Characterizing Vegetable Tissue Under Elevated Pressure Using *In Situ* Electrical Conductivity Measurement and Instrumental Analysis

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Abstract

A study was conducted to investigate the pressure-induced textural changes in selected low acid vegetable samples (carrot, potato, and red radish) using *in situ* electrical conductivity measurements. *In situ* electrical conductivity of the vegetable samples, as a function of pressure and holding time at 25ºC, was recorded every 30 s for 10 min under elevated pressures (200, 400, 600 MPa) using a custom made electrical conductivity cell. 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. The tissue disintegration index (*Z*) was calculated based on the *in situ* electrical conductivity data of the vegetable samples under pressure and those of raw samples. Sample hardness and stiffness were evaluated using an instrumental texture analyzer, and then calculated to a crunchiness index (*CI*). The relationship between *Z* and *CI* was investigated with empirical model fitting of the first and second polynomial. *Z* and *CI* had an inverse relationship within the range of experimental conditions studied. *In situ* electrical conductivity measurement was a useful tool to document the extent of vegetable tissue damage during high pressure processing.

Acknowledgement
I would like to thank my adviser Dr. V.M. (Bala) Balasubramaniam, and Dr. Sudhir Sastry for their help with this study.

1. Introduction

High pressure processing involves elevated pressures (400-600 MPa) used for microbial inactivation, enzymatic activation or inactivation, and alteration of food functionality at room temperature. High pressure effects only non-covalent bonds (hydrogen, ionic, and hydrophobic bonds) and has little effect on chemical constituents (Rastogi et al., 2007). Pressure treatment has minimal impact on food quality attributes such as flavor, color, and taste; and has minimal effects on nutrient content as compared to thermal processing. Several researchers have demonstrated microbial pasteurization or sterilization effects during pressure treatment. Specifically, the addition of heat with pressure treatment (500-700 MPa) enhanced the efficacy of spore inactivation to a greater degree than pressure treatment at room temperature (Ahn et al., 2007; Ananta et al., 2001; Okazaki et al., 2000; Rajan et al., 2006). Accordingly, high pressure processing is optimal to satisfy both emerging food safety issues and consumers' demands for minimally processed food.

Several commercial pressure-treated products, such as fruit jams, guacamole, sea food, and deli hams, are currently available. Fruits and vegetables are one of the major categories of pressure-treated products commercially available in the market (Tonello Samson, 2011). Food texture is one of the major considerations in defining the quality of fruits and vegetable. Microscopically, textural quality is also dependent on the cell wall and middle lamella polysaccharides (Van Buren, 1979). Nguyen et al. (2007) reported better hardness retention in pressure-treated carrots (at 600 MPa and 105ºC) than thermal-treated carrots (at 0.1 MPa and 100ºC). Pressure treatment before cooking (99.5ºC) was reported to improve the texture of vegetables and pressure-treated carrots

retained textural quality (Kasai et al., 1995). In the sensorial texture perception, there was no significant difference of the crunchiness between raw carrots and pressure-treated carrots (600 MPa, 2 min). Authors attributed this to pressure-induced biochemical changes. Studies done on vegetable tissue have indicated that thermal processing degrades pectin quality during cooking (Trejo Arya et al., 2007). Pectinmethylesterase (PME) activity would be the most probable reason for textural improvements under pressure (Basak and Ramaswamy, 1998). The action of PME 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 retains or increases PME activity in the vegetables (Ly-Nguyen et al., 2003). Pressure treatment increases the density of the cellular structure by eliminating air from the tissue (Basak and Ramaswamy, 1998).

However, the aforementioned studies utilized post-pressure processing evaluation of textural changes. Molecular level *in situ* evaluation may provide further insight into textural changes under pressure. Dielectric properties (electrical properties) of biological tissues provide information about tissue structure and composition (Kuang and Nelson, 1998). The electrical properties in biological matter enable the determination of morphological and structural properties cell systems (Angersbach et al., 2002). The authors evaluated the structural changes in vegetable membranes with electrical conductivity measurement after high pressure treatment. Various researchers have correlated the degree of tissue damage to changes in the electrical conductivity during ohmic heating or pulsed electric field (PEF) treatment (Bazhal et al., 2003; De Vito et al., 2008; Lebovka et al., 2002). External stress from pressure treatment could effect

membrane integrity or induce cellular leakage of electrolytes into intercellular areas (Angersbach et al., 2002; Trejo Araya et al., 2007).

