Evaluating the Feasibility of Producing Shelf-Stable Low-Acid Vegetables Through Pressure-Ohmic-Thermal Sterilization: Studies on Product Quality and Microbiological Safety

Dissertation

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Abstract

Synergy of combining pressure-electric field-heat for preserving shelf-stable low-acid foods was investigated. Our hypothesis was that synergy of pressure (rapid temperature increase with heat of compression as well as instant adiabatic cooling) can be simultaneously combined with ohmic heating (rapid internal heat generation) to produce microbiologically safe and better quality shelf stable low acid food products.

First, studies were conducted to characterize in situ electrical conductivity of selected vegetable products (carrot, potato, red radish) using a semi-custom made high pressure experimental setup. In situ electrical conductivities of vegetable samples under going pressure treatment at 200, 400, 600 MPa at 25°C (holding times up to 10 min) were estimated. The hardness and stiffness of the samples were evaluated using the instrumental texture analyzer. This information was related to the crunchiness index (CI). Pressure treatment increased electrical conductivity values of all the samples as a function of pressure and holding time. Beyond a certain threshold of pressure holding time, the electrical conductivity did not change further. Knowledge of electrical conductivity values of raw, treated and frozen-thawed products was utilized to calculate tissue disintegration index (Z). Z values were found to be inversely correlated to CI.

Quality studies of pressure ohmic thermal sterilization (POTS) were carried out using a laboratory scale prototype POTS cell that loaded into a
pressure chamber. It essentially consisted of a sample holder with a stationary electrode at one end and a movable electrode at the other end. Such design enabled the sample to receive simultaneous or sequential delivery of pressure-electric field and thermal treatment to products. POTS enabled the rapid temperature come-up time within 1.45 min to 105°C under 600 MPa through synergy between pressure and electric field. POTS had the rapidest temperature come-up time to 105°C within 1.45 min followed by ohmic heating (3.58 min) and pressure assisted thermal processing (PATP, 6.84 min). POTS treated carrots showed better crunchiness index (CI) of 0.76 as compare to PATP (CI=0.57) and ohmic heated (CI=0.62) carrots.

Finally, combined effects of pressure (600 MPa), electric field (50 V/cm), and heat (105°C) and their selected combinations were investigated for the inactivation of two pressure-thermal resistant bacterial spores of Bacillus amyloliquefaciens and Bacillus stearothermophilus. The influence of food matrices on microbial susceptibility to the treatments was tested using green pea puree (pH 6.1), carrot puree (pH 5.0), and tomato juice (pH 4.0) while sterile 0.1% NaCl solutions (pH 5.0 & 7.0) served as controls. POTS treatment inactivated B. amyloliquefaciens and B. stearothermophilus spores suspended in 0.1 % NaCl at pH 7.0 by 4.6 and 5.6 log during 30 min treatment. B. stearothermophilus spores were more susceptible to the POTS treatment than B. amyloliquefaciens spores. Increasing acidity of the food matrices accelerated the inactivation of both spores. The spores investigated followed a non-linear inactivation kinetics and Weibull model could explain the POTS spore inactivation. In conclusion, this study demonstrated POTS is a promising technology for producing microbiologically safe and better quality shelf stable low acid vegetable products.
Dedication

Dedicated to my family
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Invention disclosure


Fields of Study

Major Field: Food Science and Technology
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Consumer demand for healthy and minimally processed foods prompted the food processors and academic researchers to investigate several advanced thermal (microwave, ohmic) and non-thermal (high pressure processing, pulsed electric field processing, ozone, UV processing among others) technologies. These advanced thermal and nonthermal technologies aim to reduce undesirable effects of thermal processing in comparison to conventional thermal processing and thereby improve product quality. Among these technologies, this dissertation focuses on combining beneficial effects of two promising technologies, ohmic heating and high pressure processing.

1.1. High Pressure Processing

High pressure processing involves elevated pressure (400-600 MPa) for microbial inactivation, enzymatic activation or inactivation, and alteration of food functionality with or without the addition of external heat. Pressure treatment in general has limited effects on nutrition, color and similar quality attributes. Uniform compression heating and expansion cooling on decompression help to reduce the severity of thermal effects such as quality degradation and nutritional loss encountered with conventional processing techniques. High pressure processing is essentially a batch process, where the pre-packaged food is loaded inside a pressure vessel and treated with pressure for specific
period of time. Further, pressure treatment enabled food processors to extend shelf life of treated products without employing excessive preservatives. Several value-added pressure treated food products including seafood, meat, juices and smoothies, vegetables are commercially pasteurized and marketed in the United States since 1997. By mid of 2008, about 125 industrial HHP machines were in production for food processing worldwide. Almost 85% of these machines were installed after 2000, mainly due to major capabilities offered by the suppliers. North America, Europe, Australia and Asia lead the commercialization of high-pressure technology.

While pressure pasteurized products are microbiologically safe, pressure treatment itself cannot be used for spore inactivation. Pressure in combination with heat is required for spore inactivation. This process is called pressure-assisted thermal processing (PATP) or pressure-assisted thermal sterilization (PATS). During PATP, pre-heated (70-85°C) food material is subjected to a combination of elevated pressure (500 to 700 MPa) and temperature (90 to 120°C) for a specified holding time. PATP has shown better preservation of textural qualities in the low acid vegetable products. Minimal thermal exposure during shorter pressure holding time helped to retain product textural quality attributes in comparison to conventional retort processing where the product experience prolonged thermal exposure. However, the product quality may be impacted during preheating due to thermal exposure to harsher pre-heating temperatures (70-85°C).

1.2. Ohmic heating

Ohmic heating is one of the advanced thermal processing technologies, where the food material is made part of an electrical circuit. The internal heat is generated in the
form of an internal energy transformation (from electric to thermal) within the material. Internal heat generation is governed by a function of electric field and electrical conductivity of the food samples.

James Prescott Joule first established the principle of ohmic heating, and then the technology is variously referred to as Joule heating, electroconductive heating, electroheating, electrical resistance heating, and direct electrical resistance heating. The practical application on the foods includes pasteurization, sterilization, processing of fouling-sensitive material, blanching, thawing, and on-line detection of starch gelatinization. Currently more than 100 ohmic heaters have been installed, with systems used for whole strawberry and yogurt in Japan and low acid ready-to-eat meals in the USA.

1.3. Pressure Ohmic Thermal Sterilization

Pressure ohmic thermal sterilization technology utilizes synergistic combination of pressure and ohmic heating technologies to produce superior quality low-acid foods. During pressure-ohmic thermal sterilization, the product can be ohmically preheated and then subjected to combined pressure-ohmic-thermal treatment. This can potentially reduce thermal exposure and may help the processors to produce superior quality products. At present, prior to this study, no equipment is available for systematically evaluate combined pressure-ohmic-thermal effects on food materials. Further, very limited knowledge is available on the literature on microbial safety and instrumental quality benefits of pressure-ohmic thermally processed food materials.

1.4. Objectives
1. To develop a laboratory scale pressure-ohmic thermal sterilization apparatus for conducting pressure-thermal-ohmic experiments

2. To examine the influence of pressure on the *in situ* electrical conductivity of food samples

3. To examine the combined pressure-ohmic-thermal effect on the quality attributes of selected low acid vegetable products

4. To examine the efficacy of pressure ohmic thermal sterilization in microbial inactivation using two pressure-heat resistant bacterial spores.
Chapter 2: In Situ Thermal, Volumetric and Electrical Properties of Food Matrices Under Elevated Pressure and the Techniques Employed to Measure Them

2.1. Introduction

There has been a recent profusion in research evaluating the in situ properties of food matrices under high pressure. In theory, hydrostatic pressure is uniformly and instantaneously applied to food samples, irrespective of the geometry exposed. Isostatic principles of high pressure processing have certain advantages over thermal processing; however, it does not escape the classical limitations, imposed by heat transfer (Denys et al., 2000b). Due to the differences in compression heating various macromolecules in foods (e.g. lipids, proteins, etc.) and the differences in the physical heat-transfer boundary conditions, the temperature of food samples processed inside a pressure vessel can change. Application of high pressure processing systems requires an extensive understanding of the appropriate thermal and physical properties of the processed material, in order to effectively analyze, design and operate the systems (Denys and Hendrickx, 1999). Familiarity with the in situ properties of various foods under pressure is essential to accurately validate process uniformity and to develop the appropriate kinetics models for microbial and enzymatic inactivation. Developing in situ
measurement sensors for food properties under high pressure is challenging. Sensors need to be (i) relatively high pressure tolerant (700MPa); (ii) precise, accurate, and decisive; (iii) able to measure solid and liquid biomaterials of any transparency; and (iv) without limitations on compression media (Min et al., 2009). The current review describes thermal, volumetric, and electrical properties of food matrices and their in-situ measurement techniques employed to measure such properties. It also covers reviews relevant high pressure processing equipment including the vessels, intensifiers, and closures.

2.2. High pressure processing equipment

2.2.1 High pressure vessel construction

The high pressure vessel is one of two critical elements, in a high pressure processing system, the other being the pressure intensifier. The vessel is the most costly part of the machine (Hernando Sáiz et al., 2008). Commercial vessels range in pressure tolerance between 400 and 600 MPa, with a life span of more than 100,000 cycles (Alegre et al., 2007; Ananth et al., 1998; Linton et al., 2001; López-Caballero et al., 1999; Minerich and Labuza, 2003; Thakur and Nelson, 1998). High pressure vessel construction is classified into three types: monoblock (monolithic) vessels, multiwall vessels, and wire wound vessels. The monoblock design is a single piece of cylindrical, hollow metal, which tolerates pressure without a permanent change in its dimension (Bobrowsky, 1973). It is limited to pressures below 400 MPa and internal diameters smaller than 150 mm (Ting, 2011).
Multilayer vessels are made by layering thin plates around a central tube. The layers are then heated, tightened and welded (Towler and Sinnott, 2008). The multilayer design has a higher pressure capacity and longer lifespan than the monoblock design (Jahed et al., 2006; Mertens and Deplace, 1993). However, thermal conduction through the wall is lower than in the solid-wall vessels, so adjustments must be made for reduced heating and cooling rates (Vetter et al., 2001).

A wire wound vessel is built by winding a high-tension strip around a core to ensure optimal pressure distribution and regulated tension during winding (Eremets, 1996). The outer circumference of a wire wound vessel cylinder generates compressive residual stresses within the vessel wall, so high tensile stresses do not arise when it is subjected to internal pressure, giving wire wound vessels superior strength (Koizumi and Nishihara, 1992). In food-processing applications, the wire-wound design is preferred because it allows the equipment to leak and relieve pressure avoiding catastrophic explosions due to pressure build-up (Fellows, 2009).

2.2.2. High pressure intensifier

The pressure intensifier consists of in-line dual cylinders (small and larger diameter) where a drive piston is directly connected to an intensifier piston (Toledo, 2007). Figure 2.1 describes the typical high pressure processing equipment outfitted with a pressure intensifier, air driven hydraulic pump and air compressor. The air-operated hydraulic pump drives a large diameter piston at $P_1$ and $A_1$. The large piston is coupled to small piston at $P_2$ and $A_2$. Pressure is magnified depending on the ratio of the surface areas between the small and large piston. If the area ratio of the large piston to the small piston
is 20:1, it will intensify the lower pressure ($P_1$) on the small piston ($A_1$) at up to 20 times higher pressure ($P_2$) on the large piston ($A_2$).

2.2.3. Vessel closures

In general, vessel closure designs are classified into three types, an external frame or yoke support, a continuous thread closure or an interrupted thread closure. The external-frame type closure maintains internal pressure via a separate frame or yoke (Ting, 2011). An external-frame can obstruct the installation of thermocouple or electrical wires through the vessel’s top closure, which may make external-frame types unsuitable for in situ property studies. Continuous thread closures require a relatively long time to open and close. Interrupted thread closures allow for faster openings and closings (Fellow, 2009; Ohlsson and Bengtsson, 2009), unlike the continuous closure. For in situ property measurement studies, thermocouple or electrical wires are commonly run through the closure via conical cavities. An example of a popular electrical feed-through is the Bridgman cone-type, shown in Figure 2.2 (Sherman and Stadtmuller, 1987).

2.3. Installation of thermocouple and electrical wires into high pressure vessel

To study the in situ thermal properties under pressure a reliable measure of temperature is essential. K-type thermocouples have proven to be reliable up to 700 MPa and 100ºC, with less than 2ºC deviations influenced by elevated pressure and temperature (Bundy, 1961). Electrical wire feed through is also essential to supply electric power for in situ electrical property studies. One method for such wiring in high pressure vessels is to drill a hole right through the Bridgman-type metal cone (beryllium copper, stainless
steel, mild steel, maraging steel) and silver soldering a continuous thermocouple through the axis of the cone. This silver-soldered cone can subsequently pass through the conical cavity of the high pressure vessel closure (Sherman and Stadtmuller, 1987). The surface of the metal cone must then be electrically insulated to prevent direct contact between the high pressure thermocouple cone and vessel enclosure. Kynar heat-shrink tubing has proven effective for insulating the conical feed through, up to 1000 MPa at 25°C (Terry and Ruoff, 1972). For a high temperature application, mineral-insulated (MI) cable can be used as the high pressure feed through wires. MI cable is a variety of conductor cables (e.g. thermocouple or power supply copper wire) insulated internally by inorganic magnesium oxide powder (Miller et al., 2009) inside a metal sheath. Therefore, MI cable can be directly silver-soldered onto metal cones without further insulation.

2.4. Thermal properties: heat of compression, thermal conductivity, thermal diffusivity, and specific heat

2.4.1. Heat of compression

All compressible materials adiabatically change temperature during physical compression, depending on their compressibility and specific heat (Balasubramaniam et al., 2004; Patazca et al., 2007; Ting et al., 2002). Adiabatic temperature increases resulting from compression is related as follows.

\[ \frac{dT}{dP} = \frac{T_i \cdot \beta_p}{C_p \cdot \rho_p} \]  

(2.1)
It depends on the initial temperature ($T_i$), thermal expansivity ($\beta_p$, K$^{-1}$) at pressure, specific heat ($C_{pp}$, J/kg·K) at pressure (Pa), and density ($\rho_p$, kg/m$^3$) at pressure. Under compression, the internal energy of the system increases as the material is compressed too quickly for significant heat transfer to occur, resulting in a rapid rise in temperature (Rasanayagam et al., 2003). Table 2.1 summarizes the heat of compression (°C/MPa) of water and selected foods.

2.4.2 Thermal conductivity

The thermal conductivity of water under pressure (mega or giga pascal range) has been studied for almost 100 years (Bridgman, 1923; Kestin et al., 1984; Lawson et al., 1959). *In situ* thermal conductivity data of water under elevated pressure is available from the National Institute of Standards and Technology (NIST) reference fluid thermodynamic and transport properties-REFPROP Version 9.0 (Lemmon et al., 2010). In other hand, very limited information is available about the *in situ* thermal conductivity of food materials.

Measurements of thermal conductivity are classified as either, a steady state method or an unsteady state method (transient method). In steady state methods, a long time is required to achieve constant conditions (several hours) and therefore incompatible with perishable foods that may change chemically and physically during that time (Nesvadba, 2005). Among unsteady state methods, a line heat source probe technique has been commonly used because of its reliability, simplicity, and little limitation on sample size and geometry (Murakami et al., 1996; Reidy and Rippen, 1971; Sweat and Haugh, 1974).
The temperature rise of the probe is measured as a function time and thermal conductivity; and is calculated as follows (Nesvadba, 2005).

\[
k = \frac{Q}{4\pi\Delta T} \left[ \ln \left( \frac{t_e}{t_s} \right) \right]
\]

(2.2)

Where, \( k \) = thermal conductivity of the sample (W/m·°C), \( Q \) = heat generated by line heat source (W/m), \( t_s \) = start time (s) and \( t_e \) = end time (s) within the straight-line portion of the curve, and \( T_s \) and \( T_e \) = temperature (°C) at time \( t_s \) and \( t_e \), respectively.

This technique was adopted to measure the in situ thermal conductivity \((k_p)\) of liquid foods under pressure (Denys and Hendrickx, 1999; Ramaswamy et al., 2007; Zhu et al., 2008). Figure 2.3 shows the cross sectional view of a line heat source probe for measuring in situ thermal conductivity \((k_p)\) fabricated by Ramaswamy et al. (2007). K-type thermocouple wires along with the insulated constantan heater wire were inserted inside a stainless steel hypodermic needle tube (outer diameter: 0.71 mm; thickness: 0.15 mm). Care was taken to position the thermocouple junction in the middle of the probe tube by physical measurement. The wires were insulated to avoid any short circuits. They were connected to the power source and data acquisition system. These probes were aligned approximately in the central axis of the polycarbonate sample holder (19 mm diameter) with a movable piston. The bottom of the outer sample holder housed a removable and free moving piston, sealed with an O-ring. This allowed pressure to transmit from medium to sample through vertical motion. After pressure equilibration, a line heat source probe provided power to the sample, and temperature change was monitored as a function of time to calculate in situ thermal conductivity \((k_p)\) under
pressure. $k_p$ of selected foods, at elevated pressure and 25ºC, has been investigated by previous researchers (Nguyen et al., 2012; Ramaswamy et al., 2007; Zhu et al., 2008). In their studies, regression models were constructed to estimate the changes in \textit{in situ} thermal conductivity ($k_p$) as a function of pressure using the experimental data as follows.

$$k_p = a_0 + a_1 \cdot P + a_2 \cdot P^2 + a_3 \cdot P^3$$  \hspace{1cm} (2.3)

Table 2.2 summarizes the coefficients for the regression equation describing the thermal conductivity of water and selected foods as a function of pressure at 25ºC. Since equation 2.3 is empirical in nature, it is important that such equations are used to estimate thermal conductivity values only within the range of experimental conditions. Extrapolations may result in erroneous values. Maximum pressures in each study fell between 350 to 700 MPa depending on the processing equipment and customized sensor. All tested samples had increasing $k_p$ as a function of pressure. Under pressure, the intermolecular distance is decreased hence reducing the mean free path for the molecules, resulting in an increase in thermal conductivity (Nguyen et al., 2012). Apple juice (88% moisture) showed similar $k_p$ to that of water. Among tested food samples, clarified butter showed the maximum increase (87%) in $k_p$ at 700 MPa relative to atmospheric pressure, followed by canola oil (62% increase) and apple juice (38% increase). Water and aqueous-based materials have lower compressibility (Bridgman, 1923) than fat-based foods. Consequently, their $k_p$ values under pressure increase to a lesser extent (Ramaswamy et al., 2007). Moisture content plays an important role in the thermal conductivity of tested materials (Zhu et al., 2008).
The extent of heat exchange between the product and its surroundings is influenced in part by its thermal properties, such as its thermal conductivity and specific heat (Rasanayagam et al., 2003). Whenever foods with high thermal conductivity are pressurized, care is required to monitor any potential heat loss to the surrounding pressure medium or vessel, to validate the process uniformity and microbial safety. In an unheated/noninsulated pressure vessel, it is recognized that the coldest region is located near the wall or near vessel closures (Balasubramaniam et al., 2004). Macronutrients in food matrices, such as water, fat, protein, and carbohydrate, have different thermal conductivities under pressure. Therefore, the cold spot must be carefully considered for heterogeneous foods (Ardia et al., 2004). Insulating vessels minimizes the heat loss through inner wall. Further research is required to evaluate the in situ thermal conductivities of a broad range of food components and the effect of their mixing under pressure.

2.4.3. Thermal diffusivity

A dual needle line heat probe has been adopted to estimate in situ thermal diffusivity ($\alpha_p$) and specific heat ($C_p$) in food matrices under pressure (Nguyen et al., 2012; Zhu et al., 2007). The basic design is similar to line heat source probes, except there is a second K-type thermocouple beyond the first probe (line heater and K-type thermocouple). The second probe maintains a constant distance from the first probe. Under pressure, a line heater at the first probe heated samples and then the temperature change of a second probe thermocouple was recorded to calculate in situ thermal diffusivity as follows proposed by (Nguyen et al., 2012).
\[
\alpha_p = \frac{r^2}{4} \left( - \frac{t_m^{-1} - (t_m - t_d)^{-1}}{\ln (t_m) - \ln (t_m - t_d)} \right) \tag{2.4}
\]

Where \( r \) = the distance (m) between line heat and second thermocouple probe, \( t_d \) = duration of the heat pulse (s), and \( t_m \) = time from the start of heating to maximum temperature (s).

Previous researchers developed regression models to estimate the changes in \textit{in situ} thermal diffusivity as a function of pressure using experimental data as follows.

\[
\alpha_p = a_0 + a_1 \cdot P + a_2 \cdot P^2 + a_3 \cdot P^3 \tag{2.5}
\]

Table 2.3 tabulates the coefficients for the regression equation describing \textit{in situ} thermal diffusivity (\( \alpha_p \)) of water and selected foods as a function of pressure, at 25°C. The thermal diffusivity data seem more discrete than conductivity, but the trend of positive pressure dependency was obviously demonstrated (Zhu et al., 2007). Water showed a 22\% increase in \( \alpha_p \) at 600 MPa in comparison to its initial value at 0.1 MPa. Among tested food samples, soybean oil had the largest increase (53\%) at 600 MPa followed by cream cheese (39\%), honey (32\%), tomato puree (30\%) and guacamole (29\%). Thermal diffusivity has a significant impact on the heat flux inside food samples and the surrounding media. When there is a heat flux across the boundary of the system, the transient temperature field, which occurs within the product must be taken into account (Ardia et al., 2004). Whenever food samples of high thermal diffusivity are pressure
processed, care should be taken to minimize the heat flux during pressure holding times. Results from thermal diffusivity studies suggest that temperature profiles should be properly documented during pressure build-up, holding time, and depressurization.

