likely due to the increase in electrical conductivity at higher temperatures (Halden et al. 1990).

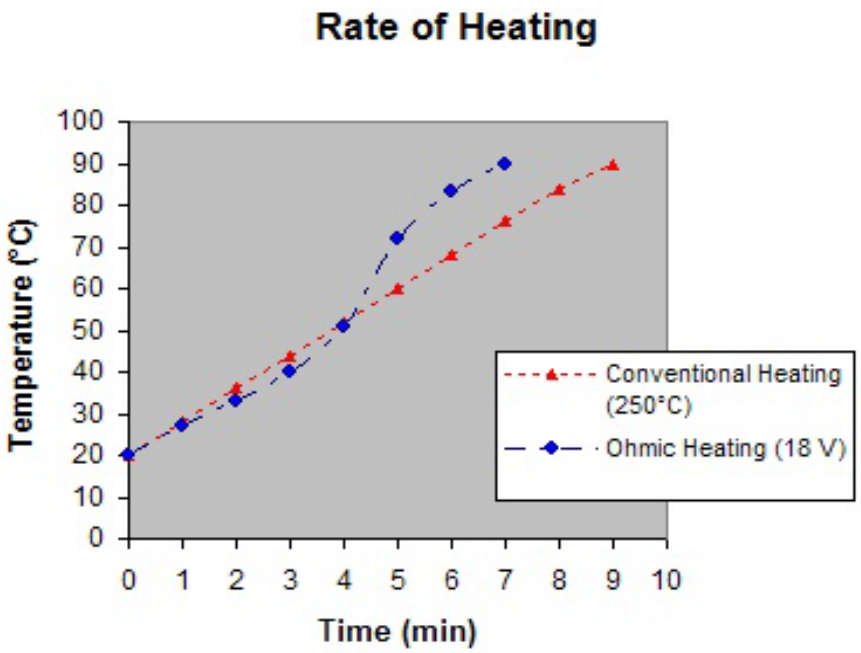

Figure 3.3 Rate of Heating Between Ohmic and Conventional Methods

Cell Count Enumeration

All samples from both treatments were plated on MRS agar plates (for ideal growth of *Lactobacillus* strains) and incubated at 35°C for 72 hr. Controls were plated for the laboratory air, pipette tips, MRS agar, sterile buffer and original inoculated solution. The plates were enumerated visually and reported as log cfu/mL.

Effect of HeatingTreatments on L. acidophilus inactivation

The ohmic heating treatment increased the temperature at a faster rate, and amplified the electrical conductivity of the solution, which most likely increased the total lethality as compared with conventional heating (*Fig. 3.4*) (*Table 3.2*). The temperature distribution within the system was also relatively uniform, another factor that most likely increased the ohmic heating treatment's effectiveness. No determination could be made in this experiment if the electric field created in the system had any effect on the lethality, although the presence of the field can only augment the microbial kill, and not inhibit it (Halden et al. 1990). Conventional heating had a noticeable effect on lethality, although not as effective as ohmic heating, most likely due to the slower rate of heating and the less uniform temperature distribution.

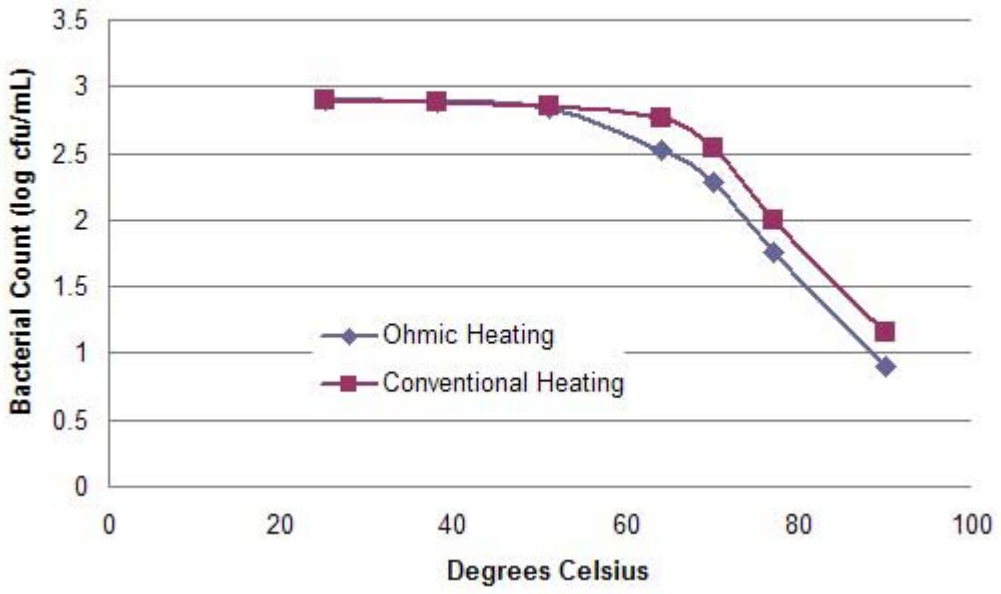

Figure 3.4 Effect of Heating Treatments on *L. acidophilus* inactivation

Table 3.2 Log cfu/mL of Heating Treatments

Ohmic Heating	Conventional Heating
2.90	2.90
2.88	2.88
2.84	2.85
2.53	2.77
2.29	2.54
1.76	2.00
0.90	1.15

Discussion

Ohmic heating is a rapid, uniform treatment to increase the temperature of a medium. This rapid heating has been shown to decrease the amount of thermal abuse on food products, creating a more desirable product. Increasing or decreasing the applied voltage, with almost instantaneous results, easily controls the method of heating. The temperature distribution within the medium is affected by density and the specific heat (measure of the heat energy required to increase the temperature of a unit quantity of a substance by a certain temperature interval) of the product (FDA-CFSAN 2000). Therefore, a homogenous mixture with a known specific heat will invariably have uniform temperature distribution throughout the system. Conventional heating is still the standard for food processing, but is less easily controlled and can have wide variations of temperature within the system since this method heats from the outside in. There will always be temperature discrepancies in the conventional heating system, no matter how well agitated or homogenous the mixture is.

Ohmic heating could be an effective future processing technique employed by food manufacturers. The additional control over the system, the rapid and uniform heating, higher quality product, and ability to regulate the electrical conductivity of a food is a more cost effective and less damaging way to kill microbes in food.

CHAPTER 4 - Conclusion

Ohmic heating is an excellent alternative food processing technique that shows much promise in the food manufacturing industry. Since the early 1990's, the technology involving ohmic heating has increased dramatically. As time goes on, this technology will become even more streamlined and efficient. In the future, it is possible that even 'green' energy, such as water, solar, or wind power could create the electricity needed for ohmic heating systems. Special applications for a wider variety of foods could be created to maximize efficiency.

Further studies are recommended to evaluate the possible use in other food applications. Fermented products like beer or wine would benefit from ohmic heating by decreasing the processing time, using the findings from Cho et al. (1996) that ohmic heating may decrease the lag period of fermentative bacteria. Baked goods like meringues and cookie dough may use ohmic heating as a method of drying and processing the eggs present in both products without affecting the final taste or structure. Lean meat products like chicken or rabbit may perhaps benefit from ohmic heating, as there is very little fat associated with these products, lessening the possibility of underprocessed fat globules. More extensive studies into pathogenic bacteria inactivation, such as *E.coli* O157:H7 or *Salmonella*, are also recommended.

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