The current study was proposed to evaluate *in situ* electrical conductivity changes in vegetable tissues undergoing high pressure processing and correlate the electrical conductivity values to instrumental quality attributes. The relationship between *in situ* assessment and instrumental analysis should provide a better understanding of textural changes in vegetables undergoing pressure treatment.

2. Materials and Methods

2.1. Sample preparation

Baby carrots, potatoes, and red radishes were purchased from a local market in large quantities to minimize the quality variations in the raw materials and stored at 4ºC for up to a week. Cylindrically-shaped extracts (11.7 mm diameter and 16 mm length) were prepared using a cork borer (Fisher Scientific, Pittsburgh, PA, USA).

2.2. Pressure generating system

A laboratory scale pressure test system (Harwood Engineering, 26190, Walpole, MA, USA) was used. The cylindrical pressure vessel interior dimensions were 25.4 mm diameter and 153 mm depth, which allows the temperature to be controlled through an external jacket. Pressurization rate was approximate 20 MPa/s. The top closure of the pressure vessel had a conical cavity for high pressure feed through wires and thermocouples. A temperature controlled propylene glycol circulated through an external

jacket to maintain the sample's temperature at 25ºC during the *in situ* electrical conductivity measurements.

2.3. In situ electrical conductivity measurement sensor

The cross sectional view of *in situ* electrical conductivity sensor was shown in Fig. 1. It was composed of an outer sample holder (19 mm diameter, 105 mm length, 43104, US Plastics, Lima, OH, USA) and inner sample holder (12.7 mm diameter, 50 mm length, 43102, US Plastics, Lima, OH, USA).

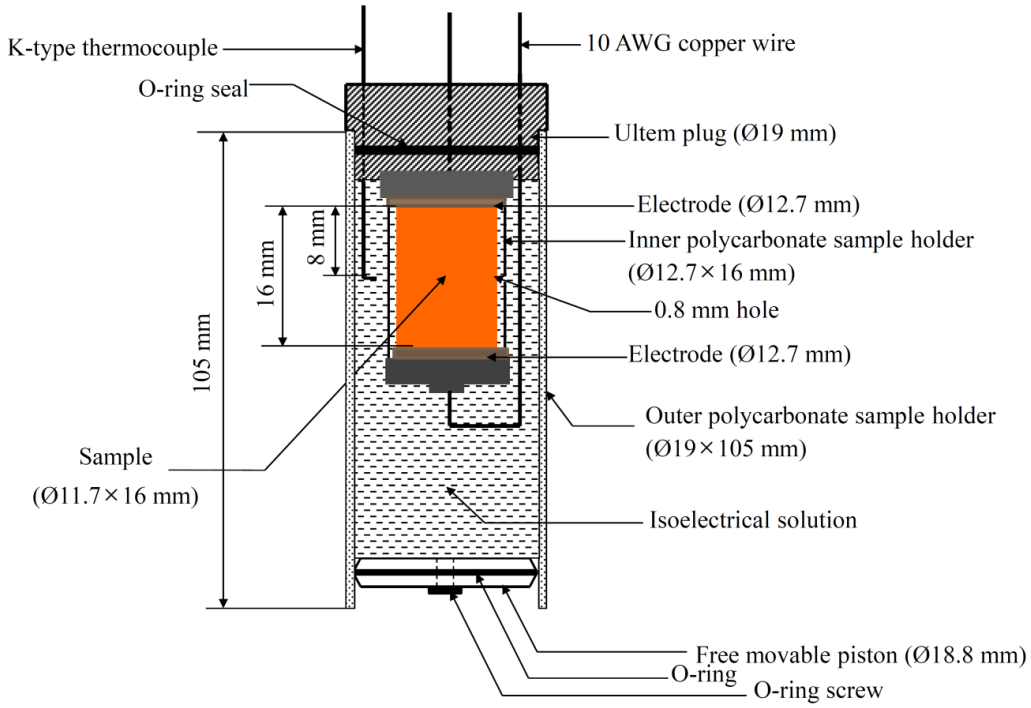


Fig. 1. Cross sectional view of the in situ electrical conductivity cell made for high pressure application.