2.4.4. Specific heat

Based on knowledge of *in situ* thermal conductivity \((k_p)\), thermal diffusivity \((\alpha_p)\), and density data for selected food materials \((\rho_p)\) (Min et al., 2010), *in situ* specific heat \((C_p)\), under pressure, was estimated as follows (Nguyen et al., 2012).

\[
C_p = \frac{k_p}{\alpha_p \cdot \rho_p}
\]  

Where, \(k_p=in situ\) thermal conductivity \((\text{W/m} \cdot \text{°C})\) under pressure, \(\alpha_p=in situ\) thermal diffusivity \((\text{m}^2/\text{s})\) under pressure, and \(\rho_p=in situ\) density under pressure \((\text{kg/m}^3)\). *In situ* specific heat \((C_p)\) changes under pressure were empirically modeled as follows, and are summarized in Table 2.4.

\[
C_p = a_0 + a_1 \cdot P + a_2 \cdot P^2 + a_3 \cdot P^3
\]  

\(C_p\) values of tested materials (water, soybean oil, honey, guacamole) had decreasing trends as a function of pressure, except guacamole. Guacamole did not have a clear \(C_p\)
trend. Initial increase in \( C_p \) of guacamole may be attributed to entrapped air bubbles in the sample during loading although effort was done to de-areate the guacamole by applying vacuum before pressure treatment (Nguyen et al., 2012). \( C_p \) is the quotient of thermal conductivity to diffusivity, and both conductivity and diffusivity had a similar increasing rate with rising pressures (Zhu et al., 2007). Specific heat changes play a key role in temperature change due to adiabatic heating. This will be discussed further in the volumetric properties section (Section 2.5).

In the previous studies, significant efforts have been made to measure the \textit{in situ} thermal properties of various food materials at elevated pressures (350-700 MPa) at room temperature ranges. Knowledge of \textit{in situ} thermal properties enables the development of numerical models that have process uniformity and microbial safety validation. Quantitative prediction of temperature distribution is required to detect the cold spots inside high pressure vessels and establish correct processing parameters. Previous researchers have tried to simulate the temperature and pressure distribution within the vessel using mathematical modeling and computational fluid dynamics (Abdul Ghani and Farid, 2007; Denys et al., 1997; Hartmann et al., 2003). However, \textit{in situ} thermal properties of real foods were not available, so researchers based values on the thermal properties of water. For an example, Abdul Ghani and Farid (2007) used computational fluid dynamics to simulate the temperature distributions of solid (beef fat)-water mixtures during conduction in the high pressure vessel. The \textit{in situ} thermal conductivity of beef fat \( (k_{bf,p}) \) under pressure was not available, so the authors estimated \( k_{bf,p} \) as adopted by Hartmann et al. (2003):
\[ k_{bf,p} = \left( \frac{k_{bf,atm}}{k_{wt,atm}} \right) k_{wt,p} \] (2.8)

Where \( k_{bf,p} = \text{in situ} \) thermal conductivity of beef fat at pressure, \( k_{bf,atm} = \text{in situ} \) thermal conductivity of beef fat at atmospheric pressure, 0.1 MPa, \( k_{wt,atm} = \text{in situ} \) thermal conductivity of water at atmospheric pressure, and 0.1 MPa, \( k_{wt,p} = \text{in situ} \) thermal conductivity of water at pressure. Validation of the computed temperature distribution was in agreement with those measured experimentally and reported in the literature. However, a real food is composed of multiple components including water, fat, carbohydrates, and protein. Heat transfer models need accurate thermal and physical property data for the products being studied at the appropriate conditions, and thus, it was necessary to determine the pressure and temperature dependence of the relevant properties, thermal conductivity, density, specific heat and thermal expansivity (Denys et al., 2000b). Experimental \textit{in situ} thermal properties of foods will contribute to validating process uniformity in mathematical simulations.

Whenever \textit{in situ} thermal properties are studied in food matrices under pressure, care should be taken to calibrate the probe due to heater wire and thermocouple movement, secondary to compression. Probe-specific calibration can be conducted using published NIST data on the thermal properties of water under pressure. Although knowledge of \textit{in situ} thermal properties from previous studies is useful for the design and operation of high pressure equipment and for validating process uniformity, published data is limited to room temperature ranges under elevated pressure. Further studies are
needed to investigate the *in situ* thermal properties of foods at combined high pressures (350-700 MPa) and temperatures (105-121°C).

2.5. Volumetric properties: density and compressibility

In the high pressure processing, measurement of a material’s volumetric properties under pressure is required to estimate compression heating of materials and to model the thermal profiles of pressurized food systems (Barbosa-Cánovas and Rodríguez, 2005; Min et al., 2009; Min et al., 2010). The compressibility of molecules and changes in volume influences the pressure-induced changes on proteins, resulting in an equilibrium shift favoring the state with the lowest overall volume (Krešić et al., 2008; Lullien-Pellerin and Balny, 2002). In the case of liquid foods under pressure, density differences occur which lead to a free convection of fluids (Abdul Ghani and Farid, 2007).

Bridgman (1909, 1931) conducted pioneering work on the compressibility of several liquids under elevated pressure. Guignon et al. (2010) investigated the volumetric properties of pressurization fluids (water, ethanol, ethylene glycol, propylene glycol, castor oil, silicon oil) up to 350 MPa. The compressibility of water is available in the NIST reference fluid thermodynamic and transport properties-REFPROP Version 9.0 (Lemmon et al., 2010). Table 2.5 summarizes the isothermal compressibility (MPa⁻¹), volume and density change of water as functions of pressure. Water reduces its initial volume by 14.8% at 600 MPa. Water properties are useful to simulate the behavior of high-moisture foods; however, some significant discrepancies may appear in modeling predictions if pure water properties are used instead of corresponding properties for the real food (Guignon et al., 2009).
Previous researchers (Aparicio et al., 2011; Denys et al., 2000a; Guignon et al., 2009; Min, et al., 2009; Min, et al., 2010) conducted experiments measuring the compressibility of several food materials under pressure. Various methods, such as the piezometer, piston-displacement method, and hydrometer, were adopted to measure the volumetric properties of food under pressure (Barbosa, 2003). Denys et al. (2000b) determined the density of tomato paste and apple pulp by measuring the amount of pressure-transfer medium pumped into the high pressure equipment. More recently, a variable volume piezometer and linear variable differential transformer were used to measure the compressibility and density of both liquid and solid foods under pressure (Aparicio et al., 2011; Guignon et al., 2009; Min et al., 2009; Min et al., 2010). Min et al. (2009) customized the variable volume piezometer for both liquid and solid foods applicable up to 700 MPa. The modified piezometer utilized the magnet coil and movable copper piston as an eddy current sensor to sense piston displacement upon pressurization. A magnet coil, wrapped around a polycarbonate sample tube, produced an electromagnetic field by means of vertical movements of the copper piston. Inductance changes and their empirical relationship to volume changes of water (published NIST data base) were determined. Subsequently, this empirical model was used to calculate the volume change of tested food samples depending on inductance changes in the variable volume piezometer under pressure as follows.

$$\rho_p = a_0 + a_1 \cdot P + a_2 \cdot P^2$$  \hspace{1cm} (2.9)
Table 2.6 summarizes the parameters of empirical models to estimate the density changes of selected foods as a function of pressure. The density of all tested foods increased as a function of pressure. The density of water increases from 997 kg/m$^3$ at 0.1 MPa to 1189 kg/m$^3$ at 700 MPa. Apple juice has the highest increase in density (17.4%) with pressure build-up, 0.1 MPa to 700 MPa. Clarified buffer (17.2%) has the second largest change in density followed by soybean oil (16.9%), chicken fat (16.2%), carrot (14.6%), chicken breast (14.1%), cheddar cheese (13.7%), deli ham (13.0%), honey (8.9%), and then salmon (8.6%). Results demonstrate that food densities under pressure, particularly for those relatively high in solids, fat, and porosity, deviate from the behavior of water (Min et al., 2010). Measured differences in food densities under pressure is attributable mostly to differences in compressibility. Published in situ properties of specific heat (Nguyen et al., 2012) and density (Min et al., 2011) can be used to derive theoretical adiabatic temperature increases ($dT$) using equation 2.1. Figure 2.4 shows the theoretical estimates for temperature increases in water, honey, and soybean oil. For the thermal expansivity ($\beta_p$, K$^{-1}$) of soybean oil and honey, there seems to be no published data; so, the $\beta_p$ of water ($\beta_{p,wt}$) under pressure was used from NIST reference fluid thermodynamic and transport properties-REFPROP Version 9.0 (Lemmon et al., 2010). Although $\beta_{p,wt}$ could differ from soybean oil and honey, reasonable values of $\beta_{p,wt}$ were applied for both soybean oil and honey. In the theoretical estimations of adiabatic temperature increases, soybean oil showed the greatest temperature increase, from 25°C at 0.1 MPa to 60°C at 600 MPa. Water and honey increased in temperature up to 43 and 48°C, respectively, with rising pressures to 600 MPa. Rasanayagam et al. (2003) reported the experimental value of adiabatic temperature increases in soybean oil up to 62.8°C at 600 MPa when it
was compressed from 25°C at 0.1 MPa. In our study, the theoretical estimation of adiabatic temperature increases had strong agreement with the published experimental values, within 2.8°C. Water had an experimental adiabatic temperature increase up to 42.4°C (initial temperature of 25°C at 0.1 MPa) with elevating pressures up to 600 MPa (Patazca et al., 2007); therefore, water’s experimental data was consistent with theoretical estimates based on in situ specific heat and density. Adiabatic temperature increase has shown its importance when evaluating microbial inactivation. A difference of 3-4°C in adiabatic heating has resulted in a difference of up to 6 log cycles of spore inactivation (Ardia et al., 2004).

In the LeChatelier’s principle, phenomena that are accompanied by a decrease in volume are enhanced by pressure, and vice-versa. Thus, under pressure, reaction equilibriums are shifted towards the most compact state, and the reaction rate constant is increased or decreased (Rastogi et al., 2007). Covalent bonds are highly incompressible (Prehoda et al., 1998) and, therefore, not influenced by elevated pressure. Whereas, high pressure stimulates some phenomena (e.g. phase transition, chemical reactivity, change in molecular configuration, chemical reaction) that are accompanied by a decrease in volume, but it opposes reactions that involve an increase in volume (Linton and Patterson, 2000; Norton and Sun, 2008).

Volumetric changes of different foods under pressure would influence protein denaturation, collagen denaturation and chemical reactions, beyond microbial inactivation. The magnitude of the standard volume change resulting from unfolding globular protein provides unique insight into packing and hydration differences between folded and unfolded proteins (Prehoda et al., 1998). The partial molar volume of the
denatured protein system decreases with increasing pressure relative to that of the native protein. In the case of collagen, in contrast to globular proteins, unfolding (collagen denaturation) results in increased partial specific volume at low pressure and decreased partial specific volume at pressures above 324 MPa (Potekhin et al., 2009). It has been shown that the transfer of hydrophobic compounds to water is accompanied by a large decrease in specific volume at low pressures (Masterton, 1954; Potekhin et al., 2009). At present, there is limited information relating volumetric properties of foods and components to chemical reactions or structural changes under pressure. Practically applied, high pressure can disrupt the three dimensional structures of larger molecules or cell structures (i.e. proteins, including enzymes, lipids, cell membranes, etc.); but have no effect on small covalently bonded molecules, such as vitamins, flavor components and some pigments. Further works, however, are needed to define this phenomenon further.

2.6. In situ electrical conductivity

The electrical behavior of food materials under pressure has, recently, been investigated to determine the effects of pressure on texture and extent of starch gelatinization. Dielectric properties of biological tissues provide information about tissue structure and composition (Kuang and Nelson, 1998). Angersbach et al. (2002) investigated pressure-induced membrane damage in potato tissues due to electrical conductivity changes (non-in situ method), immediately after pressure treatment. The trend of increasing electrical conductivity in potato was observed based on treatment intensity; thus, measuring electrical conductivity can be employed to assess the cellular status of materials under pressure. Bauer and Knorr (2004) described pressure-induced
wheat and tapioca starch gelatinization using electrical conductivity measurements (non-
*in situ* method), after pressure treatment. The electrical conductivity of pressure-treated
starches increase as a function of pressure and pressure holding time; and the curves of
electrical conductivity are well correlated to curves for degree of gelatinization and
pressurization time. Authors proposed that the rise in gelatinized starch conductivity is
attributable to an ion release and amylose leaching out of the granule. Consequently,
electrical conductivity corresponds with the degree of pressure-induced starch
gelatinization, and is an effective tool to detect the degree of gelatinization.

Earlier studies have measured *in situ* electrical conductivity of metals and several
chemical solutions (Bridgman, 1921; Bridgman, 1931; Quist et al., 1965; Quist and
Marshall, 1968; Scaife, 1974), but previous cell designs were not suitable for food or
biological materials since the electrical properties of biomaterials are field-strength
dependent (Cima and Mir, 2004; Min et al., 2007). Min et al. (2007) suggested the *in situ*
electrical conductivity cell for food applications should include: (1) an insulated electric
field (Schiefelbein et al., 1998) and allow for estimation of the cell constant under
pressure; (2) provide a uniform electric field, necessary for differentiating between
pressure and electromagnetic-induced changes in food and biological samples (Cima and
Mir, 2004); (3) uniform Joule heating of samples under pressure to enable research on the
combined effects of pressure, temperature and electric field strength on conductivity.

Min et al. (2007) and Park et al. (2012) developed an *in situ* electrical conductivity
cell for liquid and solid food applications under pressure (Fig. 2.5). Two platinum-plated
titanium cylindrical electrodes were enclosed at both ends of the inner sample holder,
separated by a 16 mm gap. The bottom of the outer sample holder housed a free movable
piston, sealed with an O-ring. The piston allowed pressure to transmit from pressure medium to sample through vertical movement. The inner sample holder had a 0.8 mm hole in the middle, allowing pressure equilibration of the sample between the inner and outer sample holders. For liquid food testing, samples were simultaneously loaded into both the inner and outer sample holder. Subsequently, liquid foods functioned as a pressure transmitting fluid inside the conductivity cell. For solid foods, samples were loaded into the inner sample holder and then isoelectrical solution was poured into the outer sample holder to enable pressure equilibration. The in situ electrical conductivity values ($\sigma_p$) for samples were determined using equation 2.10 with data for applied voltage ($V$), current ($I$), and cell constant ($ke_p$) under pressure, as follows.

$$\sigma_p = ke_p \times \frac{I}{V} \tag{2.10}$$

In situ electrical conductivity of liquid foods (orange, apple juice and tomato juice) increased as a function of pressure, peaking between 200 and 500 MPa and decreasing between 500 and 800 MPa (25°C) (Min et al., 2007). For example, the electrical conductivity of tomato juice increased from 0.61 S/m at 0.1 MPa to 0.66 S/m at 400 MPa, and then decreased to 0.58 S/m with the pressure increment at 800 MPa. Authors indicated that increasing electrical conductivity between 200 and 500 MPa would originate in ionic movement and changes in viscosity, and then the subsequent concave downward effect of electrical conductivity might cause a distortion effect on the ions that hinders mobility at the higher pressures. Tomato juice had the highest in situ electrical conductivity.
conductivity among tested samples. Authors proposed that the higher mineral content in
tomato juice (278 mg minerals/100 g juice) resulted in the highest electrical conductivity,
followed by orange juice (210 mg minerals/100 g juice) and apple juice (139.5 mg
minerals/ 100 g juice) (composition data from USDA ARS, 2006). In general, the effect
of pressure on electrical resistance in the solution is very complicated, as might be
expected from the numerous factors involved (Bridgman, 1931).

*In situ* electrical conductivity changes of selected vegetables are plotted as a
function of pressure and pressure holding time in Fig. 2.6 (Park et al., 2012). Pressure
treatment increased *in situ* electrical conductivity values for all the processed samples, as
a function of target pressure and holding time up to a certain threshold level. Beyond this
threshold level, the electrical conductivity values did not change further. For example, the
*in situ* electrical conductivity of raw carrot was 0.027±0.003 S/m, at 0.1 MPa and 25°C,
and then reached up to 0.181±0.032 S/m, at 600 MPa and 3 min holding time. No
significant increase in *in situ* electrical conductivities was observed from 3 to 10 min
holding time, at 600 MPa. Pressure treatment induces the transport of solutes from inside
to outside the cell (and vice versa) with changes in cell permeability (Préstamo and
Arroyo, 1998). Cell permeability could lead to increase *in situ* electrical conductivity
under pressure. The compressed structure and increased density seen in vegetable
samples under pressure would also, likely, increase the *in situ* electrical conductivity in
samples tested. Although *in situ* electrical conductivity changes in relationship to tissue
damage undergoing at pressure is outside the scope of this review, the stabilized electrical
conductivity seen in samples suggests that there is a certain threshold of pressure holding
time that could be used to minimize further tissue damage in vegetables. At present, the
knowledge of *in situ* electrical conductivity is not sufficient, further research needs to focus on liquid and solid foods. In the future, information on *in situ* electrical conductivity under pressure will contribute to the development of new processing technologies, which combine high pressure processing with other technologies such as ohmic heating or pulsed electric field processing.

2.7. Reaction volume and pH

Understanding the pH changes of foods under pressure could be important for controlling microbial inactivation and pH-dependent reactions during high pressure processing. The initial steps in understanding pH relative to pressure were taken by Min et al. (2011) with weak acid buffers. Weak acid buffer solutions can have pressure-dependent pH changes, due to pressure-dependent ionization equilibrium. Hypotheses state that increasing pressure increases dissociation of weak acids, as ionized products fill smaller volumes due to solvent electrostriction around resulting charged species (Haman, 1980). A consequence of pressure-dependent weak acid dissociation is pressure-dependent pH. As presented by Min et al. (2011), reaction volumes for protonsion of weak buffer solutions at different pressures can be used to calculate changes in the molal equilibrium constant for the buffer, due to pressure. Molal equilibrium constants under pressure can then be used to calculate molal pH changes due to pressure. Min et al. (2011) used a variable volume piezometer to measure *in situ* reaction volumes for protonsion of weak acid buffering agents of 2-(N-morpholino) ethanesulfonic acid, citric acid, sulfanilic acid and phosphoric acid under pressure up to 400 MPa (25°C). The methodology involved initial separation of buffering agents within the piezometer,
using gelatin capsules. Under pressure, the volume of the reactants was measured at 25°C. The contents were then heated to 40°C to dissolve the gelatin and initiate the reaction. Chamber temperature was cooled to 25°C and product volume was measured. Reaction volumes were used to calculate the pH of buffer solutions as a function of pressure. The largest pH change was seen in phosphoric acid buffer, which dropped an average of 0.25 pH units per 100 MPa, while the pH of citric acid buffer dropped by 0.13 units per 100 MPa. Sulfanilic acid buffer showed almost no pH sensitivity to pressure, whereas the pH of 2-(N-morpholino) ethanesulfonic acid buffer increased by 0.075 units per 100 MPa. These results suggest that increasing ionization of phosphoric acid and citric acid as a function of pressure increases hydrogen ion concentration, promoting pH decrease. Whereas 2-(N-morpholino) ethanesulfonic and sulfanilic acid have relative pH stability under pressure. pH changes under pressure are clearly complex, and more rigorous study may be needed to fully understand pH changes in complicated food matrices.

2.8. Conclusions

A clear grasp of in situ thermal, volumetric, electrical properties, reaction volume, and pH changes of food matrices under elevated pressure is critical for validating high pressure processing uniformity and developing suitable kinetics models of microbial inactivation. Developing appropriate sensors to measure in situ properties under pressure is challenging work, involving installation of thermocouples, electrical wires, and measurement sensors into pressure vessels. Also, it requires calibrating the process using already published data, such as NIST steam properties under pressure. Previous research has successfully evaluated the in situ thermal conductivity, thermal diffusivity, specific
heat, compressibility, density, electrical conductivity, and pH changes of selected foods up to 600-700 MPa at room temperature ranges. This information is useful for optimizing further high pressure processing with respect to process uniformity and microbial safety. Experimental in situ property data will enable the development of a mathematical model or computational simulation to validate temperature and pressure distribution for all types of high pressure processing.

At present, in situ property data for foods is limited to room temperature ranges under elevated pressure. There has been a recent surge in research, which simultaneously studies the effects of elevated pressures (400-600 MPa) and sub-retorting temperatures (105-121°C) in shelf-stable, low-acids foods in pressure assisted thermal processing (PATP). For this purpose, additional knowledge of the in situ properties of foods needed as a function of both elevated pressure and temperature. Foods are complicated matrices, composed of many macromolecules such as water, fat, carbohydrate, and protein. In situ property measurements under pressure are also needed for each respective nutrient and the effect of mixing, typical to foods.
Nomenclature

A area of the intensifier piston

a regression model parameter

Cp specific heat (kJ/kg·K)

, specific heat (J/kg·K) for adiabatic temperature increase (K)

I current (A)

k thermal conductivity (W/m·ºC)

ke cell constant for electrical conductivity (m⁻¹)

P pressure (MPa)

, pressure (Pa) for adiabatic temperature increase

Q linear heat generation (W/m)

r the distance between line heat and second thermocouple probe

duration of heat pulse

e end time

T temperature (K or ºC)

i initial

t time (s)

m time from the start of heating to the temperature reaching maximum

V voltage (V)

p pressure

α thermal diffusivity (m²/s)

β thermal expansivity (K⁻¹)

ρ density (kg/m³)

σ electrical conductivity (S/m)


Norton, T, & Sun, D.W. (2008). Recent advances in the use of high pressure as an effective processing technique in the food industry. *Food and Bioprocess Technology*, 1, 2–34.


<table>
<thead>
<tr>
<th>Substances</th>
<th>Temperature increase (ºC) per 100 MPa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water(^1)</td>
<td>2.9</td>
</tr>
<tr>
<td>Salmon fish(^1)</td>
<td>3.0</td>
</tr>
<tr>
<td>Extracted beef fat(^1)</td>
<td>6.3</td>
</tr>
<tr>
<td>Linoleic acid(^1)</td>
<td>5.9</td>
</tr>
<tr>
<td>Soybean oil(^1)</td>
<td>6.3</td>
</tr>
<tr>
<td>Olive oil(^1)</td>
<td>7.2</td>
</tr>
<tr>
<td>Cream cheese(^2)</td>
<td>4.7</td>
</tr>
<tr>
<td>Egg yolk(^2)</td>
<td>4.3</td>
</tr>
<tr>
<td>Egg white(^2)</td>
<td>2.8</td>
</tr>
<tr>
<td>Egg whole(^2)</td>
<td>3.3</td>
</tr>
<tr>
<td>Hass avocado(^2)</td>
<td>3.7</td>
</tr>
<tr>
<td>Whole milk(^2)</td>
<td>3.2</td>
</tr>
<tr>
<td>Skim milk(^2)</td>
<td>3.0</td>
</tr>
<tr>
<td>Honey(^2)</td>
<td>2.9</td>
</tr>
</tbody>
</table>

\(^1\) Rasanayagam et al., 2003  
\(^2\) Patazca et al., 2007

Table 2.1. Heat of compression (ºC/100 MPa) for selected foods at initial temperature of 25ºC.
1) Empirical model was constructed in this study based on NIST reference fluid thermodynamic and transport properties-REFPROP Version 9.0 (Lemmon et al., 2010)

2) Ramaswamy et al., 2007, measured up to 700 MPa at 25ºC

3) Zhu, et al., 2008, measured up to 350 MPa at 25ºC

4) Nguyen, et al., 2012, measured up to 600 MPa at 25ºC

Table 2.2. Coefficients of regression equation (Equation 2.3) for *in situ* thermal conductivity ($k_p$, W/m·ºC) of water and selected food materials as a function of pressure (MPa) at 25ºC.

<table>
<thead>
<tr>
<th>Material</th>
<th>$a_0$</th>
<th>$a_1$</th>
<th>$a_2$</th>
<th>$a_3$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water$^{3)$</td>
<td>0.607</td>
<td>0.0005</td>
<td>-0.0000007</td>
<td>0.0000000066</td>
<td>1.00</td>
</tr>
<tr>
<td>Apple juice$^{2)$</td>
<td>0.6003</td>
<td>0.000329</td>
<td>.</td>
<td>.</td>
<td>0.92</td>
</tr>
<tr>
<td>Canola oil$^{2)$</td>
<td>0.1987</td>
<td>0.000176</td>
<td>.</td>
<td>.</td>
<td>0.99</td>
</tr>
<tr>
<td>Clarified butter$^{3)$</td>
<td>0.2356</td>
<td>0.000293</td>
<td>.</td>
<td>.</td>
<td>0.91</td>
</tr>
<tr>
<td>Cheddar cheese$^{3)$</td>
<td>0.351</td>
<td>0.000266</td>
<td>-0.00000017</td>
<td>.</td>
<td>0.90</td>
</tr>
<tr>
<td>Chicken breast$^{3)$</td>
<td>0.522</td>
<td>0.0003778</td>
<td>-0.000000347</td>
<td>.</td>
<td>0.88</td>
</tr>
<tr>
<td>Potato$^{3)$</td>
<td>0.588</td>
<td>0.000363</td>
<td>-0.00000058</td>
<td>.</td>
<td>0.99</td>
</tr>
<tr>
<td>Tomato puree$^{4)$</td>
<td>0.531</td>
<td>0.0003</td>
<td>.</td>
<td>.</td>
<td>0.96</td>
</tr>
<tr>
<td>Honey$^{4)$</td>
<td>0.329</td>
<td>0.0001</td>
<td>.</td>
<td>.</td>
<td>0.95</td>
</tr>
<tr>
<td>Guacamole$^{4)$</td>
<td>0.421</td>
<td>0.0002</td>
<td>.</td>
<td>.</td>
<td>0.97</td>
</tr>
<tr>
<td>Cream cheese$^{4)$</td>
<td>0.363</td>
<td>0.0002</td>
<td>.</td>
<td>.</td>
<td>0.95</td>
</tr>
</tbody>
</table>
An empirical model was constructed in this study based on NIST reference fluid thermodynamic and transport properties—REFPROP Version 9.0 (Lemmon et al., 2010)

Nguyen, et al., 2012, measured up to 600 MPa at 25ºC

Zhu, et al., 2007, measured up to 350 MPa at 5ºC

<table>
<thead>
<tr>
<th>Material</th>
<th>$a_0$</th>
<th>$a_1$</th>
<th>$a_2$</th>
<th>$a_3$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water$^1$</td>
<td>0.146</td>
<td>0.0001</td>
<td>-0.0000003</td>
<td>0.0002</td>
<td>1.00</td>
</tr>
<tr>
<td>Soybean oil$^2$</td>
<td>0.077</td>
<td>0.00002</td>
<td>0.00000008</td>
<td></td>
<td>0.94</td>
</tr>
<tr>
<td>Honey$^2$</td>
<td>0.093</td>
<td>0.000001</td>
<td>0.00000008</td>
<td></td>
<td>0.95</td>
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<tr>
<td>Tomato puree$^2$</td>
<td>0.130</td>
<td>0.0001</td>
<td>-0.00000006</td>
<td></td>
<td>0.85</td>
</tr>
<tr>
<td>Guacamole$^2$</td>
<td>0.122</td>
<td>0.00007</td>
<td>-0.00000002</td>
<td></td>
<td>0.83</td>
</tr>
<tr>
<td>Cream cheese$^2$</td>
<td>0.111</td>
<td>0.00006</td>
<td>0.00000002</td>
<td></td>
<td>0.92</td>
</tr>
<tr>
<td>Potato$^3$</td>
<td>0.145</td>
<td>0.0000821</td>
<td>-0.0000000255</td>
<td></td>
<td>0.63</td>
</tr>
</tbody>
</table>

1) Empirical model was constructed in this study based on NIST reference fluid thermodynamic and transport properties—REFPROP Version 9.0 (Lemmon et al., 2010)

2) Nguyen, et al., 2012, measured up to 600 MPa at 25ºC

3) Zhu, et al., 2007, measured up to 350 MPa at 5ºC

Table 2.3. Coefficients of regression equation (Equation 2.5) for in situ thermal diffusivity ($\alpha_p$, m$^2$/s) of water and selected food materials as a function of pressure (MPa).
1) Empirical model was constructed in this study based on NIST reference fluid thermodynamic and transport properties-REFPROP Version 9.0 (Lemmon et al., 2010)

2) Nguyen, et al., 2012, measured up to 600 MPa at 25ºC

Table 2.4. Coefficients of regression equation (Equation 2.7) for in situ specific heat ($C_p$, kJ/kg·K) of water and selected food materials as a function of pressure (MPa) at 25ºC.

<table>
<thead>
<tr>
<th>Material</th>
<th>$a_0$</th>
<th>$a_1$</th>
<th>$a_2$</th>
<th>$a_3$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water$^1$</td>
<td>4.181</td>
<td>-0.0024</td>
<td>0.000005</td>
<td>-0.00000004</td>
<td>1.00</td>
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<tr>
<td>Soybean oil$^2$</td>
<td>2.493</td>
<td>-0.000008</td>
<td>-0.000001</td>
<td>.</td>
<td>0.83</td>
</tr>
<tr>
<td>Honey$^2$</td>
<td>2.432</td>
<td>0.00007</td>
<td>-0.000001</td>
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<td>0.96</td>
</tr>
<tr>
<td>Guacamole$^2$</td>
<td>3.190</td>
<td>0.0025</td>
<td>-0.00001</td>
<td>0.00000001</td>
<td>0.75</td>
</tr>
<tr>
<td>Pressure (MPa)</td>
<td>Isothermal compressibility (MPa$^{-1}$)</td>
<td>Volume (m$^3$/kg, 10$^{-3}$)</td>
<td>Density (kg/m$^3$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>---------------------------------------</td>
<td>-------------------------------</td>
<td>-----------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>0.00045246</td>
<td>1.003</td>
<td>997</td>
<td></td>
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</tr>
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<td>50</td>
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<td>0.982</td>
<td>1018</td>
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</tr>
<tr>
<td>100</td>
<td>0.00035723</td>
<td>0.964</td>
<td>1038</td>
<td></td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>0.00032168</td>
<td>0.947</td>
<td>1056</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>0.00029215</td>
<td>0.933</td>
<td>1072</td>
<td></td>
<td></td>
</tr>
<tr>
<td>250</td>
<td>0.00026746</td>
<td>0.920</td>
<td>1087</td>
<td></td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>0.00024666</td>
<td>0.908</td>
<td>1101</td>
<td></td>
<td></td>
</tr>
<tr>
<td>350</td>
<td>0.00022895</td>
<td>0.898</td>
<td>1114</td>
<td></td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>0.00021371</td>
<td>0.888</td>
<td>1127</td>
<td></td>
<td></td>
</tr>
<tr>
<td>450</td>
<td>0.00020045</td>
<td>0.879</td>
<td>1138</td>
<td></td>
<td></td>
</tr>
<tr>
<td>500</td>
<td>0.00018882</td>
<td>0.870</td>
<td>1149</td>
<td></td>
<td></td>
</tr>
<tr>
<td>550</td>
<td>0.00017851</td>
<td>0.862</td>
<td>1160</td>
<td></td>
<td></td>
</tr>
<tr>
<td>600</td>
<td>0.00016931</td>
<td>0.855</td>
<td>1170</td>
<td></td>
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</tr>
<tr>
<td>650</td>
<td>0.00016102</td>
<td>0.848</td>
<td>1180</td>
<td></td>
<td></td>
</tr>
<tr>
<td>700</td>
<td>0.00015354</td>
<td>0.841</td>
<td>1189</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^{1}$NIST reference fluid thermodynamic and transport properties-REFPROP Version 9.0 (Lemmon et al., 2010)

Table 2.5. Volumetric properties of water as a function of pressure (MPa) at 25ºC.
Empirical model was constructed in this study based on NIST reference fluid thermodynamic and transport properties-REFPROP Version 9.0 (Lemmon et al., 2010)

Min, et al., 2009, at 25°C, measured up to 700 MPa at 25°C

Min, et al., 2010, at 25°C, measured up to 700 MPa at 25°C

Table 2.6. Coefficients of regression equation (Equation 2.9) for in situ density ($\rho_p$, kg/m$^3$) of water and selected food materials as a function of pressure at 25°C.
Figure 2.1. A cross sectional view of a high pressure intensifier and its principle.

\[
P_1 \times A_1 = P_2 \times A_2
\]
\[
A_1 >> A_2
\]
\[
P_2 >> P_1
\]
Figure 2.2. The Bridgman cone-type electrical lead-through. C indicates a thin conical shell of pipestone and I indicates electrical insulation (from Sherman & Stadtmuller, 1987).
Figure 2.3. Schematic diagram of the high pressure experimental set-up (a) and thermal conductivity probe (b) (Ramaswamy et al., 2007).
Figure 2.4. Theoretical estimates of temperature increase based on published *in situ* property data of specific heat (Nguyen et al., 2012) and density (Min et al., 2010). ○: water; □: honey, △: soybean oil.
Figure 2.5. Cross sectional view of the *in situ* electrical conductivity cell made for high pressure application.
Figure 2.6. *In situ* electrical conductivity of the vegetable samples as a function of pressure and holding time: (a) carrot; (b) potato; (c) red radish ( ◇ 0.1 MPa, □ 200 MPa, △ 400 MPa, ○ 600 MPa). The dotted vertical line indicates the come-up time. Pressurization rate was approximately 20 MPa/s.
Chapter 3: Estimating Pressure Induced Changes in Vegetable Tissue Using *In Situ* Electrical Conductivity Measurement and Instrumental Analysis

Abstract

Pressure-induced textural changes in selected vegetables (carrot, potato, and red radish) were investigated using *in situ* electrical conductivity measurement. Electrical conductivity of the vegetables was recorded *in situ* up to 10 min under elevated pressures (200, 400, 600 MPa at 25°C) using a custom-fabricated experimental setup. The tissue disintegration index (Z) was determined for raw, processed and frozen-thawed samples. The hardness and stiffness of the samples were evaluated using the instrumental texture analyzer. This information was related to the crunchiness index (CI). Pressure treatment increased electrical conductivity values of all the samples as a function of pressure and holding time. Beyond a certain threshold of pressure holding time, the electrical conductivity did not change further. *In situ* electrical conductivity measurement was a useful tool to document the extent of textural changes during high pressure processing.

*Key words:* high pressure processing, *in situ* electrical conductivity, vegetables, texture, quality
3.1. Introduction

High pressure processing involves treating food products at elevated pressures (400-600 MPa) with or without heat addition. Pressure treatment affects only non-covalent bonds (hydrogen, ionic, and hydrophobic bonds) and has little effect on chemical constituents. Since products have minimal thermal exposure, pressure treatment reduces the thermal impact on food quality attributes such as flavor, color, and nutrient content (Balasubramaniam and Farkas, 2008). Vegetable is one of the major categories of pressure-treated products commercially available in the market (Tonello, 2011).

Food texture is a major consideration in defining the quality of fruits and vegetables. Microscopically, textural quality is also dependent on impact of process treatment on plant tissue. Plant tissue is composed of cells, which are surrounded by cell membranes. Cell membranes are of low electrical conductivity; whereas, the material inside the cells and surrounding are of high electrical conductivity (Nelson, 1973). Dielectric properties of biological tissues provide information about tissue structure and composition (Kuang and Nelson, 1998). Impedance measurement provides information about physiological changes in outer or inner cells, and on membranes as an equivalent circuit model; thus, explanations for the impedance spectra could be provided at the cellular level (Inaba et al., 1995; Wu et al., 2008). The electrical properties of biological matter enable the determination of morphological and structural properties cell systems (Angersbach et al., 2002). These authors evaluated the structural changes in vegetable membranes with electrical conductivity measurement after high pressure treatment. Various studies investigated the relation between dielectric properties and structure of plant cells (Bazhal et al., 2003; Benavente et al., 1998; De Vito et al., 2008; Greenham,
1966; Inaba et al., 1995; Jackson and Harker, 2000; Lebovka et al., 2002; Luscher et al., 2005; Wu et al., 2008; Zhang and Willison, 1991). Pressure-induced external stress could change the membrane integrity or induce cellular leakage of electrolytes into intercellular areas (Angersbach et al., 2002; Trejo Araya et al., 2007). Very limited information is available relating in situ electrical conductivity changes of vegetable tissue under pressure to product texture. The objectives of the current study were to evaluate in situ electrical conductivity changes in vegetable tissues undergoing high pressure processing; and to determine their relationship to instrumental measurements of vegetable texture.

3.2. Materials and methods

3.2.1. Sample preparation

Vegetable samples (baby carrots, potatoes, and red radishes) were purchased from a local market in large quantities. Sufficient quantities were to minimize quality variations in the raw materials. Samples were stored at 4ºC for up to a week. Experiments utilized vegetable samples cut into cylinders (11.7 mm diameter and 16 mm length) using a cork borer (Fisher Scientific, Pittsburgh, PA, USA).

3.2.2. Pressure generating system

A laboratory scale pressure test system (Model 26190, Harwood Engineering, Walpole, MA, USA) was used. The pressure generating system (1000 MPa) was equipped with a diaphragm pump (SC-67-6000W-100, SC Hydraulic, Brea, CA, USA), a hydraulic vane pump (TMB-002-21R-1-00, Denison Hydraulics, Marysville, OH, USA), and an intensifier (SA-10-6-.875FGD-150K, Harwood Engineering, Walpole, MA, USA). The cylindrical pressure vessel interior dimensions were 25.4 mm diameter and 153 mm
depth. Product temperature could be controlled through an external jacket. Pressurization rate was approximately 20 MPa/s. The top closure of the pressure vessel had conical cavities for high pressure feed through wires and thermocouples.

3.2.3. *In situ* electrical conductivity cell

The *in situ* electrical conductivity cell is described in Fig. 3.1. It was composed of outer (19 mm diameter, 105 mm length, 43104, US Plastics, Lima, OH, USA) and inner (12.6 mm diameter, 50 mm length, 43102, US Plastics, Lima, OH, USA) sample holders. Two platinum-plated titanium cylindrical electrodes (12.6 mm diameter) were enclosed at both ends of the inner sample holder, separated by 16 mm distance. The back half of the electrode stepped down to 6.9 mm diameter and housed an O-ring to fit securely into the electrode plug (Ultem®, SABIC Plastics, Pittsfield, MA, USA). AWG #14 copper wires were soldered on each electrode and passed through the bored outer sample holder plug (Ultem®, SABIC Plastics, Pittsfield, MA, USA). The end of the copper wires were soldered to the high pressure electrical feed through and electrically insulated using polyolefin heat shrink tubing (Q2-Z-1/8, Qualtek Electronics, Mentor, OH, USA). A type K thermocouple (0.51 mm diameter, TFAL/CY-020, Omega engineering, Stamford, CT, USA) was positioned between the outer and inner sample holders to measure the temperature under pressure. The thermocouple was positioned to avoid physically damaging the vegetable sample. The cavities of electrical wire in the sample holder were sealed with epoxy (Devcon, Danvers, MA, USA) to prevent the pressure medium from entering the sample holder. The bottom of the outer sample holder housed a removable and free moving piston, sealed with an O-ring. This allowed pressure to transmit from medium to sample through vertical motion. The inner sample holder had a 0.8 mm hole
in the middle which allowed pressure equilibration between the vegetable sample and the pressure transmitting fluid of isoelectrical solution.

3.2.4. Preliminary experiments

Preliminary experiments compared the electrical conductivity values of 0.01 and 0.1 molal KCl at 25°C (reference solutions) estimated using our current experimental setup against published data at atmospheric pressure (Lide, 2006). Our measurements (0.142±0.010 and 1.290±0.080 S/m for 0.01 and 0.1 molal KCl at 25°C, respectively) were different within 1% of the published data. Cell constant \(k\) for our conductivity cell at atmospheric pressure was determined to be 127.33 m\(^{-1}\) at 0.1 MPa. Subsequently, additional corrections were carried out to estimate the cell constant values under pressure taking into account the compressibility of polycarbonate sample holder (Min et al., 2007; Warfield, 1967). The cell constants \(k_p\) under pressure were estimated as 124.49, 122.93, and 121.29 m\(^{-1}\) at 200, 400, and 600 MPa, respectively. Additional preliminary experiments verified the impact of sample temperature increase due to ohmic heating as well as high pressure processing. Application of moderate electric fields could increase sample temperature due to ohmic heating. Ohmic heating could induce the breakdown of the cellular structure in the plant tissues due to combined effects of electroporation and thermal heating (Lee and Jun, 2011). This phenomenon can influence electrical conductivity values of the test samples and product quality (Bazhal et al., 2003; Kulshrestha and Sastry, 2003; Wang and Sastry, 2002; Weaver and Chizmadzhev, 1996). It was observed that low electric field (0.75 V/cm) used in this study did not significantly increase product temperature or its electrical conductivity values (Fig. 3.2a). Palaniappan
and Sastry (1991) reported that the electrical conductivity of carrot increases from 0.033 S/m at 30°C to 0.034 S/m at 32°C based on a linear model of electrical conductivity.

Attention was also paid to control the thermal effects caused by heat of compression of test samples when exposed to high pressure processing (Fig. 3.2b). Prior to loading into the pressure vessel, the vegetable samples were chilled (7-19°C) so that sample temperature under pressure was at about 25°C±2°C.

3.2.5. Preparation of vegetable samples for electrical conductivity measurement

A cylindrically-shaped vegetable sample (11.7 mm diameter, 16 mm length, 2 g) was loaded into the inner polycarbonate sample holder. The vegetable sample (Ø11.7 mm) was smaller than the inner sample holder (Ø12.6 mm), thereby preventing any physical damage to the vegetable tissue during the loading procedure. After loading the vegetable sample, an isoelectrical solution was poured into the outer sample holder to allow for pressure equilibration and to ensure contact resistance between electrode and sample (Sastry, 2005). The isoelectrical solution was made to have the same electrical conductivity as each vegetable sample, using demineralized water (97801, OSU Chemistry Store, Columbus, OH, USA) and NaCl (Fisher Chemical, Certified, ACS, Pittsburgh, PA, USA). The volume ratio of sample to isoelectrical solution was calculated as 0.86:0.14 (v/v) in the inner sample holder. A removable and free moving piston was installed into the outer sample holder. An O-ring screw sealed weep hole enabled removal of air bubbles existing in the in situ electrical conductivity cell.

3.2.6. In situ electrical conductivity measurement of vegetable samples under pressure
In situ electrical conductivity of each vegetable was continuously measured at three different pressure levels, 200, 400, and 600 MPa, at 25°C for a 10 min holding time. Considering compression heating (CH) of elevated pressure, vegetable samples were initially precooled (7-19°C, \(T_i\)) at atmospheric pressure (\(P_{atm}\)) to maintain a sample temperature of 25°C (\(T_p\)) after reaching the target pressure (\(P_p\)) during the experiment. \(\Delta T_H\) is the temperature gain of the precooled sample from the surrounding medium during sample loading time into the pressure chamber.

\[
T_i = T_p - \left[CH \times (P_p - P_{atm}) + \Delta T_H\right]
\]  

(3.1)

In situ electrical conductivity was determined from the voltage and current data as (Rieger, 1994):

\[
\sigma = \frac{L \cdot I}{A \cdot V} = k \cdot \frac{I}{V}
\]  

(3.2)

A function generator (GFG-8216A, Chino Inc., Torrance, CA, USA) provided about 1.1-1.2 V root mean square (RMS) AC sinusoidal potential to both electrodes in the circuit. Electric field strength in the vegetable sample was calculated as 0.75 V/cm considering sample length (16 mm). This low voltage gradient was aimed to minimize ohmic heating and electropermeabilization effects from the applied electrical field. The
frequency was set as 1 kHz to prevent the double layer capacitance and polarizing effect on electrodes and inside samples (Braunstein and Robbins, 1967). An oscilloscope (TDS 5052, Tektronix, Beaverton, OR, USA) monitored the voltage across the samples, and current was calculated from the voltage across a current-sensing resistor (MP725, Caddock Electronics Inc., Roseburg, OR, USA) of 150 Ω (±1% tolerance). This experimental setup was double checked using verified sample resistors in the range of 100-10000 Ω. Voltage and current data were recorded every 30 s to evaluate the influence of pressure holding time on the in situ electrical conductivity. All in situ electrical conductivity measurements were performed at least five times to determine the effects of sample-to-sample variations. The resistance of the total circuit was measured by contacting both electrodes with an LCZ meter (4276A, Agilent Technologies, Santa Clara, CA, USA), at atmospheric pressure and 1 kHz. Total resistance was determined as 0.1 Ω and then this value was subtracted from the measurement.