Two platinum-plated titanium cylindrical electrodes were enclosed at both ends of the inner sample holder, separated by 16 mm distance. Ten AWG copper wires were soldered on each electrode and passed through the bored outer sample holder plug (Ultem®, GE Plastics, Pittsfield, MA, USA). The end of the copper wires were soldered to the high pressure electrical feed through and electrically insulated using heat shrink tubing (FIT 105, Alpha Wires, Elizabeth, NJ, 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 position of the thermocouple was aimed to avoid physically damaging the vegetable sample. The cavities of electrical wire in the sample holder were sealed with epoxy (Devcon, 1233, Glenview, IL, USA) to prevent 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 pressure medium to sample through vertical moving. 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 NaCl isoelectrical solution. Isoelectrical solution was made to have the same electrical conductivity as each vegetable sample, with demineralized water (97801, OSU Chemistry Store, Columbus, OH, USA) and NaCl (Fisher Chemical, Certified ACS, Pittsburgh, PA, USA). The isoelectrical solution had two main purposes. First, the solution allowed pressure equilibration between the inner and outer sample holders through a 0.8 mm hole on the inner sample holder. It also assured the contact resistance between electrode and sample (Sastry, 2005). The volume ratio of sample to isoelectrical solution was calculated as 0.85:0.15 (*v*/*v*) in the inner sample holder.

2.4. In situ electrical conductivity measurement of the 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 25ºC for a 10 min holding time. Considering compression heating (*CH*) of elevated pressure, vegetable samples were initially precooled (7-19ºC, T_i) at atmospheric pressure (P_{atm}) to maintain a sample temperature of 25ºC (T_p) after reaching the target pressure (P_p) during the experiment. ΔT_H is the temperature gain of the precooled sample from the surrounding medium during sample loading time into the pressure chamber.

$$T_i = T_p - \left(CH \times \frac{P_p - P_{atm}}{100} + \Delta T_H \right) \quad (1)$$

The *in situ* electrical conductivity values (σ) of the samples were determined using equation 2 from the knowledge of applied voltage (V), current (I), and cell constant (k_p) under pressure (Rieger, 1994). k_p was estimated from volumetric compressibility of polycarbonate polymers (Min et al., 2007; Warfield, 1967).

$$\sigma = k_p \times \frac{I}{V} \quad (2)$$

Figure 2 described the experimental set-up for *in situ* electrical conductivity measurement under pressure. A function generator (GFG-8216A, Chino Inc., 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 the ohmic heating and electropermeabilization effect from the applied electrical field. The frequency of AC sinusoidal was set as 1 kHz to prevent the double layer capacitance and polarizing effect on electrodes and inside the samples (Braunstein and Robbins, 1967; Lebovka et al., 2008).