3.2.7. Pressure treatment of vegetable samples for instrumental texture analysis

Five cylindrical vegetable samples (Ø11.7×16 mm) were packaged with a NaCl isoelectrical solution into one flexible pouch, which was then heat-sealed. The volume ratio of vegetable sample to NaCl isoelectrical solution was 0.86:0.14 (v/v), to maintain the same experimental condition to in situ electrical conductivity measurement. Packaged vegetable samples were pressure-treated at 200, 400, and 600 MPa at 25ºC for 1, 5, and 10 min holding times (Model 26190, Harwood Engineering, Walpole, MA, USA). Sample packages were respectively precooled to 7, 13, and 19ºC depending upon target pressure of 200, 400, and 600 MPa, to compensate for compression heating. Experiments were conducted at a process temperature of 25ºC±2ºC. After pressure
treatment, vegetable samples were taken out of the pouch, and lightly wiped with a paper towel in preparation for instrumental texture analysis. Two replications for each flexible pouch were performed comprising of 10 measurements in the instrumental texture analysis, after pressure treatment.

3.2.8. Tissue disintegration index ($Z$)

The tissue disintegration index ($Z$) was calculated from measured in situ electrical conductivity, as follows.

\[
Z = \frac{\sigma_{tret} - \sigma_{raw}}{\sigma_{ft} - \sigma_{raw}}
\]  

(3.3)

$Z$ indices can provide an estimation of the cellular structure damage in ohmic heating and pulsed electric field treatments (Bazhal et al., 2003; De Vito et al., 2008; Lebovka et al., 2002; Lebovka et al, 2005; Rogov and Gorbatov, 1974). Intact tissue (raw sample) has a $Z$ index of 0, and then approaches 1 with the development of tissue damage. For the estimation of $Z$ value of completely damaged vegetable tissues, tests utilized frozen-thawed samples. Each vegetable sample was frozen at -18°C and thawed at room temperature. This cycle was repeated twice.

3.2.9. Instrumental texture analysis

The impact of pressure treatment on texture of pressure treated samples was analyzed using a puncture test. The test utilized a texture analyzer (TA-XT2, Stable
micro system, Surrey GU7 1YL, UK). A 2 mm diameter probe axially punctured the
cylindrical vegetable sample up to 9 mm depth with a load cell of 25 kg± 5 g at a cross
head speed of 1 mm/s. The force-deformation curve was obtained. A puncture test of
each experiment was performed approximately 10 times to characterize biological
variation between samples.

3.2.10. Crunchiness index (CI)

The crunchiness index (CI) was calculated from the puncture test results proposed
by Nguyen et al. (2010). Texture parameters were extracted from experimental force-
deformation curve using Matlab software (Version 7.9.0.529, Mathworks Inc., Natick,
MA, USA). CI considers both textural parameters of the maximum puncture force (F)
and the slope (Grad) in the force-deformation index. F and Grad indicate the hardness
and stiffness (modulus) of the vegetable, respectively (Bourne, 1982; Dobraszczyk and
Vincent, 1999; Gonzalez, 2009; Mohsenin, 1986). Both parameters were compared to
those of raw samples to calculate the CI, as shown in equation (3.4). Intact tissue
indicates a CI of 2 which decreases to 0 with the development of tissue damage. Previous
research has reported that the combination of both parameters into a unified parameter
(CI) gives a better overall indication of textural transformations during pressure treatment
(Nguyen et al., 2010). Specifically, the slope at 20% (Grad20%) of the maximum puncture
in the force-deformation curve provides the best discrimination among processed samples’
textural qualities and accordance with sensory evaluations. Therefore, Grad20% was
selected to evaluate the CI in our study.
\[ CI = \frac{F_{\text{ret}}}{F_{\text{raw}}} + \frac{\text{Grad}_{20\%\text{ret}}}{\text{Grad}_{20\%\text{raw}}} \]  

(3.4)

3.2.11. Statistical analysis

The data were analyzed using SAS, 9.1.3, software (SAS Inst. Inc., Cary, NC, USA). Fisher’s least-significant difference (LSD) procedures were used for a multiple comparison among treatments at the 95% confidence interval (P<0.05). To evaluate the relationship between \( Z \) and \( CI \), experimental results were empirically fitted to the first and second order polynomial regression using SAS, as shown in equations (3.5, 3.6). Figure 3.3 summarizes the outlining sequential experimental procedure. Empirical modeling provided estimated coefficients (\( \beta_0, \beta_1, \beta_2 \)) and probabilities of each coefficient (Pr value).

\[ CI = \beta_0 + \beta_1 \cdot Z \pm \varepsilon \]  

(3.5)

\[ CI = \beta_0 + \beta_1 \cdot Z + \beta_2 \cdot Z^2 \pm \varepsilon \]  

(3.6)

3.3. Results and discussion

3.3.1. Influence of pressure treatment on \textit{in situ} electrical conductivity of the vegetable samples
Figure 3.4 shows the influence of elevated pressure and pressure holding time on the *in situ* electrical conductivity of each vegetable sample. Pressure treatment increased electrical conductivity values of all the processed samples as a function of target pressure and holding time up to a certain threshold level. Beyond this threshold level, the electrical conductivity values did not change further depending on samples. The standard deviation in the *in situ* electrical conductivity measurement ranged 10-15% of its original value (data not shown). For carrot samples, electrical conductivity of raw carrots was $0.027 \pm 0.003 \text{ S/m}$, at 0.1 MPa and 25ºC, and then reached up to $0.185 \pm 0.023 \text{ S/m}$, at 600 MPa and 10 min holding time. Depressurization of the test samples did not alter the *in situ* electrical conductivity values of the all the samples tested (data not shown). Wang and Sastry (1997) reported the electrical conductivity of raw carrot as 0.033 S/m, at 0.1 MPa and 30ºC. Angersbach et al. (2002) proposed the increasing membrane damage of high pressure-treated plant tissues (100-400 MPa at 25ºC, 10 min) using electrical conductivity measurement at 0.1 MPa after pressure treatment. The authors state that pressure treatment, even at moderate pressures of 200-300 MPa, causes irreversible damage of subcellular membranes in plant cells. Pressure treatment induces the transport of solutes from inside to outside the cell (and vice versa) with changed cell permeability (Préstamo and Arroyo, 1998). Cell membranes have high impedance at the frequencies of kHz level (Repo et al., 2000). Trejo Araya et al. (2007) reported cellular leakage in pressure-treated carrot at 500 MPa. This cellular leakage is considered one of the potential sources for increased electrical conductivity under pressure. Pressure treatment increases the density of the cellular structure by eliminating air from the tissue during decompression (Basak and Ramaswamy, 1998). At pressures greater than 100 MPa, air
likely dissolves in the first 100 MPa (Min et al., 2010). This compressed structure under pressure would also likely increase the \textit{in situ} electrical conductivity of vegetable samples tested.

Increasing \textit{in situ} electrical conductivity of carrot was stabilized after approximately 3 min holding time at each pressure level (Fig. 3.4a). The stabilized electrical conductivity suggests that there is a certain threshold of pressure holding time to minimize further tissue damage in vegetables. As mentioned above, temperature control is an important consideration in the electrical conductivity measurement of biological tissue. In our experiment, temperatures reached 26-27°C during the compression period, and then stabilized to 25°C within a 1 min holding time at elevated pressure. Although the temperature decreased from 26-27°C to 25°C during initial pressure holding time (Fig. 3.2b), electrical conductivity continuously increased up to 3 min and then stabilized. Thus, we concluded that a temperature deviation of $\pm 2^\circ$C did not contribute on the changes in the \textit{in situ} electrical conductivity at the elevated pressures. Among the samples tested, frozen-thawed carrots had the highest electrical conductivity of $0.581\pm0.054$ S/m, indicating severe tissue damage. Freezing and thawing vegetable samples primarily caused physical tissue damage rather than biochemical changes in the vegetable tissue such as gelatinization. These observations were consistent with earlier researchers. In frozen-thawed vegetable tissues, cell membranes and associated compartments are disrupted. This leads to a dramatic change in electrical impedance characteristics in the plant tissues; most of the reactance component is lost, and the resistance declines dramatically (Harker and Dunlop, 1994; Harker and Forbes, 1997;
Jackson and Harker, 2000). These phenomena propose extensively increased electrical conductivity of frozen and thawed vegetables.

Potato samples showed a similar trend in \textit{in situ} electrical conductivity changes under pressure to that of carrot (Fig. 3.4b). Initially, the electrical conductivity of raw potato was 0.051±0.007 S/m, and then it reached 0.124±0.002 S/m at 600 MPa and 10 min holding time. Angersbach et al. (2002) reported the electrical conductivity of raw potato as 0.054 S/m at 0.1 MPa. In our study, the highest electrical conductivity of 0.686±0.031 S/m was observed in the frozen-thawed potato, indicating complete tissue damage. Freezing and thawing almost completely eliminate the electrical impedance with damaged potato cell membrane (Dejmek and Miyawaki, 2002). Elevated pressure and prolonged holding time increased \textit{in situ} electrical conductivity in potato tissues. Changes in cell walls, membranes and compositions of cell contents are said to be factors influencing electrical conductivity changes, associated with cell wall breakdown, tissue damage, and tissue softening (Wang and Sastry, 1997). Dörnenburg and Knorr (1992) proposed that the increased membrane permeability of potato tissues is due to high pressure processing. In the potato tissue, pressure-induced starch gelatinization would also impact the electrical conductivity of the sample. Gebhardt et al. (2007) proposed onset of gelatinization occurs in potato starch granules at 650 MPa using \textit{in situ} observation of the diamond anvil cell. The starch gelatinization of plant tissues increases the electrical conductivity in pressure treatment and ohmic heating (Bauer and Knorr, 2004; Wang and Sastry, 1997). Although an evaluation of starch gelatinization was outside the scope of our study, it would be an important consideration in understanding the textural changes of potato tissue under pressure.
Electrical conductivity of raw red radish was 0.016±0.002 S/m, and then increased up to 0.159±0.013 S/m at 600 MPa and 10 min holding time (Fig. 3.4c). Frozen-thawed radish had the maximum electrical conductivity of 0.279±0.002 S/m. Previous researchers reported an increase in electrical conductivity of Japanese white radish after a pressure treatment of 400 MPa at 25ºC for 10 min (Imai et al., 1995; Yamamoto et al., 1992); and proposed that pressure-induced membrane damage and ion leakage would increase electrical conductivity.

3.3.2. Influence of pressure and holding time on instrumental texture analysis

The force-deformation curve of the puncture test is given in Fig. 3.5. Puncture tests were conducted after pressure treatments at 200, 400, and 600 MPa for 1, 5, and 10 min (25ºC). Figure 3.5 shows the force-deformation curve at 10 min holding time. Puncture tests were performed approximately 10 times at each condition. Only the representative force-deformation curve is presented, which best explains the mean results. The curve reached the maximum puncture force with tissue rupture, and then decreased. Decreased puncture force and slope were observed in pressure-treated carrots as compared to raw samples. In carrot tissue, membrane disruption induces instant firmness loss, reducing cell turgor pressure (Greve et al., 1994). During high pressure processing, turgidity loss and cellular changes can take place (Trejo Araya et al., 2007). The cellular changes can include: cell conformation changes, cell elongation, cell separation or debonding and/or cell wall disruption. In our study, 400 and 600 MPa treatments resulted in significantly less hardness and stiffness in the carrot tissue (P<0.05). As expected, frozen-thawed samples had the greatest textural damage. Freezing and thawing plant tissues induces a gradual breakdown in the organization of the protoplasmic structure. In
most cases, it results in the rupture of the plasmalemma with the subsequent loss of turgor pressure in cells (Delgado and Rubiolo, 2005). Potato exhibited similar trends in the force-deformation curve to those of carrot. The greatest hardness and stiffness were observed in the raw potato, followed by pressure treatment and then the frozen-thawed samples, in decreasing order.

Notably, red radish had different trends in the force-deformation curve to those of carrot and potato. Pressure-treated red radishes had significantly higher puncture forces in the force deformation curve than those of controls (P<0.05). This is in accordance with previous research (Nguyen et al., 2010) where authors reported that pressure treatment at 600 MPa and room temperature increased the puncture force values of red radishes. Visually, the pressure-treated red radishes were more rubbery, had a soaked appearance, and deformed structure as compared to carrots and potatoes. Diffusion of red pigment into the internal tissue was also observed in the pressure-treated red radish. Dowgiallo (2005) proposed that a more malleable material will require a higher cutting force, as part of the cutting force is used to deform the material. The more rubbery texture of the pressure-treated red radish would induce higher puncture force than for the raw-sampled red radish. In this study, the textural changes in pressure treated samples were primarily related as a function of electrical conductivity and instrumental texture analysis.

It is worth noting that further influences on product texture is possible through pressure induced biochemical changes. The pressure treatment can play a role in improving product texture under pressure by acting on enzymes (Basak and Ramaswamy, 1998). Depending upon the level of pressure-heat intensity, the action of pectin methylesterase on pectin results in demethylated pectin, forming a complex with Ca^{2+} ion
and contributing to an enhancement in texture (Anthon et al., 2005; Kato et al., 1997; Lee et al., 1979; Nguyen et al., 2007). Pressure treatment prior to cooking (99.5°C) was reported to improve the texture of vegetables and pressure-treated carrots retained textural quality (Kasai et al., 1995). High pressure induces lowered β-elimination susceptibility and enhanced pectin cross-linkage (De Roeck et al., 2010). High pressure induced absence of pectin solubilisation caused by β-eliminative depolymerisation resulted in better firmness when compared to thermally processed carrot dices (Knockaert et al., 2011). More research is needed to relate biochemical changes with in situ electrical conductivity values and instrumental texture analysis of the test samples.

3.3.3. Influence of pressure and pressure holding time on tissue disintegration index (Z) and cruchiness index (CI)

Changes in the tissue disintegration index (Z) and cruchiness index (CI) of each vegetable sample are shown in Fig. 3.6-3.8. In carrot and potato samples, there were overall trends of increasing Z and decreasing CI values, with elevated pressure and prolonged holding time. As expected, frozen-thawed carrot had the highest Z value and lowest CI value, which means complete tissue damage. As mentioned earlier, Z=0 indicates intact tissue and approaches 1 with the development of tissue damage. Whereas, CI=2 indicates intact tissue and decreases to 0 with textural failure. For example, 200 MPa pressure treatments at 25°C for 1 min yielded Z values of 0.06 and 0.01 for carrot and potato, respectively. The corresponding CI values were 1.66 and 1.57, respectively. 600 MPa pressure treatments at 25°C for 10 min showed Z values of 0.29 and 0.12 for carrot and potato which corresponds to the CI values of 1.12 and 0.93, respectively. In the red radish samples, Z increased in accordance with elevated pressure and prolonged
holding time, similar to carrot and potato; whereas, CI did not have clear trends influenced by pressure and holding time.

The effects of elevated pressure on increasing Z values in carrot samples was statistically significant (P<0.05, Fig. 3.6). Whereas, no statistical significance was correlated among pressure holding times at 200 and 400 MPa. No significance of holding time was attributable to stabilized in situ electrical conductivity within approximately 3 min holding time at each pressure level (Fig. 3.4a). Major textural changes in carrot are expected to occur during the initial pressure holding time (3 min) based on the calculated Z values. Increasing Z indices could also imply more compactness in the vegetable tissues except for tissue damage. For the CI of pressure-treated carrots, the effects of elevated pressure were statistically significant between 200 and 400 MPa treatments (P<0.05); whereas, it had a minor effect at 400 and 600 MPa. These results imply the threshold of pressure level which does not lead to the further textural changes. The effects of holding time were only statistically significant at 200 MPa (P<0.05). The results of the CI were mostly in accordance with the results for Z, indicating a threshold of pressure holding time at which it did not induce more textural changes. Specifically, elevated pressure was more influential on Z than holding time. Previous researchers proposed that pressure softening is only pressure-level dependent due to the instantaneous nature of the initial loss (Basak and Ramaswamy, 1998).

In the potato samples, elevated pressure significantly increased Z values (P<0.05, Fig. 3.7). Angersbach et al. (2002) reported that high pressure induced-membrane damage areas are correlated with the level of pressure applied to potato samples. Whereas, the effect of holding time on Z values was not statistically clear between 5 and 10 min at
each pressure level. The results also suggest a threshold of pressure holding time for further textural changes in the potato tissues. The CI of pressure-treated potato samples showed a decreasing trend at elevated pressure and prolonged holding time. In the statistical analysis, no significant effect of elevated pressure was observed between 400 and 600 MPa for CI. Statistically, the influence of holding time was not clear at 200 MPa; whereas, at higher pressures (400 and 600 MPa) significantly decreased CI was observed at 10 min holding time (P<0.05).

The effect of elevated pressure on Z values in red radish samples were not clear between 200 and 400 MPa treatments (Fig. 3.8). Ueno et al. (2009), previously, reported that cellular membrane structures in Brassica rapa root would be partially destroyed at 200 MPa, and completely destroyed over 400 MPa (radish as Brassicaceae family). In our study, prolonged holding time continuously increased Z values of red radish at 400 and 600 MPa (P<0.05). Based on the Z results, textural changes in red radish tissues consecutively occur at elevated pressure. These were not in accordance with the findings in pressure-treated carrot and potato samples. Clear trends of pressure and holding time effect were not observed in the CI results. Pressure-treated red radish shows higher puncture force (hardness) than control samples (Fig. 3.5c). This was contradictory to our findings in carrot and potato samples. Okazaki et al. (1998) reported that radish became harder after pressure treatment at 200-400 MPa and 25°C using instrumental texture analysis. The authors proposed that high pressure significantly delays softening rates in radish samples, and the effects of higher pressure are greater in association with inhibited β-elimination effect. In our study, the CI considered both the hardness and stiffness of vegetable samples. Although the hardness (maximum puncture force) of pressure-treated
radish increased, stiffness (slope) of the force-deformation curve decreased, indicating pressure-induced texture failure. Specifically, the \( CI \) of frozen-thawed red radish was 1.30, which was considerably higher than those of frozen-thawed carrot and potato. In the force-deformation curve of frozen-thawed red radish, puncture force had a similar magnitude as control samples. Radish and carrot tubers greatly differ in their histological structure and biological function (Herppich et al., 2003ab). Tubers of the biennial carrots have a clearly defined structure consisting of periderm, cortex (phloem) and core (xylem) tissue. Each tissue has parenchyma cells of different sizes and mechanical properties. In contrast, radish tubers mainly consist of large, thin-walled parenchyma cells. Differences in plant tissue cell structure would result in differences in resistances to pressure treatment. This might be attributable to the resistance of different vegetable cell layers to pressure treatment (Gonzalez, 2009; Nguyen et al., 2010).

3.3.4. Empirical model relating \( Z \) and \( CI \)

The relationship between \( Z \) and \( CI \) was fitted to a first and second order polynomial (3.5, 3.6), and then tabulated in Table 3.1. In the first order polynomial, all of the vegetable samples showed the negative linear coefficient (\( \beta_1 \)) indicating an inverse relationship between \( Z \) and \( CI \). The highest coefficient of determination (\( R^2 \)) was calculated as 0.87, in the carrot sample, followed by the potato and red radish sample, in decreasing order. Carrot and potato samples had significant linear coefficient (\( \beta_1 \)) in the probability test (P<0.05). Whereas, the first order polynomial of red radish did not demonstrate a close inverse relationship between \( Z \) and \( CI \) with low \( R^2 \) and a lack of significance in the probability test.
Table 3.2 summarizes the results of the second order polynomial between Z and CI. Second order fittings improved the empirical model with higher $R^2$ values. They also proved the inverse relationship between Z and CI with a negative linear coefficient ($\beta_1$). Although quadratic coefficient ($\beta_2$) indicated a positive coefficient, no significance was found in the probability test ($P>0.05$). Neither first nor second order empirical models demonstrated a close relationship between Z and CI in red radish samples. Red radish demonstrated its own characteristics in the force-deformation curve of the puncture test. This would cause the lack of correlation between Z and CI in the radish samples. From the empirical models of carrot and potato, the Z value of the in situ electrical conductivity measurement was a useful tool to estimate the pressure-induced textural changes of the vegetable tissue in relationship to the instrumental texture analysis. Texture is a very complex quality that is related to the physiological status and the mechanical properties of a product (von Willert et al., 1995). Different texture attributes can also be measured objectively by different destructive and non-destructive methods (Herppich et al., 2003b).

For example, Abbott et al. (1995) investigated the correlation coefficient ($R^2$) between sonic vibration characteristics and puncture firmness tester in the apple. The values ranged from 0.5 to 0.9, depending on cultivar and sonic resonance. In our study, a good relationship between in situ electrical conductivity and instrumental texture analysis was found in carrot and potato samples; whereas, red radish showed relatively weak strength.

3.4. Conclusions

In situ electrical conductivity measurement and its tissue disintegration index (Z) provide information about textural changes in vegetable tissues undergoing high pressure
treatment. Overall, increasing trends of in situ electrical conductivity were observed as a function of pressure and holding time up to a certain threshold level. The electrical conductivity values did not change further depending on samples after specific holding time. Z was influenced by elevated pressure and prolonged holding time. Certain threshold of pressure and holding time was expected not to induce further textural changes of vegetable tissue. Empirical model fitting demonstrated the inverse relationship for carrot and potato between Z and CI. In situ electrical conductivity measurement was a useful tool for understanding the pressure-induced textural changes of vegetable tissues.
Nomenclature

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A$</td>
<td>cross sectional area ($m^2$)</td>
</tr>
<tr>
<td>$CH$</td>
<td>heat of compression ($^\circ$C/100MPa)</td>
</tr>
<tr>
<td>$CI$</td>
<td>crunchiness index</td>
</tr>
<tr>
<td>$F$</td>
<td>maximum puncture force (N) of the force deformation curve</td>
</tr>
<tr>
<td>$Grad$</td>
<td>slope of the force deformation curve</td>
</tr>
<tr>
<td>$I$</td>
<td>current (A)</td>
</tr>
<tr>
<td>$k$</td>
<td>cell constant ($m^{-1}$)</td>
</tr>
<tr>
<td>$L$</td>
<td>length (m)</td>
</tr>
<tr>
<td>$P$</td>
<td>pressure (MPa)</td>
</tr>
<tr>
<td>$T$</td>
<td>temperature ($^\circ$C)</td>
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<tr>
<td>$V$</td>
<td>voltage (V)</td>
</tr>
<tr>
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<td>volume ($m^3$)</td>
</tr>
<tr>
<td>$Z$</td>
<td>tissue disintegration index</td>
</tr>
<tr>
<td>$\beta$</td>
<td>parameters in the empirical models</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>electrical conductivity (S/m)</td>
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Subscripts

<table>
<thead>
<tr>
<th>Symbol</th>
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</tr>
</thead>
<tbody>
<tr>
<td>atm</td>
<td>atmospheric condition (0.1 MPa)</td>
</tr>
<tr>
<td>ft</td>
<td>frozen-thawed sample</td>
</tr>
<tr>
<td>$H$</td>
<td>heat transfer from the surroundings</td>
</tr>
<tr>
<td>$i$</td>
<td>initial condition</td>
</tr>
<tr>
<td>$p$</td>
<td>pressurized condition</td>
</tr>
<tr>
<td>raw</td>
<td>raw sample</td>
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<tr>
<td>tret</td>
<td>treated sample</td>
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<td>$0,1,2$</td>
<td>parameters of intercept and slope in the empirical model fitting</td>
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<td>20%</td>
<td>slope at 20% of the maximum puncture force</td>
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Table 3.1. Estimated coefficients and probability test of the fitted first-order polynomial parameters between $Z$ and $CI$ ($CI = \beta_0 + \beta_1 \cdot Z \pm \varepsilon$).

<table>
<thead>
<tr>
<th></th>
<th>Carrot</th>
<th></th>
<th>Potato</th>
<th></th>
<th>Radish</th>
<th></th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Coefficients</td>
<td>$Pr &gt;</td>
<td>t</td>
<td>$</td>
<td>Coefficients</td>
<td>$Pr &gt;</td>
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<tr>
<td>$\beta_0$</td>
<td>1.622*</td>
<td>0.0001</td>
<td>1.412*</td>
<td>0.0001</td>
<td>1.572*</td>
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<td>$\beta_1$</td>
<td>-1.511*</td>
<td>0.0001</td>
<td>-1.232*</td>
<td>0.0025</td>
<td>-0.369</td>
<td>0.1164</td>
</tr>
</tbody>
</table>

$R^2$ values  
Carrot: 0.87  
Potato: 0.66  
Radish: 0.25

SSEY ($\varepsilon$):  
Carrot: 0.17  
Potato: 0.27  
Radish: 0.18

* Significant at 95% confidence interval
Table 3.2. Estimated coefficients and probability test of the fitted second-order polynomial parameters between $Z$ and $CI$ ($CI = \beta_0 + \beta_1 \cdot Z + \beta_2 \cdot Z^2 \pm \varepsilon$).

<table>
<thead>
<tr>
<th></th>
<th>Carrot</th>
<th></th>
<th>Potato</th>
<th></th>
<th>Radish</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta_0$</td>
<td>1.759*</td>
<td>0.0001</td>
<td>1.661*</td>
<td>0.0001</td>
<td>1.753*</td>
<td>0.0001</td>
</tr>
<tr>
<td>$\beta_1$</td>
<td>-2.701*</td>
<td>0.0044</td>
<td>-6.874*</td>
<td>0.0035</td>
<td>-1.586*</td>
<td>0.0368</td>
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<tr>
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<td>0.1122</td>
<td>5.479</td>
<td>0.0097</td>
<td>1.197</td>
<td>0.0798</td>
</tr>
<tr>
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<td>0.86</td>
<td></td>
<td>0.50</td>
<td></td>
</tr>
<tr>
<td>SSEY ($\varepsilon$)</td>
<td>0.15</td>
<td></td>
<td>0.19</td>
<td></td>
<td>0.16</td>
<td></td>
</tr>
</tbody>
</table>

* Significant at 95% confidence interval
Figure 3.1. Cross sectional view of the *in situ* electrical conductivity cell made for high pressure application.
Figure 3.2. Verification of ohmic heating effect (temperature: \(\text{---}\), electrical conductivity: \(\text{--}\)) at 0.1 MPa (a) and temperature versus pressure history of carrot during electrical conductivity measurement at 600 MPa (b) (temperature: \(\text{-----}\), pressure: \(\text{-----}\)).
Figure 3.3. Flow chart outlining sequential experimental procedure.

Vegetable samples: carrot, potato, red radish

Cylindrical shaping (Ø11.7×16 mm)

Continuous monitoring of in situ electrical conductivity under pressure (200-600 MPa at 25°C for 10 min)

Pressure treatment in isoelectrical solution at 200, 400, 600 MPa, 25°C for 1, 5, 10 min

Texture profile (hardness and stiffness)

Tissue disintegration index (Z)

Crunchiness index (CI)

Data analysis
1) Multiple comparison of the mean Z and CI values (Fisher’s least significant difference method)
2) Empirical model fitting between Z and CI
Figure 3.4. *In situ* electrical conductivity of the samples as a function of pressure and holding time: (a) carrot; (b) potato; (c) red radish (◇ 0.1 MPa, □ 200 MPa, Δ 400 MPa, ○ 600 MPa). The dotted vertical line indicates the come-up time.
Figure 3.5. Sample force-deformation curves for (a) carrot, (b) potato, and (c) red radish.
Figure 3.6. Changes in $Z$ (a) and $CI$ (b) of carrot as a function of pressure and holding time: ■ 1 min; □ 5 min; □□ 10 min. Values with different letters are significantly different (P<0.05). $Z$ and $CI$ of frozen-thawed carrot were 1.00±0.10 and 0.19±0.03, respectively.
Figure 3.7. Changes in $Z$ (a) and $CI$ (b) of potato as a function of pressure and holding time:  ■ 1 min;  □ 5 min;  △ 10 min. Values with different letters are significantly different ($P<0.05$). $Z$ and $CI$ of frozen-thawed potato were $1.00\pm0.05$ and $0.27\pm0.04$, respectively.
Figure 3.8. Changes in $Z$ (a) and $CI$ (b) of red radish as a function of pressure and holding time: ■ 1 min; □ 5 min; ▴ 10 min. Values with different letters are significantly different ($P<0.05$). $Z$ and $CI$ of frozen-thawed red radish were $1.00\pm0.01$ and $1.30\pm0.20$, respectively.
Chapter 4: Quality of Pressure Ohmic Thermal Sterilized (POTS) Shelf-Stable Low Acid Carrots

Abstract

Pressure ohmic thermal sterilization (POTS) involves ohmic heating of the foods by applying an electric field under elevated pressure. POTS technology, developed in this study, enabled the volumetric heating up to 105°C and adiabatic cooling of food samples by utilizing the synergy between pressure (600 MPa) and electric field (30 V/cm). Experiments were conducted using a custom made POTS apparatus that utilized carrot as the model food system. The samples were processed at a target temperature of 105°C at 600 MPa and processed for 0, 1, 3, and 5 min by regulating the pressure and electric field (30 V/cm). Carrot samples processed by ohmic heating (0.12 MPa, 30 V/cm, 105°C) and pressure-assisted thermal processing (PATP; 600 MPa, 30 V/cm, 105°C) served as controls. Among the technologies investigated, POTS had the fastest thermal come-up time (1.45 min) followed by ohmic heating (3.58 min) and PATP (6.84 min; preheating time of ≈ 6 min). Results indicate the POTS samples had the least textural damage due to minimal thermal exposure. POTS samples had higher crunchiness index (CI) of 0.76 compare to PATP (CI=0.57) and ohmic heated (CI=0.62) carrots. The treatment conditions of all the technologies (POTS, PATP, and ohmic heating) impacted tissue
stiffness more than hardness. All the technologies investigated had minimal impact on product color. This study demonstrates the potential to produce high quality shelf stable low-acid vegetable products using POTS.

Key words: ohmic heating, pressure-assisted thermal processing, pressure ohmic thermal sterilization (POTS), carrot, quality

4.1. Introduction

Consumers demand healthy and minimally processed low-acid shelf stable foods with better quality and nutritional attributes as well as microbial safety. Although conventional retorting and canned products have a proven track for microbial safety, products are exposed to intensive heat over a long time. This further resulted in undesired quality and nutritional loss. In response to consumer demands, the food processors are investigating a number of advanced thermal (ohmic heating and microwave heating) and non-thermal based (pressure-assisted thermal processing; PATP) technologies for preserving fresh-like quality attributes. Both ohmic and microwave heating basically utilize the volumetric heating of food materials using electric field or microwave energy, respectively (Sastry, 2008). While a volumetric heating of advanced thermal processing methods helps to minimize quality degradation during processing, the products are conventionally cooled by slow conduction and convection. PATP involves the simultaneous application of elevated pressures (500–700 MPa) and sub-retorting temperatures (90–120ºC) to a pre-heated food (Rajan et al., 2006a; Rastogi et al., 2010).
One of the unique advantages of PATP is the ability to increase product temperature quasi-instantaneously with the heat of compression, which reduces the severity of the thermal effect. In addition the product is instantly cooled to initial temperature (70-85°C) upon depressurization. Various researchers demonstrated microbial safety and quality of PATP products (Ahn et al., 2007; Nguyen et al., 2010; Paredes-Sabja et al., 2007; Rajan et al., 2006a; Trejo Araya et al., 2007). However, product quality degradation during pre-heating is a concern. This study focused on evaluating the use of pressure-assisted ohmic thermal sterilization (POTS) which synergistically combine the benefits of PATP (rapid temperature come-up with heat of compression and instant adiabatic cooling with decompression) and ohmic heating (rapid and uniform internal heat generation). Our hypothesis was that synergy between the pressure and the electric field will enable rapid heating and cooling of the treated samples with minimal thermal abuse. Accordingly, the objective of our study is to compare selected quality attributes of carrots treated by three sterilization technologies (POTS, PATP and ohmic heating).

4.2. Materials and methods

4.2.1. Sample preparation

Carrots were purchased from a local market in large quantities to minimize quality variations in the raw materials and stored at 4°C for up to a week.

4.2.2. Pressure generating system

4.2.2.1. Pressure generating system for POTS
A laboratory scale pressure test system (26190, Harwood Engineering, Walpole, MA, USA) was used. The pressure generating system rated up to 1000 MPa with a pressure intensifier (SA-10-6-875FGD-150K, Harwood Engineering, Walpole, MA, USA). The pressurization rate was approximately 30 MPa/s. The cylindrical pressure vessel’s interior dimensions were 25.4 mm diameter and 153 mm depth. Food-grade propylene glycol (60% concentration, SAFE-T-THERM®, Houghton Intl. Inc., Valley Forge, PA, USA) was used as the pressure-transmitting medium. More details of the experimental setup are available elsewhere (Min et al., 2007).

4.2.2.2. Pressure generating system for PATP

A laboratory scale high-pressure kinetics tester (PT-1, Avure Technologies, Kent, WA, USA) was used for PATP treatments. The pressure generating system rated up to 700 MPa by an intensifier (M-340 A, Flow International, Kent, WA, USA) up to 700 MPa. The rate of pressurization was approximate 30 MPa/s. The cylindrical pressure vessel (54-mL of the internal volume) was immersed in a temperature-controlled propylene glycol bath (Avatar Corporation, University Park, IL, USA). The target process temperature of 105°C was kept during the pressure-holding period by setting the glycol bath at the desired process temperature. Food-grade propylene glycol (Safe 620TY, Houghton Intl. Inc., Valley Forge, PA, USA) was used as the pressure-transmitting fluid. Additional details are available elsewhere (Rajan et al., 2006a).

4.2.3. Pressure ohmic thermal sterilization (POTS) cell and experimental set-up description
Figure 4.1 describes the customized movable electrode POTS cell developed in this study. The POTS cell consisted of the cylindrical polycarbonate sample holder (15.9 mm inner diameter, 19.1 mm outer diameter, 45 mm length, 43104, US Plastics, Lima, OH, USA), a stationary electrode at one end and a movable electrode at the other end. Both stationary and movable electrodes were made of platinum-plated titanium. Previous studies have shown the minimal electrochemical reactivity of the platinum-plated titanium electrode and it’s utility as electrode material at low frequency ohmic heating (Samaranayake et al., 2005; Tzedakis et al., 1999). The stationary electrode was 16.8 mm diameter on the face. It was fitted into an ultem electrode plug (Ultem®, GE Plastics, Pittsfield, MA, USA) with O-ring seal (No.008, AS568A standard). An O-ring tightened brass screw was threaded into the electrode plug, and then jointed to the back side of the electrode for electrical contact to a high-pressure feed through wire. The movable electrode had a face diameter of 15.6 mm and a weep hole thread was bored on the center. The weep hole functioned to remove air bubble during POTS cell sample loading. The ultem electrode plug housed a movable electrode, which had the central cavity for the O-ring tightened weep hole screw. The high pressure feed through wire and K-type thermocouple were silver soldered to the steel cone. A Teflon-coated K-type thermocouple (0.51 mm diameter, TFAL/CY-020, Omega engineering, Stamford, CT, USA) mounted at the geometric center of the carrot was used to measure the process temperature during POTS.

An isolation transformer (25491 S-83871, OLSUN, Richmond, IL, USA) and variable transformer (POWERSTAT®, Bristol, CT, USA) provided the electric field strength of 15-30 V/cm across the samples at 60 Hz under 600 MPa. Measured voltage,
current, and temperature were recorded every 3 s using a data logger (34970A, Agilent Technologies, Santa Clara, CA, USA).

4.2.4. Sample loading into POTS cell

The carrots were cut into cylindrical pieces (15 mm diameter, 30 mm length, 5 g) using a cork borer (Fisher scientific, Pittsburg, PA, USA). A cylindrical shaped carrot (15 mm diameter, 30 mm length, 5 g) was loaded into the polycarbonate POTS cell after installing the stationary electrode. K-type thermocouple (0.51 mm diameter, TFAL/CY-020, Omega engineering, Stamford, CT, USA) was mounted at the geometric center of the sample and then an isoelectrical solution was poured into the extra space in the POTS cell. The isoelectrical solution matched the electrical conductivity of the carrot ($\sigma=0.027\pm0.003$ S/m, 0.01 m NaCl) using demineralized water (97801, OSU Chemistry Store, Columbus, OH, USA) and NaCl (Fisher Chemical, Certified, ACS, Pittsburgh, PA, USA). The solution helped direct contact resistance between the electrode and sample, and functioned as a pressure-transmitting medium inside of the POTS cell. The isoelectrical solution matched the electrical conductivity of the carrot ($\sigma=0.027\pm0.003$ S/m, 0.01 m NaCl) using demineralized water (97801, OSU Chemistry Store, Columbus, OH, USA) and NaCl (Fisher Chemical, Certified, ACS, Pittsburgh, PA, USA). The solution helped direct contact resistance between the electrode and sample, and functioned as a pressure-transmitting medium inside of the POTS cell. The volume ratio of sample to isoelectrical solution was calculated as 0.90: 0.10 ($v/v$) inside of the POTS cell, at 0.1 MPa. The movable electrode was mounted at the end of the POTS cell maintaining.

Knowing the compressibility of carrot and water was necessary to discern the appropriate initial position of the movable electrode before pressurization. Compressibility of carrot and water were estimated at 0.000118 MPa$^{-1}$ and 0.000244 MPa$^{-1}$ at 600 MPa, respectively (Min et al., 2009). In this experimental set-up, a major portion of the compression occurs with the vertical displacement of the movable
electrode and isotropic compression, similar to the work of Min et al. (2009). The reduced distance between the stationary and movable electrodes under pressure was considered during data analysis.

4.2.5. POTS experimental procedure

During POTS, sample temperatures were elevated up to 105°C with the application of an electric field at 600 MPa. Firstly, samples were pressurized up to 600 MPa (30 MPa/s) through the vertical displacement of the movable electrode inside the pressure chamber, and temperature increased from 25°C to 43-45°C with the heat of compression during this period. Secondly, a constant AC electric field strength of 30 V/cm at 60 Hz was applied across the sample for ohmic heating up to 105°C at 600 MPa. The target temperature of 105°C was held for 0, 1, 3, and 5 min of POTS processing by regulating the electric field (15-30 V/cm). After treatment, samples were immediately cooled in an ice-water mix and kept under refrigerated conditions until analysis (within 2hr of treatment). Recorded voltage and current data were related to the changes in the in situ electrical conductivity of carrot (σ_{isp}) at elevated pressures and temperatures. Electrical conductivity (σ) can be determined from the cell constant (k), current (I), and voltage (V) according to Rieger (1994), as seen in equation 4.1.

\[
\sigma = k \times \frac{I}{V} = \frac{L}{A} \times \frac{I}{V} \tag{4.1}
\]
*In situ* electrical conductivity of POTS carrots was estimated from the cell constant ($k_p$), voltage ($V_p$) and current data ($I_p$) under pressure. As seen in equation 4.2, $k_p$ was calculated based on the electrode distance ($L_p$) and inner cross sectional area ($A_p$) of the polycarbonate POTS cell at 600 MPa.

\[
k_p = \frac{L_p}{A_p}
\]  

(4.2)

$L_p$ was determined to be 0.03 m by the vertical displacement of the movable electrode. $A_p$ was calculated from the volumetric change ratio of polycarbonate polymers at atmospheric and elevated pressures (Min et al., 2007; Warfield, 1967) as follows.

\[
A_p = \left( \frac{V_p}{V_{\text{atm}}} \right)^{2/3} \times A_{\text{atm}}
\]  

(4.3)

$k_p$ of the polycarbonate POTS cell at 600 MPa was determined to be 149.67 m$^{-1}$, and then the *in situ* electrical conductivity ($\sigma_{isp}$) of carrot under pressure was calculated as follows in equation 4.4.
Each POTS experimental condition was repeated at least five times to characterize any biological variations between samples.

4.2.6. Ohmic heating cell and experimental set-up at nominal pressure (0.12 MPa)

Ohmic heating was conducted at a nominal pressure of 0.12 MPa to prevent the boiling of cellular fluid in the carrot tissue. The same polycarbonate ohmic cell for POTS was used. Carrot sample (15 mm diameter, 30 mm length, 5 g) was loaded into the polycarbonate ohmic cell with surrounding isoelectrical solution ($\sigma=0.027\pm0.003$ S/m, 0.01 m NaCl). The volume ratio of sample to isoelectrical solution was calculated as 0.90:0.10 (v/v) inside of the ohmic cell. The ohmic cell was placed in a pyrex glass T-tube (201 mm length, 51 mm inside diameter), and then 0.12 MPa of pneumatic pressure was supplied from an air compressor. The polycarbonate ohmic cell had a 2 mm hole at the middle point for thermocouple installation and nominal pressure transmission. The same experimental set-up (isolation transformer, current sensing resistor, safety fuse and grounding) described with POTS was utilized.

4.2.7. Ohmic heating experimental procedure

In the ohmic heating, sample temperature ohmically increased up to 105ºC by a constant AC electric field of 30V/cm and 60 Hz at a nominal pressure of 0.12 MPa, and then target temperature (105ºC) was held for 0, 1, 3, and 5 min through adjusting the

$$
\sigma_{isp} = k_p \times \frac{I_{isp}}{V_{isp}} = \frac{L_p}{A_p} \times \frac{I_{isp}}{V_{isp}}
$$

(4.4)
electric field (10-30 V/cm). After treatment, samples were immediately cooled in an ice-water mix, and kept under refrigerated condition until analysis (within 2 hrs of treatment). Recorded voltage \( V \) and current \( I \) data were related to the changes in the \textit{in situ} electrical conductivity of carrot during ohmic heating. The cell constant \( k_{atm} \) at nominal pressure was estimated to be same to that at 0.1 MPa and calculated as 151.57 m\(^{-1}\). The \textit{in situ} electrical conductivity \( \sigma_{iso} \) of ohmic heating at elevated temperature and nominal pressure (0.12 MPa) was calculated as follows in equation 4.5.

\[
\sigma_{iso} = k_{atm} \times \frac{I_{iso}}{V_{iso}} = \frac{L_{atm}}{A_{atm}} \times \frac{I_{iso}}{V_{iso}} 
\]  

(4.5)

Each ohmic heating experiment was repeated five times to characterize any biological variations between samples.

4.2.8. PATP experimental procedure

PATP experiments were conducted using the earlier published procedures of the laboratory (Nguyen et al., 2007). Cylindrical carrot sample (15 mm diameter, 30 mm length, 5 g) was placed inside a 10-mL polypropylene syringe (Model 309604, Becton Dickinson and Co., Franklin Lakes, NJ, USA) with isoelectrical solution and then a free movable piston was installed. In the PATP experiment, isoelectrical solution was used as a pressure-transmitting fluid inside the syringe to maintain identical experimental conditions to POTS and ohmic heating experiments. The syringe containing the carrot
was preheated to the desired initial preheating temperature ($T_i$, 67°C) in a water bath at 68°C (Isotemp 128, Fisher Scientific, Pittsburg, PA, USA) considering the heat of compression ($CH$), and then loaded into the pressure vessel. After preheating, the syringe containing the carrot was immediately loaded into a pressure vessel and pressure was built up to 600 MPa (30 MPa/s). During the compression period up to 600 MPa, the isoelectrical solution inside of the syringe pressurized the carrot samples. Sample temperature rose to a final target temperature ($T_f$) of 105°C with the heat of compression. The target temperature of 105°C was held for 0, 1, 3, and 5 min at 600 MPa. After treatment, samples were immediately cooled in an ice-water mix, and kept under refrigerated condition until analysis (within 2 hrs post-treatment). To evaluate the effects of preheating on carrot qualities, only samples preheated up to 67°C for 0 and 5 min holding times were prepared. Each PATP experimental condition was repeated at least five times to characterize any biological variations between samples.

4.2.9. Quality attributes

4.2.9.1. Puncture test and crunchiness index ($CI$) calculation

For instrumental texture analysis, a puncture test was conducted using a texture analyzer (TA-XT2, Stable micro system, Surrey GU7 1YL, UK). A 2 mm diameter probe axially punctured the cylindrical vegetable sample up to 9 mm deep with a load cell of 25 kg± 5 g at a cross head speed of 1 mm/s. The force-deformation curve was obtained. The force-deformation curve of the puncture test was fitted with a third order polynomial and texture parameters of hardness and stiffness (modulus) were extracted using Matlab software (Version 7.9.0.529, Mathworks Inc., Natick, MA, USA). Hardness represents
the force required to compress a food between the molars, and stiffness expressed the ratio between stress and strain as deformation or elastic modulus of the materials (Dobraszczyk and Vincent, 1999). Hardness and stiffness were determined from the maximum puncture force ($F$) and the slope ($Grad$) in the force-deformation curve, respectively (Bourne, 2002; Dobraszczyk and Vincent, 1999; Gonzalez, 2009; Mohsenin, 1986). Specifically, the slope at 20% ($Grad_{20\%}$) of the maximum puncture in the force-deformation curve has the best discrimination among processed samples’ textural qualities and has good accordance with sensory evaluations (Nguyen et al., 2010). Therefore, $Grad_{20\%}$ was selected to evaluate the stiffness in our study. Earlier researchers reported crunchiness index ($CI$) can be used to characterize intensity of different treatment on product texture (Nguyen et al., 2010). The crunchiness index ($CI$), is given below.

\[
CI = \frac{F_{\text{tret}}}{F_{\text{raw}}} + \frac{Grad_{20\%\text{tret}}}{Grad_{20\%\text{raw}}}
\]  

(4.6)

Intact tissue indicates the $CI$ of 2, which decreases to 0 with the development of textural changes. In this publication, the ratio of maximum puncture force ($F_{\text{tret}}/F_{\text{raw}}$) was designated as hardness index ($HI$) and the ratio of slope in the force deformation curve ($Grad_{20\%\text{tret}}/Grad_{20\%\text{raw}}$) is defined as stiffness index ($SI$). It is worth to note that the intact raw tissue without any treatment likely have $HI$ and $SI$ values of 1. $CI$ was useful to
evaluate the impact of different treatment intensities on product hardness and stiffness (Nguyen et al., 2010).

4.2.9.2. Color measurement

Sample color was measured using a colorimeter (CR210, Minolta, Osaka, Japan). The colorimeter was calibrated using a white standard plate \((L^*=97.83, a^*=0.43, b^*=1.98)\). CIELAB \(L^*, a^*,\) and \(b^*\) values are represented as the indicators of lightness, redness and yellowness, respectively. Total discoloration \((\Delta E)\) was calculated as follows in equation 4.7.

\[
\Delta E = \sqrt{(L_{\text{tret}}^* - L_{\text{raw}}^*)^2 + (a_{\text{tret}}^* - a_{\text{raw}}^*)^2 + (b_{\text{tret}}^* - b_{\text{raw}}^*)^2}
\]  

(4.7)

4.2.9.3. Tissue disintegration index \((Z)\)

\(Z\) indices are widely used estimates for the tissue damage of vegetable products during ohmic heating and pulsed electric field treatments (Bazhal et al., 2003; De Vito et al., 2008; Lebovka et al, 2005; Rogov and Gorbatov, 1974). The tissue disintegration index \((Z)\) was calculated from changes in the electrical conductivity of carrot samples \((\sigma_{\text{tret}})\) after POTS, ohmic heating, and PATP compared to those of raw \((\sigma_{\text{raw}})\) and frozen-thawed samples \((\sigma_{\text{ft}},\) supposed complete damage) as follows. To induce complete tissue
damage, each vegetable sample was frozen at -18°C and thawed at room temperature. This cycle was repeated two times to ensure complete tissue damage.

\[ Z_{tret} = \frac{\sigma_{aim} - \sigma_{raw}}{\sigma_{fin} - \sigma_{raw}} \]  \hspace{1cm} (4.8)

Intact tissue (raw sample) has a \( Z_{tret} \) index of 0, which approaches to 1 with the development of tissue damage.

4.2.10. Analysis of the treatment intensity: Pressure and thermal dosage

Pressure and thermal dosage were calculated to compare the process intensity between POTS, ohmic heating, and PATP. Pressure dosage \( (P_d, \text{MPa} \cdot \text{s}) \) and thermal dosage \( (T_d, \text{°C} \cdot \text{s}) \) were defined as the amount of pressure and heat applied to a sample from the initial \( (t_i) \) to final time \( (t_f) \) during treatment, respectively. \( P_d \) (4.9) and \( T_d \) (4.10) were estimated with an integration of pressure versus time and temperature versus time plot, as follows.

\[
P_d = \int_{t_i}^{t_f} P dt = \frac{(P_0 + P_1) \cdot \Delta t}{2} + \frac{(P_1 + P_2) \cdot \Delta t}{2} + \frac{(P_2 + P_3) \cdot \Delta t}{2} + \cdots + \frac{(P_{n-1} + P_n) \cdot \Delta t}{2} \]  \hspace{1cm} (4.9)

\[
T_d = \int_{t_i}^{t_f} T dt = \frac{(T_0 + T_1) \cdot \Delta t}{2} + \frac{(T_1 + T_2) \cdot \Delta t}{2} + \frac{(T_2 + T_3) \cdot \Delta t}{2} + \cdots + \frac{(T_{n-1} + T_n) \cdot \Delta t}{2} \]  \hspace{1cm} (4.10)
The above function was calculated using trapezoidal numerical integration of MATLAB software (Version 7.9.0.529, Mathworks Inc., MA, USA).

4.2.11. Statistical analysis

Fisher’s least-significant difference (LSD) procedures were used for a multiple comparison among treatments at the 95% confidence interval (P<0.05). In the process intensity and quality analysis, each POTS, ohmic heating, and PATP treatment was replicated 5 times at each experimental condition.

4.3. Results and discussion

4.3.1. Pressure, temperature, electric field, and electrical conductivity histories during POTS, ohmic heating and PATP

Figure 4.2 presents the pressure, temperature and electric field histories of carrot sample during POTS. Data are shown for the 3 min holding time as a representative example. In the POTS, sample temperatures increased from 25°C to 43-45°C via compression heat during pressure build up to 600 MPa (Fig. 4.2-a, A↔B). Subsequently, sample was ohmically heated to 105°C at 600 MPa with an applied AC electric field (30 V/cm) at 60 Hz (Fig. 4.2-b, B↔C). POTS resulted in a mean temperature come-up time of 1.45±0.31 min from 25°C to 105°C at 600 MPa. During extended pressure and temperature holding times, the target temperature was held at 105°C (Fig. 4.2-a, C↔D) by regulating the electric field strength (15-30 V/cm) at 600 MPa (Fig. 4.2-b, C↔D). The current ranged from 0.1 to 0.6 A depending on the sample temperature and electric field
adjustment. After each POTS holding time (0, 1, 3, and 5 min), temperature decreased to 70–73°C with decompression to 0.1 MPa (Fig. 4.2-a, D↔E) and interrupted electric fields (Fig. 4.2-b, D↔E). The use of thermocouple probes in an ohmic heating environment can lead to problems such as electrical discharges and signal perturbations inside the heating device (Zell et al., 2009). In this study, electrical insulation of the thermocouple feed through wire was critical to prevent direct contact between the thermocouple wire and top closure of the pressure vessel. It was checked using a digital multimeter (Model 179, Fluke, Everett, WA, USA) every POTS run. The electrical resistance of mega ohm levels between thermocouple and vessel closure was selected for criteria of good insulation.

Figure 4.3 compares the temperature profiles during POTS (600 MPa, 30 V/cm, 105°C), PATP (600 MPa, 0 V/cm, 105°C), and ohmic heating (0.12 MPa, 30 V/cm, 105°C). Among the technologies, POTS had minimal temperature rise time (1.45±0.31 min), followed by ohmic heating (3.58±0.81 min) and PATP (6.84±0.49 min). PATP also included a pre-heating step (≈ 6 min) prior to PATP treatment. Ohmic heating takes relatively longer to elevate temperatures from an initial 25°C to a 45°C compared to that of POTS. Figure 4.4 compares the changes in in situ electrical conductivity versus temperature, between POTS and ohmic heating. Relatively low electrical conductivities (0.032–0.110 S/m) were observed at the initial stage of ohmic heating (Fig. 4.4, A↔B). A rapid temperature rise, above sample temperatures of 50°C (B↔C), was possible with significantly increased electrical conductivity.

Whereas, in POTS, sample temperature initially increased from 25°C to 43–45°C within 20 s with the heat of compression during pressure build up to 600 MPa (Fig. 4.2-a, A↔B). Therefore, POTS-treated samples rapidly passed through the low range of
electrical conductivity stage, without ohmic heating, and then was ohmically heated at the higher electrical conductivity ranges under pressure (Fig. 4.2-b, B↔C). Heat generation rates obtained during ohmic heating are proportional to the electrical conductivity at a constant voltage gradient (Lima et al., 2001). The electrical conductivities of fruit and vegetable tissues linearly increase with temperature, during ohmic heating (Sarang et al., 2008). The rate of ohmic heating is directly proportional to the square of the electric field strength and the electrical conductivity (Shynkaryk et al., 2010). In the PAPT, relatively slow temperature rises were attributed to a slow preheating period (~5.9 min at 0.1 MPa) before loading the sample into the pressure vessel.

4.3.2. Effect of POTS, preheating, PATP, and ohmic heating on the force-deformation curve in the puncture test and textural parameters

Figure 4.5 presents the typical force-deformation curve of the carrot after preheating, PATP, POTS, and ohmic heating. Depending upon the intensity of the treatment, different treatments decreased puncture force and slope in comparison to that of raw samples (11.3 N).

Table 4.1 summarizes the influence of different treatments on textural parameters of HI (hardness index), SI (stiffness index), and CI (crunchiness index). Intact (raw) tissue indicates the HI and SI of 1, which decreases to 0 with the development of tissue damage. CI of intact tissue, estimated from both HI and SI, expresses 2, and then approaches to 0 with the development of textural transformations.

POTS and ohmic heating preserved better HI as compared to those of PATP. POTS, ohmic, and PATP decreased HI of carrot by 34, 44, and 55 % of its initial value at 5 min
holding time, respectively. POTS showed overall higher HI than ohmic heated carrot except for 0 min holding time. POTS minimized thermal abuse because of its rapid temperature rise through combined effects of pressure and electric field. In addition, the rapid cooling utilized in POTS carrots was possible with adiabatic cooling during decompression to 0.1 MPa. Minimized thermal abuse of POTS would mainly contribute the retention of hardness. Earlier studies reported the firming texture of vegetable products followed by high pressure treatment (Basak and Ramaswamy, 1998; Kasai et al., 1995). High pressure processing contributes the unaltered hardness of carrots with inhibited β-elimination of pectin. Beyond biochemical reaction, elevated pressure induces more compact tissue structure in vegetables with elimination of air and increased density. Density of carrot increased from 1047 kg/m³ at 0.1 MPa and to 1195 kg/m³ at 600 MPa (Min et al., 2010). In our study, ohmic treated carrot also showed a rapid temperature rise through internal heat generation without thermal lag. HI of carrot was decreased by only 5% during ohmical temperature come-up to 105°C within 3.58±0.81 min. Further hardness loss gradually occurred by 44% during 5 min holding time. In our best knowledge, limited number of studies directly compared textural qualities of vegetable products between ohmic heating and conventional thermal processing at equivalent temperature (105°C) and holding time (0-5 min). Thermal only treatment (105°C, 0.1 MPa) decreased the hardness of carrot by ≈95%, possibly due to slow temperature come-up time (47.5 min) (Nguyen et al., 2010). On the contrary, in our study, ohmic heated carrot at 105°C for 5 min showed minimal hardness loss (44%). Ohmic heating is a very effective technology of agricultural products to improve texture through rapid heating (Imai et al., 1995). At moderate electric field ohmic heating under 100 V/cm, electric
field does not induce the high degree of tissue damage associated with electroporation (Lebovka et al., 2005) as compared to thermal effects. In our study, it is postulated that textural transformation of carrot samples would mainly result from elevated temperature rather than moderate electric field (30 V/cm) during ohmic heating. Yang et al. (1997) reported a good retention of textural qualities of vegetable products (carrot, potato, mushroom, green pea, and corn) suspended in the soups during ohmic heating at sterilization temperature (≈130°C) for 34-70 s holding time. Preheating decreased HI of carrot by 14-16%; however, there was no significant difference in HI between 0 and 5 min preheating holding time (P>0.05). PATP decreased the HI of carrot by 55% during 5 min holding time. Preheating at 85°C decreased the puncture force (hardness) of carrot (Nguyen et al., 2010). When the carrot pieces were only preheated as a treatment (60°C, 40 min), the hardness already decreased to about 60% of its original hardness (Lemmens et al., 2009). This suggests that carrot tissue is initially damaged during the preheating period before PATP. In the thermal dosage, PATP showed the highest thermal dosage among treatments, which is attributable to a slow preheating period (≈5.8 min). Preheating incurred a thermal dosage of 19074±688°C·s during a temperature rise to 67°C for 5.8 min. This amounted to 80, 64, 43, and 33% of the thermal dosages associated with PATP treatments at 0, 1 min, 3 min, and 5 min, respectively. Subsequently, PATP treated samples would result in a lower HI when compared to other treatments.

All of the treatments significantly decreased the SI (stiffness) by 87-94% as compared to that of raw sample (Table 4.1). High pressure treated carrot samples became more rubbery (i.e., reduced stiffness) but retained hardness (Nguyen et al., 2010). In the vegetable products, a decrease in stiffness could be due to a shift toward a more rubbery
state or a reduction of turgor pressure (Georget et al., 2004). Thermally processed carrots exhibited significantly smaller modulus of deformability and stiffness values than raw carrots (Ahmed et al., 1991). Thermal softening of carrots results from membrane damage and the associated turgor pressure loss (De Roeck et al., 2010; Greve et al., 1994). In our study, preheating (67°C) was enough to induce significant changes in the SI before PATP. Stiffness of carrots was found to decrease within 5 s during cooking (Georget et al., 2004). Stiffness of carrots decreased by 88% during 5 min thermal processing at 0.1 MPa (Ormerod et al., 2004). These authors proposed that lowering of stiffness in the thermal processing is primarily due to the reduction in the strength of the binding between the cells. The thermal degradation of the pectin may also cause the porosity of the tissue to increase, since the pectin matrix is believed to control cell wall porosity and this may be reflected in the lower stiffness of the tissue (Carpita and Gibeaut, 1993). In our study, it is postulated that major textural transformation was resulted from changes in stiffness rather than hardness when vegetable products are treated under elevated pressure, electric field, or heat.

Previous research has reported that the combination of both hardness (HI) and stiffness (SI) into a unified parameter, crunchiness index (CI), gives a better overall indication of textural transformations during pressure treatment (Nguyen et al., 2010). Preheating, in our study, decreased CI from 2 close to 1 before PATP, which means half of the crunchiness was lost in the preheating stage. It is postulated that preheating initiates the tissue damage (half loss of CI); and, therefore, PATP results in more textural failure than POTS and ohmic heating. There was no significant difference between the CI of POTS and ohmic heated carrots (P>0.05). This implies there are no detrimental effects
due to combined pressure, electric field, and thermal energy (POTS) on textural qualities compared to only using electric field and thermal energy (ohmic heating). In the POTS and ohmic heating, $CI$ gradually decreased as a function of holding time. This was not true of PATP, where there was no significant difference in $CI$ among the different holding times (1, 3, and 5 min) in the PATP carrots. Previously researchers have proposed that pressure softening is only pressure-level dependent due to the instantaneous nature of the initial loss (Basak and Ramaswamy, 1998). In our study, major tissue disintegration would primarily result from elevated temperature (105°C) rather than electric field (30 V/cm) and pressure treatment (600 MPa). Only an ohmic heating frequency of 60 Hz was investigated. Further studies are required to investigate the effects of ohmic heating frequencies (kHz and MHz levels) and varying electric field strength on textural qualities and heating characteristics during POTS.

4.3.3. Effect of POTS, preheating, PATP, and ohmic heating on color

Table 4.2 summarizes the influence of different treatments on color values. Raw samples had $L^*$, $a^*$ and $b^*$ values of 56.10, 27.34 and 48.00, respectively. Goncalves et al. (2010) reported $L^*$, $a^*$ and $b^*$ values in raw carrot as 53.20, 21.0 and 49.64, respectively. Decreasing $L^*$ values were observed with preheating, PATP and POTS-treated carrots compared to raw and ohmic heated carrots. This has previously been observed with PATP and reduced $L^*$ values when compared to raw samples (Nguyen et al., 2010). In our study, $a^*$ values were not significantly different between treatments or when compared to raw samples. Carotenoids are important for the orange, yellow and red colors in fruits and vegetables. Carotenoids are fairly pressure stable (Bhale, 1997; de
Ancos et al., 2000; Fernandez Garcia et al., 2001); and carotenoid pigments are fairly stable up to sterilization temperature (Abbatemarco and Ramaswamy, 1994; Jen, 1989). Pasteurization as well as mild temperature sterilization caused only limited β-carotene isomerization; whereas, temperatures at which significant disruption of the food matrix occurred (>120°C), increased β-carotene isomerization was observed (Lemmens et al., 2010; Marx et al., 2003). High pressure processing also have shown the limited negative effect on β-carotene concentration and isomerization in carrots (Knockaert et al., 2011; McInerney et al., 2007). Our results of a* values suggests that β-carotene in carrot samples were not significantly influenced by either pressure, electric field, or temperature, or their combination effects.

Preheating induced lower b* values than any other treatments. During blanching (70-90°C), as processing time and temperature increased, carrots became darker, corresponding to a decreased b* value (Goncalves et al., 2010). Longer holding times resulted in more total discoloration (ΔE); however, there was no significant difference between treatments. In our study, no distinct discoloration effects were observed with combined electric field and pressure in the POTS treated carrots as compared to other treatments.

4.3.4. Effect of POTS, preheating, PATP, and ohmic heating on tissue disintegration index (Z_tret)

Figure 4.6 showed the changes in Z_tret, which were estimated with electrical conductivity measurement of carrot at 0.1 MPa and 25°C after each treatment. There was no significant difference in tissue disintegration (Z_tret) between POTS and PATP. The
results suggest that no combined effect of pressure, electric field, and thermal energy (POTS) on textural failure in the carrot tissue as compared to that of pressure and thermal energy (PATP). Ohmic heating induced lower $Z_{tret}$ value (lesser tissue damage) than those of POTS and PATP; however, it was statistically significant only at 5 min holding time ($P<0.05$). Preheating increased $Z_{tret}$ up to 0.2, which indicates preheating is a source of textural degradation before pressure treatment. Increasing $Z_{tret}$ indices resulting from POTS and PATP could imply more dense structure of carrot tissue after pressure treatment beyond tissue damage. Pressure treatment is known to increase the cellular density of vegetables by eliminating air from the tissue during decompression (Basak and Ramaswamy, 1998).

4.3.5. Pressure and thermal dosage

Figure 4.7 compares the calculated pressure and thermal dosage among POTS, PATP, ohmic heating, and preheating treatments. POTS showed significantly higher-pressure dosage (MPa·s) than PATP. During POTS, sample was ohmically heated to 105°C within $1.45\pm0.31$ min under 600 MPa. Therefore, POTS carrots had longer pressure holding time, which amounts to a temperature rise time of $1.45\pm0.31$ min at 600 MPa as compared to that of PATP.

The rapid temperature rise generated in POTS resulted in a lower total thermal dosage than ohmic heating. POTS combines the heat of compression and electric field for ohmic heating at 600 MPa. This combination accelerated the temperature rise using the synergy of pressure, electric field, and thermal energy. PATP showed the highest thermal dosage, followed by preheating, ohmic heating, and POTS. The highest thermal dosage of
PATP resulted from a slow preheating (≈5.8 min) at 0.1 MPa. Preheating induced a thermal dosage of 19074±688°C·s during a temperature gradient to 67°C. This accounted for 80%, 64%, 43%, and 33% of the thermal dosages seen with PATP treatments at 1s, 1 min, 3 min, and 5 min, respectively. In our POTS experimental conditions, the combined effect of pressure, electric field and thermal energy minimized thermal abuse in samples.

4.4. Conclusions

In this study, a POTS movable electrode cell and relevant equipment were successfully developed to heat (30 V/cm) carrot samples ohmically up to 105°C under an elevated pressure of 600 MPa. POTS resulted in a significantly rapid temperature rise when compared to ohmic heating and PATP. Minimized thermal abuse was detected with POTS due to the combining of pressure and electric field, and the resultant rapid heating. In addition, rapid cooling of POTS carrot samples was possible with adiabatic cooling during decompression to 0.1 MPa. POTS had better textural retention compared to carrots treated with PATP. Our findings indicated that the requisite preheating at 0.1 MPa induces the textural changes before PATP. Whereas, POTS did not require a slow preheating period, eliminating a major source of thermal abuse. POTS did not show a synergistic effect of electric field and pressure on textural degradation compared to treatments with only electric field application (ohmic heating). Specifically, pressure treated carrots showed better retention of hardness. This study demonstrates the potential use of POTS technology for producing a high quality, shelf stable, low-acid vegetable product using a synergy of pressure, electric field, and thermal energy.
Nomenclature

\( A \) cross sectional area (m\(^2\))

\( a^* \) redness

\( b^* \) yellowness

\( CH \) heat of compression (\(^\circ\)C/MPa)

\( CI \) crunchiness index

\( \Delta E \) total discoloration

\( F \) maximum puncture force (N) of the force deformation curve

\( Grad \) slope of the force deformation curve

\( HI \) hardness index

\( I \) current (A)

\( k \) cell constant (m\(^{-1}\))

\( L \) length (m)

\( L^* \) lightness

\( P \) pressure (MPa)

\( SI \) stiffness index

\( T \) temperature (\(^\circ\)C)

\( t \) time (s)

\( V \) voltage (V)

\( v \) volume (m\(^3\))

\( Z \) tissue disintegration index

\( \sigma \) electrical conductivity (S/m)

\( \text{Subscript} \)

\( atm \) atmospheric pressure, 0.1 MPa

\( d \) dosage

\( f \) final

\( ft \) frozen-thawed sample

\( i \) initial

\( is \) in situ

\( o \) ohmic

\( p \) elevated pressure, 600 MPa

\( raw \) raw sample

\( tret \) treated sample

\( 0,1,2,3,\ldots,n \) subinterval in trapezoidal numerical integration

\( 20\% \) slope at 20\% of the maximum puncture force
References


<table>
<thead>
<tr>
<th>Textural parameters</th>
<th>Holding time (min)</th>
<th>Control</th>
<th>Preheating</th>
<th>PATP</th>
<th>POTS</th>
<th>Ohimc</th>
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Table 4.1. Comparison of textural parameters (hardness index, stiffness index, crunchiness index) of carrots influenced by POTS (600 MPa, 30 V/cm, 105°C), ohmic heating (0.12 MPa, 30 V/cm, 105°C), preheating (0.1 MPa, 0 V/cm, 67°C), and PATP (600 MPa, 0 V/cm, 105°C). a–f Means (±Standard deviation) with a different letter in each index are significantly different at P<0.05.
Table 4.2. Effect of preheating (0.1 MPa, 0 V/cm, 67°C), POTS (600 MPa, 30 V/cm, 105°C), PATP (600 MPa, 0 V/cm, 105°C), and ohmic heating (0.12 MPa, 30 V/cm, 105°C) on the color values and discoloration (ΔE) of carrots. *Means (±Standard deviation) with a different letter in each index are significantly different at P<0.05.
Figure 4.1. Cross sectional view of the movable electrode POTS cell.
Figure 4.2. Evolution of sample temperature versus pressure (a) and electric field (b) during POTS at 600 MPa and 30 V/cm electric field application (temperature: - - - - , pressure: - - - - , electric field: --- ).
Figure 4.3. Comparison of sample temperature during POTS (600 MPa, 30 V/cm, 105°C), PATP (600 MPa, 0 V/cm, 105°C), and ohmic heating (0.12 MPa, 30 V/cm, 105°C); POTS: ——; PATP: ---; ohmic heating: ————.
Figure 4.4. Electrical conductivity changes of carrot during POTS (♦) at 600 MPa and 30 V/cm and ohmic heating (○) at 0.12 MPa and 30 V/cm.
Figure 4.5. Force-deformation curves of preheated (0.1 MPa, 0 V/cm, 67°C), POTS (600 MPa, 30 V/cm, 105°C), PATP (600 MPa, 0 V/cm, 105°C), and ohmic heating (0.12 MPa, 30 V/cm, 105°C) treated carrots.
Figure 4.6. Effect of preheating (0.1 MPa, 0 V/cm, 67°C), POTS (600 MPa, 30 V/cm, 105°C), PATP (600 MPa, 0 V/cm, 105°C), and ohmic heating (0.12 MPa, 30 V/cm, 105°C) on tissue disintegration index ($Z_{tret}$) of carrots estimated after treatments.

- 0 min.
- 1 min.
- 3 min.
- 5 min.

Means (±Standard deviation) with a different letter are significantly different at P<0.05.
Figure 4.7. Comparison of pressure dosage (a) and thermal dosage (b) on carrots among preheating (0.1 MPa, 0 V/cm, 67°C), POTS (600 MPa, 30 V/cm, 105°C), PATP (600 MPa, 0 V/cm, 105°C), and ohmic heating (0.12 MPa, 30 V/cm, 105°C).

Means (±Standard deviation) with a different letter are significantly different at P<0.05.
Chapter 5: Combined Pressure-Heat-Electric Field Inactivation of \textit{Bacillus amyloliquefaciens} and \textit{Bacillus stearothermophilus} Spores

Abstract

This study was conducted to investigate the impact of pressure ohmic thermal sterilization (POTS) treatment on inactivation of \textit{Bacillus amyloliquefaciens} and \textit{Bacillus stearothermophilus} spores. 0.1\% NaCl solutions (pH 5.0 & pH 7.0), green pea puree (pH 6.1), carrot puree (pH 5.0), and tomato juice (pH 4.1) were inoculated with spores and each set (initial count in the suspension, $2.5 \times 10^8$ cfu/ml) was loaded in a custom made POTS cell. Samples were ohmically (50 V/cm) treated at 105$^\circ$C - 600 MPa. \textit{B. amyloliquefaciens} and \textit{B. stearothermophilus} spores suspended in 0.1 \% NaCl (pH 7.0) were inactivated by 4.6 and 5.6 log during 30 min holding time, respectively. \textit{B. amyloliquefaciens} and \textit{B. stearothermophilus} spores suspended in tomato juice at pH 4.1 were reduced by 3.1 and 4.8 log during 10 min holding time. Lowering pH of food matrices accelerated the inactivation for both spores. Weibull model better described the inactivation kinetics of both spores than linear model. POTS showed its potential to inactivate the pressure-thermal resistant bacterial spores.

\textit{Key words:} pressure ohmic thermal sterilization (POTS), ohmic heating, electric field, \textit{Bacillus amyloliquefaciens}, \textit{Bacillus stearothermophilus}, low-acid food
5.1. Introduction

High pressure processing (up to 600 MPa, at or near ambient temperature) is effective in inactivating a variety of pathogenic and spoilage vegetative bacteria (Balasubramaniam and Farkas, 2008). However, bacterial spores are more pressure resistant at or near ambient temperatures (Patteron, 2005). The pressure in combination with elevated temperatures (100-120°C) can be used for efficient inactivation of bacterial spores. Many researchers demonstrated that combined pressure-heat treatment can be lethal to bacterial spores such as *Bacillus amyloliquefaciens* and *Bacillus stearothermophilus* (Ahn et al., 2007; Rajan et al., 2006ab; Ratphitagsanti et al., 2009; Ratphitagsanti et al., 2010).

Similarly, the application of electric field during ohmic heating also induces the lethal effect to bacterial spores. The basic principle of ohmic heating relies on the dissipation of electrical energy into heat, resulting in internal energy generation followed by temperature increase (Sastry, 2003). It is generally accepted that microbial efficacy of ohmic processing is primarily thermal in nature (Sastry, 2008). Beyond thermal effects, electric current also induces additional sublethal injuries of microorganisms (Palaniappan et al., 1990). For example, electric current would induce the germination of spores and result the enhanced lethality beyond thermal effects (Cho et al., 1999).

At present, very limited knowledge is available for combined lethal effects of pressure, electric field, and heat on bacterial spores. The objective of this study is to investigate the effects of POTS on synergistic inactivation of pressure thermal resistant *B. amyloliquefaciens* and *B. stearothermophilus* spores through selected combinations of
pressure, electric field, and heat. The endospores of *B. amyloliquefaciens* and *B. stearothermophilus* were selected as target microorganism. *B. amyloliquefaciens* spores are chosen due to its high pressure-thermal resistance (Rajan et al., 2006a; Margosch et al., 2004b). *B. stearothermophilus* spores are commonly implicated in spoilage of canned food known as 'flat sour', and spores of this organism are traditionally used in the evaluation of the sterilizing processes of low-acid canned foods (López et al., 1996ab).

5.2. Materials and methods

5.2.1. Preparation of spores

*B. amyloliquefaciens* TMW 2.479 Fad 82 spores were selected due to their resistance to combined pressure-heat treatment (Rajan et al., 2006a; Ratphitagsanti et al., 2009). The preparation procedure of *B. amyloliquefaciens* spore crop preparation was similar to our previous study (Ratphitagsanti et al., 2009). Briefly, the cultures were aerobically incubated in trypticase soy broth supplemented with 0.1% yeast extract (TSBYE; Difco, Becton Dickinson, Spakrs, MD, USA) at 32°C for 24 hr. Cultures from the second transfer in TSBYE was used in spore production. 100 µl of *Bacillus amyloliquefaciens* culture was spread on trypticase soy agar supplemented with 0.6% yeast extract (TSAYE; Difco, Becton Dickinson, Spakrs, MD, USA). Plates were incubated at 32°C for 10 to 14 days. The spores were harvested after 95% of the sporulation (determined using a microscope). 10 ml of sterile distilled water was poured on the surface of inoculated plates, and then spores were scrapped with a sterile glass rod. The recovered spores were washed five times by repeated centrifugation in sterile water with various speeds ranging from 2,000 to 8,000 × g for 20 min at each speed and 4°C.
Spore pellets were resuspended in sterile deionized water to obtain approximately $10^9$ spores per ml. The suspension was sonicated for 10 min (SM275HT, Crest, ETL Testing Laboratory, Corland, NY) to remove clumps and heated at 80°C for 10 min to destroy any remaining vegetative cells. The spore suspension was stored at 4°C until used.

Spore suspensions of *B. stearothermophilus* ATCC 7953 were prepared as described by Sala et al. (1995) and Rajan et al. (2006b). Sporulation was carried out on a nutrient agar (NA; Difco, Becton Dickinson, Spakrs, MD, USA) with supplement of 500 mg/kg dextrose (BD Difco) and 3 mg/kg manganese sulfate (Fisher Scientific, Pittsburgh, PA, USA). The inoculated plates were incubated at 55°C for 7 to 10 days before harvesting the spores. When they reached more than 95% sporulation, the plates were flooded with water and the spores were scraped, washed five times by differential centrifugation ranged from 2,000 to 8,000 × g for 20 min each at 4°C, and resuspended in the sterile deionized water. The suspension was treated with lysozyme (100 mg/ml for 30 min) and trypsin (200 mg/ml for 2 h) to reduce interfering cell debris. After the enzyme treatments, the spore suspension was washed 4–5 times more and then the spore pellet was diluted to approximately $10^9$ cfu/ml using deionized water. The spore suspension was stored at 4°C until used.

5.2.2. Preparation of spore carrier solution and food matrices

The effects of POTS treatment on the two bacterial spores were investigated using NaCl solutions with different pH 7.0 and 5.0 as well using three different food matrices. 0.1% NaCl solution was prepared using demineralized water (pH 7.0, OSU Chemistry Store, Columbus, OH, USA) and NaCl (Fisher Chemical, Certified, ACS, Pittsburgh, PA,
USA). Food grade citric acid (anhydrous powder, FCC; Tate & Lyle, Decatur, IL, USA) was used to adjust the pH of sterilized 0.1% NaCl solution to prepare spore carrier solution with two different pH levels (7.0, 5.0). The pH adjusted 0.1 NaCl solution was sterilized by filtration with 0.22 μm-pore-size membrane filter (Fisher scientific, Pittsburg, PA, USA). Citric acid is a weak organic acid often used as food additives in foods (Casadei et al., 2000; Ratphitagsanti et al., 2010). The pH values of the solutions were measured using a pH meter (AccumetR_model 15, Fisher scientific, Pittsburg, PA, USA). Food matrix effects were tested using carrot puree, green pea and tomato juice. Carrot puree (Gerber products company, Fremont, MI, USA), green pea (Gerber products company, Fremont, MI, USA), and tomato juice (Campbell Soup Company, Camen, NJ, USA) samples needed for the experiments were purchased from a local market in large quantities. The pH and electrical conductivity values of the carrier solution and the representative foods were summarized in Table 5.1.

5.2.3. Pressure generating system

A laboratory scale pressure test system (26190, Harwood Engineering, Walpole, MA, USA) was used. The pressure generating system rated up to 1000 MPa with a pressure intensifier (SA-10-6-.875FGD-150K, Harwood Engineering, Walpole, MA, USA). Pressurization rate was approximate 30 MPa/s. Food grade propylene glycol (60% concentration, SAFE-T-THERM®, Houghton Intl. Inc., Valley Forge, PA, USA) was used as a pressure transmitting medium. The vessel top closure (C-7435, Harwood Engineering, Walpole, MA, USA) had conical cavities for high pressure feed through electrical wires and thermocouple.
5.2.4. Microbial POTS cell and experimental set-up description

Figure 5.1 describes the customized movable electrode POTS cell designed for microbial inactivation experiment. POTS cell utilized food grade components. Outer polyethylene double pouch helped to prevent surrounding pressure medium enter into the POTS cell during the experiments. POTS cell essentially consisted of the cylindrical polycarbonate spore suspension holder (12.7 mm inner diameter, 15.9 mm outer diameter, 43.6 mm length, 43103, US Plastics, Lima, OH, USA), a stationary electrode at one end, and a movable electrode at the other end. Both stationary and movable electrodes were made of platinum plated titanium to minimize electrochemical reactions of the titanium electrode (Samaranayake and Sastry, 2005; Tzedakis et al., 1999). The stationary electrode was 12.7 mm diameter on the face. It was fitted into an ultem electrode plug (Ultem®, GE Plastics, Pittsfield, MA, USA) with a buna O-ring seal (No.007, AS568A standard). Platinum plated stainless steel screw (M2.5-0.45, metric, ANSI/ASME) was threaded into the electrode plug, and then jointed to the back side of the stationary electrode for electrical contact. The movable electrode had a face diameter of 12.6 mm, and weep hole thread (M 2.5-0.45, metric, ANSI/ASME) was bored on the center. The weep hole allowed the removal of air from the POTS cell after spore suspension loading. The ultem electrode plug housed a movable electrode, which had a central cavity for a O-ring tightened weep hole screw. Electrical wire crimp-on snap connector was soldered on both ends of stationary and movable electrode. This design facilitated the connection to conical high pressure feed through wire of vessel closure. The high pressure feed through wire (B-11632, Harwood Engineering, Walpole, MA, USA) and K-type thermocouple (B-5491CD, Harwood Engineering, Walpole, MA, USA) were silver soldered to the high
pressure steel cone. The high pressure steel cone was jointed into conical cavity of the pressure vessel closure which can hold the internal pressure up to 800 MPa. The steel cone of the high pressure feed through wire and K-type thermocouple were electrically insulated using customized conical polypropylene tubing. Each soldered part was electrically insulated using heat shrink tubing and epoxy to prevent short circuiting and current loss to the pressure transmitting fluid. Teflon coated K-type thermocouple (0.51 mm diameter, TFAL/CY-020, Omega engineering, Stamford, CT, USA) mounts at the inner wall of polycarbonate sample holder to monitor the temperature profiles during POTS. The position of thermocouple was selected as the coldest region over all of the POTS cell geometry in the consideration of heat loss to surrounding. A variable transformer (POWERSTAT®, Bristol, CT, USA) provided the electric field of 50 V/cm across the spore suspension during POTS studies. A voltage (VTR-002B, OhioSemitronics Inc., Hilliard, OH, USA) and current transducer (CTR-010X5, OhioSemitronics Inc., Hilliard, OH, USA) were utilized to monitor the voltage and current profiles. Measured voltage, current, and temperature were recorded to data logger (34970A, Agilent Technologies, Santa Clara, CA, USA). Isolation transformer (25491 S-83871, OLSUN, Richmond, IL) was utilized to minimize the electric field effect on thermocouple during ohmic heating. Safety fuse (2.5 A, 270-1008, Radioshack, Forth Worth, TX, USA) and grounding were installed to prevent the electrical shock hazards in case of short circuit under pressure.

5.2.5. Calculation of POTS cell parameters
During POTS experiments, the voltage, current data were recorded to monitor *in situ* electrical conductivity of test samples ($\sigma_i$). $\sigma_i$ was determined from the cell constant ($k_p$), current ($I$), and voltage ($V$) according to Rieger (1994).

\[
\sigma_i = k_p \times \frac{I}{V_p} = \frac{L_p}{A_p} \times \frac{I}{V_p}
\]

The cell constant ($k_p$) was calculated based on the electrode distance ($L_p$) and inner cross sectional area ($A_p$) of polycarbonate POTS cell as follows.

\[
A_p = \left( \frac{V_p}{V_{atm}} \right)^{2/3} \times A_{atm}
\]

Initial electrode distance ($L_{atm}$) was 0.0145 m at 0.12 MPa. Compressibility of test suspensions was assumed to be similar to that of water. Compressibility of water is readily available from NIST Reference Fluid Thermodynamic and Transport Properties Data Base (Lemmon et al., 2010) and used in this calculation. Accordingly, under pressure, the electrode gap might have theoretically reduced to 0.0127 m. Cross sectional area of the POTS cell ($A_p$) was calculated taking into account the volumetric ratio of
polycarbonate tube at atmospheric and target pressure (Min et al., 2007; Warfield, 1967). Above calculations estimated the polycarbonate POTS cell $k_p$ at 600 MPa as 95.16 m$^{-1}$.

Similarly, the cell constant at atmospheric pressure ($k_{atm}$) of the test cell was calculated as 114.46 m$^{-1}$.

**5.2.6. Spore suspension loading into POTS cells**

Prior to experiments, POTS electrode and polycarbonate sample holder were sterilized in an autoclave. 0.2 ml of each spore suspension (2.5 ×10$^9$ CFU/mL) and 1.8 ml of sterilized 0.1% NaCl or food matrix solution were aseptically loaded into the POTS cells. Before loading the spore suspension, it was sonicated for 10 min to separate the clumped spores (Ratphi tagsanti et al., 2009). The movable electrode was mounted at the end of the POTS cell maintaining initial distance of 0.0145 mm from the stationary electrode. The weep hole was finally sealed with an O-ring (No.003, AS568A standard) tightened screw (M2.5-0.45, metric, ANSI/ASME) after the sample loading and air removal. O-ring assembly (No.012, AS568A standard) on the groove of the movable plug enabled hermetrical travel of the movable electrode along the vertical direction in the POTS cell under pressure.

**5.2.7. POTS experimental procedure**

First, the sample (held at 25ºC) was pressurized up to 600 MPa. Then, constant electric field strength of 50 V/cm (AC electric field at 60 Hz) was applied on the samples; subsequently, temperature rose to the target temperature of 105ºC at 600 MPa. The samples were then treated at 105ºC for various holding times (0, 1, 5, 10, 20, 30 min) through adjusting the electric field (0-50 V/cm). A holding time of 0 min means that
sample temperature ohmically reached 105°C at 600 MPa, and then was immediately removed for enumeration of surviving spores. After specific holding time, samples were depressurized. The spore suspensions were kept at refrigerated conditions until enumeration (usually within 2 hours after treatment). POTS cell was also used to conduct ohmic heating experiments at 105°C for various holding times (0, 1, 5, 10 min). Nominal pressure of 0.12 MPa was generated to prevent the boiling of spore suspension at 105°C.

5.2.8. Enumeration of surviving spores

POTS treated *B. amyloliquefaciens* and *B. stearothermophilus* spore suspensions were enumerated (with and without heat shock) to evaluate the POTS treatment effect on spore germination. First, 1 ml of the POTS treated spore suspension was serially diluted in sterilized 0.1% peptone water (9 ml). For *B. amyloliquefaciens*, the aliquots of the appropriate dilutions were pour-plated on TSAYE. The plating was done in duplicates. The plates were incubated at 32°C for 48 hr. *B. stearothermophilus* spores were pour-plated on nutrient agar (NA) and then incubated at 55°C for 48 hr. Colonies were counted (*N_{POTS}* ) on a dark field Quebec colony counter (Leica Microsystems, Richmond Hill, Ontario, Canada).

To understand the extent of spore germination during POTS, a portion of the first dilution of POTS spore suspension was heat shocked at 80°C for 15 min to kill the germinated and sensitized spores that might have occurred during the treatment (Ratphitagsanti et al., 2010; Rodriguez-Palacios and LeJeune, 2011). Subsequently, the heat shocked suspension was enumerated (*N_{POTS+HT}* ) as before. The percentage of germinated spore (*G_s*) was estimated using the following relationship.
5.2.9. Modeling of inactivation kinetics

The kinetics of the microbial reduction by POTS were explained as follows.

5.2.9.1. First-order kinetics

First-order model assumes a linear relationship between microbial population reduction during the process time at constant treatment (pressure, heat, electric field), where $N_0^*$ is the spore count after POTS (temperature and pressure) and ohmic (temperature) come-up time, $N$ the number of survivors after holding time, $t$ the holding time, $D$ the decimal reduction time (min).

$$
\log \frac{N}{N_0^*} = \frac{-t}{D} 
$$

(5.4)

5.2.9.2. Weibull model

Weibull model assumes that cells and spores in a population have different resistances and a survival curve is just the cumulative form of a distribution of lethal agents (Chen and Hoover, 2003), where $b$ and $n$ are the scale and shape factors, respectively (Peleg and Cole, 1998).
\[
\log \frac{N}{N_0} = -bt^n
\]  \hspace{1cm} (5.5)

The advantages of this model are that it explains both downward concave survival curves \((n > 1)\) and upward concave curves \((n < 1)\) while providing simplicity and robustness (Buzrul and Alpas, 2004).

5.2.10. Statistical analysis

Each POTS treatment was repeated three times at each holding time condition. The data were analyzed using SAS, 9.1.3, software (SAS Inst. Inc., Cary, NC, USA). Fisher’s least-significant difference (LSD) procedures were used for a multiple comparison among treatments at the 95% confidence interval \((P<0.05)\).

5.3. Results and discussion

5.3.1. Pressure, temperature, and electric field history during POTS and ohmic heating

Figure 5.2 presents representative pressure, temperature and electric field histories of \(B.\ amyloliquefaciens\) spore suspended 0.1\% NaCl solution at pH 7.0 during POTS. During pressure come-up time, the sample temperature increased from 25°C to 43-45°C (Fig. 5.2a, A↔B). No electric field was applied across the sample during this time interval. Upon reaching the target pressure, sample was ohmically heated up to 105°C at 600 MPa (Fig. 5.2a, B↔C) through application of AC electric field strength (50 V/cm) at 60 Hz (Fig. 5.2b, B↔C). Thus, it only took about 1.26±0.05 min for the sample to
increase from 25°C to 105°C due to the synergy coming from thermal effects of compression heat during high pressure processing and internal heat generation as a result of ohmic heating. During extended pressure and temperature holding time, the target temperature of 105°C was maintained by controlling the electric field strength (Fig. 5.2b, C↔D). After specific POTS holding time, temperature rapidly decreased to 59-62°C through decompression to 0.1 MPa (Fig. 5.2a, D↔E). During the decompression, the electric field was also turned off (Fig. 5.2b, D↔E). It is worth pointing out that care was taken to minimize / eliminate electric field noise on the thermocouple reading using an isolation transformer in the circuit. In addition, an electrically insulated thermocouple was installed through the top closure (Fig. 5.1). A digital multimeter (Model 179, Fluke, Everett, WA, USA) measured the resistance between thermocouple feed through wire and top closure every POTS run. The mega ohm levels of electrical resistance were selected as a criteria indicating good insulation. Whenever the resistance decreased below mega ohm level, thermocouple feed through wires were re-insulated and installed. Application of elevated pressures was useful for reducing the thermal come-up time during POTS (1.26±0.05 min) than ohmic heating (2.12±0.16 min) (Fig. 5.3a). The in situ electrical conductivity of the test sample during POTS was higher than ohmic heating alone (Fig. 5.3b). The rate of ohmic heating is directly proportional to the square of the electric field strength and the electrical conductivity (Shynkaryk et al., 2010). Min et al. (2007) reported increased in situ electrical conductivity of food materials under elevated pressure. Authors hypothesized that ionic composition, ionic movement and viscosity of the liquid would increase the electrical conductivity of liquids at high pressure. In both POTS and ohmic heating, electrical conductivity linearly increased as a function of
elevated temperature up to 105°C. Earlier researchers reported a linear increase of electrical conductivity in fruit juices and purees with increase in temperature during ohmic heating (Icier and Illicali, 2005; Palaniappan and Sastry, 1991). The increased electrical conductivity of liquids at elevated temperature was attributed to the reduced drag of ion movements (Palaniappan and Sastry, 1991).

5.3.2. Effect of POTS on the inactivation of *B. amyloliquefaciens* and *B. stearothermophilus* spores suspended in 0.1% NaCl solution

Figure 5.4 shows the survival rates of *B. amyloliquefaciens* and *B. stearothermophilus* spores suspended in 0.1% NaCl solution at pH 7.0 subjected to POTS treatment. *B. amyloliquefaciens* and *B. stearothermophilus* spores were inactivated by 0.1 and 1.7 logs during POTS come-up time (1.26±0.05 min). At the end of 30 min POTS holding time, *B. amyloliquefaciens* and *B. stearothermophilus* spores were inactivated by 4.6 and 5.6 log. Within the range of experimental conditions of the study, combining pressure, electric field and thermal effects appears to be synergistically effective in inactivating bacterial spores. POTS yielded more lethal effects on *B. stearothermophilus* spores than *B. amyloliquefaciens* spore. Similar phenomena were observed in the resistance of *B. amyloliquefaciens* and *B. stearothermophilus* spores to pressure assisted thermal processing (Margosch et al., 2004a).

*B. amyloliquefaciens* and *B. stearothermophilus* spores suspended in 0.1% NaCl solution at pH 7.0 subjected to POTS treatment (600 MPa, 105°C, 50 V/cm) had *D* values of 7.03 and 8.51 min, respectively (Table 5.2). Even though *B. stearothermophilus* spore is highly thermally resistant, application of elevated pressures appears to decrease
spore resistance (Rajan et al., 2006b). Ahn et al. (2007) reported the $D$ value of *B. amyloliquefaciens* as 11.2 min suspended in deionized water during thermal processing at 0.1 MPa and 105°C. *Bacillus amyloliquefaciens* spores suspended in egg patties showed $D$ value of 24 min during thermal processing at 0.1 MPa and 105°C. Feeherry et al. (1987) reported the $D$ value of *B. stearothermophilus* spores as 62.04 min in the thermal processing at 0.1 MPa and 112.8°C.

In this study, Weibull model provided better estimation of inactivation kinetics with higher $R^2$ and $A_f$ close to 1 as compared to first order kinetic model within the range of our experiment. *B. stearothermophilus* spore showed higher scale factor ($b$ values) of 2.52 compared to that of *B. amyloliquefaciens* spore as 0.74. Rajan et al. (2006b) reported $b$ and $n$ values of *B. stearothermophilus* spore as 1.96 and 0.30 during pressure assisted thermal processing at 600 MPa and 105°C, respectively.

Enhanced sporicidal effects of POTS were observed in both spores by lowering the pH of 0.1% NaCl solution to 5.0 using 100 mM citric acid (Fig. 5.5). POTS at pH 5.0 reduced the population of *B. amyloliquefaciens* and *B. stearothermophilus* spores by 3.0 and 4.1 log during 10 min holding time, respectively. Citric acid (100 mM, pH 5.0) was found to be the most effective in enhancing spore inactivation during PATP (Ratphitagsanti et al., 2010). Increased lethality of spores at lower pH environment during high pressure processing might have resulted from promoted reversible exchange of endogenous ions ($\text{Ca}^{2+}, \text{Na}^+, \text{K}^+, \text{Mg}^{2+}, \text{Mn}^{2+}$) with exogenous protons, transforming the native spores into the so called H-spores which have exchangeable cations replaced with $\text{H}^+$ (Marquis and Bender, 1985; Paredes-Sabja et al., 2007; Rode and Foster, 1966). The undissociated form of the acid is more cell-wall permeable, which later dissociates within
the cell wall of microorganism, resulting in cell death (Balasubramanian and Balasubramaniam, 2010; Beales, 2003).

At 105°C, ohmic heating did not show a significant lethal effect on both spores. Populations of *B. amyloliquefaciens* and *B. stearothermophilus* spores were reduced by 1.11±0.05 and 1.08±0.19 log during the ohmic heating (0.12 MPa, 105°C) for 10 min.

Weak acid buffer solutions can have pressure-dependent pH changes due to pressure-dependent ionization equilibria (Min et al., 2011). The authors reported that citric acid buffer (570 mM) lowers its pH from 4.5 at 0.1 MPa to 4.0 at 400 MPa possibly due to increased ionization of citric acid and hydrogen ion concentration. In this study, due to technical difficulties, the *in situ* pH values of spore suspension were not measured under combined pressure, electric field, and elevated temperature during POTS.

5.3.3. Effect of POTS on the inactivation of *B. amyloliquefaciens* and *B. stearothermophilus* spores suspended in selected food matrices

Figure 5.6 shows the log inactivation of *B. amyloliquefaciens* and *B. stearothermophilus* spores suspended in green pea puree, carrot puree, and tomato juice after 10 min holding time of POTS. For all the food matrices tested, POTS was more sporicidal on *B. stearothermophilus* than *B. amyloliquefaciens*. Lethal effect of POTS was the most significant for both spores suspended in tomato juice at pH 4.1 (P<0.05). Spores in acidic tomato juice were highly susceptible than the other food matrices during POTS. It was reported that acidification of the heating medium from 7.0 to 5.0 causes a 3-fold reduction of the thermosteresistance of *B. stearothermophilus* spores (López et al., 1996ab). It was also known that spores became more resistant in low-acid foods than in
high acid foods during heat and high pressure treatment (Vercammen et al., 2011). Foods may contain number of organic and amino acids that can act as buffering agents with different pKa values (Ratphitagsanti et al., 2010). In low-pH foods, heating is more lethal to microorganisms in the presence of acetic, propionic acid, and lactic acids than phosphoric or citric acid at the same pH (Ray, 2004). Adjusting the product formulation by modifying its pH can be effective in enhancing spore lethality during high-pressure processing (Balasubramanian and Balasubramaniam, 2010). In this study, carrot puree at pH 5.0 shows the log inactivation by 2.80 and 4.11 for *B. amyloliquefaciens* and *B. stearothermophilus* spores after 10 min of POTS holding time. The populations of *B. amyloliquefaciens* and *B. stearothermophilus* spores suspended in 0.1% NaCl at pH 5.0 were reduced by 2.95 and 4.06 log inactivation at the equivalent POTS holding time.

5.3.4. Germination of survived *B. amyloliquefaciens* and *B. stearothermophilus* spores after POTS and ohmic heating

Table 5.3 summarizes the percentage of germinated spores suspended in various medium after POTS and ohmic heating. POTS treated *B. stearothermophilus* spores showed higher germination rate than those of *B. amyloliquefaciens* at earlier stages. POTS induced more than 90% germination of *B. stearothermophilus* spores suspended in 0.1% NaCl solution (pH 7.0 and 5.0) at 0 min holding time. The higher germination of *B. stearothermophilus* spores at the initial holding time suggests its susceptibility to POTS as compared to *B. amyloliquefaciens* spores. Ohmic heating of 10 min induced lower germination for both spores as compared to those of POTS at equivalent holding time. In our POTS study, synergistic effect of pressure (600 MPa) was observed and there was enhanced germination of *B. amyloliquefaciens* and *B. stearothermophilus* spores when
the electric field (50 V/cm) and pressure (600 MPa) were combined. Subjecting the spores to lower pressures (100–200 MPa) at ambient temperatures can result in spore germination, possibly due to activation of the germinant receptors (Wuytack et al., 2000). However, at higher pressures (500–600 MPa), spores that lack nutrient receptors trigger germination rapidly, suggesting that these pressures somehow open the spore’s Ca\(^{2+}\)–dipicolinic acid (DPA) channels (Paidhungat et al., 2002; Setlow, 2003). Spores of various *Bacillus* species are also germinated by pressures of 100 to 600 MPa (Gould and Sale, 1970; Knorr, 1999; Paidhungat et al., 2002; Wuytack et al., 2000). Once germinated, the spores lose their characteristic resistance and can be inactivated by a subsequent lethal treatment such as pressure or heat, and high pressure processing (Vercammen, 2011). In our study, more rapid germination of *B. amyloliquefaciens* spores were observed at 0.1% NaCl solution at pH 5.0 than at pH 7.0. Vercammen et al. (2011) reported the germination of *B. coagulans* spores being stimulated at low pH during high pressure (100–800 MPa) and heat treatments (40ºC). Addition of organic acids such as citric acid may facilitate the germination in PATP-treated *B. amyloliquefaciens* spores (Ratphitagsanti et al., 2009). Our earlier studies showed no significant germination of *B. amyloliquefaciens* spores suspended in deionized water at pH 7.0 during PATP at 600 MPa and 105ºC (Ratphitagsanti et al., 2009; Ratphitagsanti et al., 2010). Whereas, our POTS study showed significant germination of both *B. amyloliquefaciens* and *B. stearothermophilus* spores. Spore germination in the presence of electric field under ohmic heating conditions was previously reported (Cho et al., 1999; Lyng and Mckenna, 2011).
*B. amyloliquefaciens* and *B. stearothermophilus* spores suspended in food matrices showed lower germination than those of 0.1% NaCl solution. Various food constituents such as fat, proteins, minerals and sugars can increase microbial resistance to pressure (Black et al., 2007; Molina-Hoppner et al., 2004; Rendueles et al., 2011). It has been previously reported that the presence of solutes such as sugars at high levels, i.e., >10%, increases microbial baroresistance (Black et al., 2007; Palou et al., 1997). In general, dormant spore is germinated, and then subsequent inactivation occurs by cortex hydrolysis and release of the dipicolonic acid (DPA) (Black et al., 2007; Ratphitagsanti et al., 2009; Wuytack et al., 2000). It is hypothesized that the constituents in the food matrices functioned as inhibiting agents for germination to POTS treatment. In the effects of pH, tomato juice (pH 4.1) and carrot puree (pH 5.0) showed higher germination of spores and subsequently enhanced lethality than green pea puree at higher pH of 6.1. The organic acids (acetic acid, citric acid, lactic acid) induced germination of *B. amyloliquefaciens* spores in the pressure assisted thermal processing (Ratphitagsanti et al., 2009).

5.4. Conclusions

*B. amyloliquefaciens* and *B. stearothermophilus* spores suspended in 0.1% NaCl at pH 7.0 were inactivated by 4.6 and 5.6 log during 30 min treatment of POTS (600 MPa, 50 V/cm, and 105°C). Whereas, ohmic heating at 0.1 MPa and 105°C under similar holding times did not show significant lethal effects of both spores. Increasing acidity appears to synergistically reduce the spore resistance during POTS. POTS treatment in the food matrices (green pea puree, carrot puree, and tomato juice) also showed the
sporicidal effects for *B. amyloliquefaciens* and *B. stearothermophilus* spores. *B. stearothermophilus* spores were more rapidly germinated at the initial holding time of POTS as compared to those of *B. amyloliquefaciens* spores. This would result in more susceptibility of *B. stearothermophilus* spores to POTS than *B. amyloliquefaciens* spores. Weibull model better described the inactivation kinetics of both spores than the linear model used in this study. In summary, POTS treatment can be effective in inactivating the pressure-thermal resistant bacterial spores.
### Nomenclature

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tbody>
<tr>
<td>$A$</td>
<td>cross sectional area ($m^2$)</td>
</tr>
<tr>
<td>$b$</td>
<td>scale factor in Weibull model</td>
</tr>
<tr>
<td>$D$</td>
<td>$D$ values (min)</td>
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<tr>
<td>$G$</td>
<td>germination</td>
</tr>
<tr>
<td>$I$</td>
<td>current (A)</td>
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<tr>
<td>$k$</td>
<td>cell constant ($m^{-1}$)</td>
</tr>
<tr>
<td>$L$</td>
<td>length (m), electrode distance (m)</td>
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<tr>
<td>$N$</td>
<td>number of spores (CFU/ml)</td>
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<tr>
<td>$P$</td>
<td>pressure (MPa)</td>
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<tr>
<td>$t$</td>
<td>time (min)</td>
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<tr>
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<tr>
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<tr>
<td>$\sigma$</td>
<td>electrical conductivity ($S/m$)</td>
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**Superscript**
- $n$ shape factor in Weibull model

**Subscript**
- $\text{atm}$ atmospheric pressure, 0.1 MPa
- $\text{HT}$ heat shock treatment
- $\text{is}$ in situ
- $\text{POTS}$ pressure ohmic thermal sterilization
- $p$ pressure
- $s$ spore
- $0$ initial spore count
References


<table>
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<tr>
<th>Suspension medium</th>
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<th>Electrical conductivity (S/m)</th>
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<td>5.0</td>
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Table 5.1. Electrical conductivity (S/m) and pH of spore suspended in 0.1% NaCl solution and food matrices before POTS.
Table 5.2. Kinetic parameters of the first order and Weibull models for POTS inactivation of *B. amyloliquefaciens* and *B. stearothermophilus* spores suspended in 0.1% NaCl solution (pH 7.0 & 5.0)*.

* POTS holding time did not include the pressure and temperature come-up time.
<table>
<thead>
<tr>
<th>Spore medium</th>
<th>Treatment</th>
<th>Holding time (min)</th>
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<th>B. stearothermophilus</th>
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<td>0.1% NaCl, pH 5.0</td>
<td>POTS</td>
<td>0</td>
<td>44.1±17.9^DE</td>
<td>94.1±0.8^AB</td>
</tr>
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<td></td>
<td>1</td>
<td>45.5±14.4^DE</td>
<td>93.4±5.3^AB</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>84.1±2.2^B</td>
<td>93.9±2.2^AB</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>89.9±5.4^AB</td>
<td>93.7±0.2^AB</td>
<td></td>
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<tr>
<td></td>
<td>Ohmic</td>
<td>10</td>
<td>3.9±0.8^F</td>
<td>50.2±8.5^D</td>
</tr>
<tr>
<td>Green pea puree, pH 6.10</td>
<td>POTS</td>
<td>10</td>
<td>34.6±5.6^E</td>
<td>69.1±18.8^C</td>
</tr>
<tr>
<td>Carrot puree, pH 5.04</td>
<td>POTS</td>
<td>10</td>
<td>51.2±8.6^D</td>
<td>69.1±9.9^C</td>
</tr>
<tr>
<td>Tomato juice, pH 4.08</td>
<td>POTS</td>
<td>10</td>
<td>52.2±6.3^D</td>
<td>65.9±8.9^C</td>
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Table 5.3. Percent germination in the surviving *B. amyloliquefaciens* (*N_0*: 2.5×10^8 cfu/ml) and *B. stearothermophilus* (*N_0*: 2.5×10^8 cfu/ml) spore populations suspended in 0.1% NaCl solution (pH 7.0 and 5.0), green pea puree, carrot puree, and tomato juice after POTS (600 MPa, 50 V/cm, 105ºC) and ohmic heating (0.12 MPa, 50 V/cm, 105ºC).

^A^–^F^Means (±Standard deviation) with a different letter are significantly different at P<0.05.
Figure 5.1. Cross sectional view of the movable electrode POTS cell designed for microbial inactivation studies.
Figure 5.2. Pressure, temperature, and electric field histories of *B. amyloliquefaciens* spores (N₀: 2.5×10⁸ cfu/ml) suspended in 0.1% NaCl solution during pressure ohmic thermal sterilization (temperature: — , pressure: - - - , electric field: - · - ·); A↔B: pressure come-up to 600 MPa; B↔C: application of electric field (50 V/cm) to ohmically increase the product temperature to 105ºC at 600 MPa; C↔D: POTS holding time at 105ºC and 600 MPa; D↔E: Decompression to 0.1 MPa with interrupted electric field.
Figure 5.3. Evolution of sample temperature (a) during POTS (600 MPa, 50 V/cm, 105°C, - - -) and ohmic heating (0.12 MPa, 50 V/cm, 105°C, — — —), and comparison of in situ electrical conductivity (b) between POTS (- - -) and ohmic heating ( — — —).
Figure 5.4. Experimental and predicted (as fitted by linear and Weibull model) survivor fraction of spores suspended in 0.1% NaCl solution at pH 7.0 during POTS (600 MPa, 50 V/cm, 105°C): (a) *B. amyloliquefaciens* (*N₀*: 2.5×10⁸ cfu/ml); (b) *B. stearothermophilus* (*N₀*: 2.5×10⁸ cfu/ml). Experimental: ○; Linear model: −−−−; Weibull model: −−−−.
Figure 5.5. Experimental and predicted (as fitted by linear and Weibul model) survivor fraction of spores suspended in 0.1% NaCl solution at pH 5.0 during POTS (600 MPa, 50 V/cm, 105°C): (a) *B. amyloliquefaciens* (*N₀*: 2.5×10⁸ cfu/ml); (b) *B. stearothermophilus* (*N₀*: 2.5×10⁸ cfu/ml). Experimental: ○; Linear model: ---; Weibull model: - - - - - - - - .
Figure 5.6. Log inactivation ($N/N_0$) of *B. amyloliquefaciens* ($\square$, $N_0$: $2.5 \times 10^8$ cfu/ml) and *B. stearothermophilus* ($\blacksquare$, $N_0$: $2.5 \times 10^8$ cfu/ml) spores suspended in green pea puree, carrot puree, and tomato juice after POTS holding time of 10 min (600 MPa, 50 V/cm, 105ºC).
Chapter 6: Conclusions

The study investigated combined pressure-heat-electrical field effects on selected low-acid foods and bacterial spores.

**In situ electrical conductivity changes in the vegetable tissues under pressure and instrumental texture analysis**

- *In situ* electrical conductivity changes of the vegetable samples (carrot, potato, red radish) were characterized at elevated pressure (200, 400, 600 MPa, and 25ºC). *In situ* electrical conductivities of all the tested vegetable samples increased as a function of pressure and holding time up to a certain threshold level. The electrical conductivity values did not change further depending on samples after specific holding time.

- Tissue disintegration indices (Z) in carrot and potato samples increased as a function of pressure up to 600 MPa. Z values of carrot and potato did not increase further after 5 min holding time. The effect of elevated pressure on Z values in red radish samples were not clear within the experimental conditions tested.

- *Crunchiness index* (CI) of carrot and potato decreased as a function of elevated pressure due to decrease in hardness and stiffness of pressure treated samples.
Empirical model fitting demonstrated the inverse relationship between $Z$ and $CI$ for carrot and potato. The relationship between $Z$ and $CI$ was fitted to a first and second order polynomial.

In situ electrical conductivity measurement was a useful tool for understanding the pressure-induced textural changes of vegetable tissues.

**Pressure Ohmic Thermal Sterilization (POTS): quality attributes of carrot**

- A prototype laboratory scale pressure ohmic thermal sterilization (POTS) apparatus was developed to investigate its potential for shelf stable low acid vegetable products and their quality attributes. Pressure ohmic thermal sterilization (POTS) uses the combined effects of pressure (600 MPa), electric field (30 V/cm), and heat (105°C) and their selected combinations. The technology combines the synergy of pressure (rapid temperature come-up with heat of compression as well as instant adiabatic cooling with decompression) and ohmic heating (uniform and rapid temperature increase).

- Within the experimental conditions of the study, POTS carrots showed better textural retention as compared to those of PATP treated carrots.

- There were no significant differences in color (CIELAB $L^*$, $a^*$, and $b^*$ values) among POTS, ohmic, and PATP treated carrots.

- This study demonstrates the potential use of POTS technology for producing a shelf stable low acid vegetable product using a synergy of pressure, electric field, and heat.
Combined pressure-electric field-heat inactivation of *Bacillus amyloliquefaciens* and *Bacillus stearothermophilus* spores

- The influence of pressure-electric field-heat and their combination on the inactivation of bacterial spores were investigated. Pressure thermal resistant bacterial spores of *B. amyloliquefaciens* and *B. stearothermophilus* were suspended in green pea puree (pH 6.1), carrot puree (pH 5.0), and tomato juice (pH 4.0) while sterile 0.1% NaCl solutions (pH 5.0 & 7.0) served as controls.
- *B. amyloliquefaciens* and *B. stearothermophilus* spores suspended in 0.1% NaCl at pH 7.0 were respectively inactivated by 4.6 and 5.6 log during 30 min treatment of POTS (600 MPa, 50 V/cm, and 105°C).
- POTS was more sporicidal on *B. stearothermophilus* spores than *B. amyloliquefaciens* spores. Whereas, ohmic heating at 0.12 MPa for 10 min did not significantly reduce both spores. Within the range of experimental conditions of this study, combined pressure-electric field-thermal effects resulted in synergistic inactivation.
- Increasing acidity of 0.1% NaCl solution to pH 5.0 synergistically reduce the spore resistance during POTS treatment.
- 10 min of POTS treatment reduced the *B. amyloliquefaciens* spores levels in green pea, carrot puree and tomato juice by 2.4, 2.8 and 3.1 logs, respectively. *B. stearothermophilus* spores were more susceptible to the treatment than *B. amyloliquefaciens* spores. *B. stearothermophilus* spore suspended in tomato juice was inactivated by 4.8 after 10 min POTS treatment. Spores in acidic tomato juice
were highly susceptible than the other food matrices during POTS. Weibull model better described the inactivation kinetics of both spores than linear model.
Bibliography


Norton, T, & Sun, D.W. (2008). Recent advances in the use of high pressure as an effective processing technique in the food industry. *Food and Bioprocess Technology*, 1, 2–34.