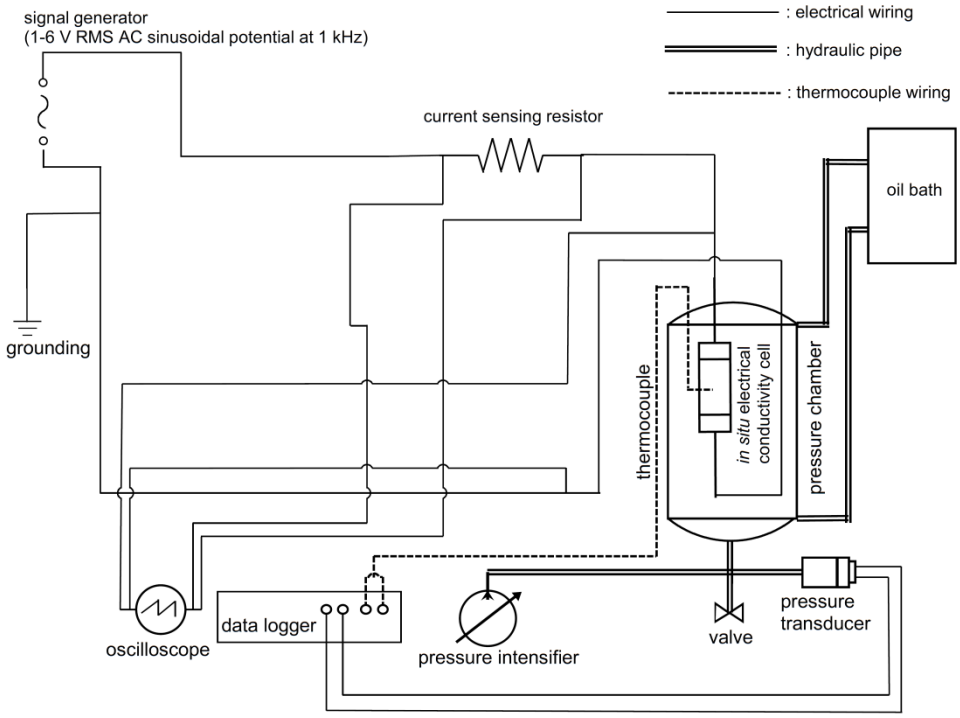


Fig. 2. Schematic diagram of the experimental set up for *in situ* electrical conductivity measurement under pressure.

An oscilloscope (TDS 5052, Tektronix, Beaverton, OR, USA) monitored the voltage across the samples, and current was calculated as the voltage across 150 Ω of the current-sensing resistor (WSC 2515, Vishay Inc., Malvern, PA, USA). This experimental set-up 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 minimize the effects of biological sample-to-sample variations.

2.5. Tissue disintegration index (Z)

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

$$Z = \frac{\sigma_{tret} - \sigma_{raw}}{\sigma_{ft} - \sigma_{raw}} \quad (3)$$

Z indices are well known to estimate 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; Lebovka, 2007a; Lebovka, 2007b; Lebovka, 2007c; Lebovka et al, 2008). Intact tissue (raw sample) has a *Z* index of 0, and then approaches 1 with the development of tissue damage. To induce tissue damage, each vegetable sample was frozen at -18ºC and thawed at room temperature. This cycle was repeated two times to ensure complete tissue damage.

2.6. Pressure treatment for instrumental texture analysis

Five cylindrical vegetable samples (Ø 11.7×16 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 0.85:0.15 (*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 (26190, Harwood Engineering, Walpole, MA, USA). Sample packages were precooled to compensate for compression heating. Precooling maintained a sample temperature of 25±2ºC, during pressure treatment. After pressure treatment, vegetable samples were taken out of the pouch, and lightly wiped with a paper towel for instrumental texture analysis. Two replications for each flexible pouch were performed comprising of 10 measurements in the instrumental texture analysis, after pressure treatment.

2.7. Instrumental texture analysis: Puncture test

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. A puncture test of each experiment was performed approximately 10 times to minimize biological variation between samples.

2.8. Crunchiness index (CI)

The crunchiness index (*CI*) was calculated from the puncture test results proposed by Nguyen et al. (2010). The force-deformation curve of the puncture test was fitted with a third order polynomial, and then texture parameters were extracted using Matlab software (Version 7.9.0.529, Mathworks Inc., MA, USA) as shown in equation 4. *CI* considered both textural parameters of the maximum puncture force (*F*) and the slope (*Grad*) in the force-deformation index. *F* and *Grad* indicate the hardness of the vegetable and stiffness (modulus), respectively

(Bourne, 2002; Dobraszczyk and Vincent, 1999; Gonzalez, 2009; Mohsenin, 1986). Both parameters were compared to those of raw samples to calculate the *CI*. Intact tissue indicates a *CI* of 2 which decreases to 0 with the development of tissue damage. Previous researcher 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% ($Grad_{20\%}$) of the maximum puncture in the force-deformation curve provides the best discrimination among processed samples' textural qualities and accordance with sensory evaluations. Therefore, $Grad_{20\%}$ was selected to evaluate the *CI* in our study.

$$CI = \frac{F_{tret}}{F_{raw}} + \frac{Grad_{20\%tret}}{Grad_{20\%raw}} \quad (4)$$

2.9. Statistical analysis and empirical modeling fitting

The data was analyzed using SAS, 9.1.3, software (SAS Inst. Inc., Cary, N.C., USA). Fisher's least-significant difference (LSD) procedures were used for a multiple comparison among treatments at the 5% confidence level ($P<0.05$). To evaluate the relationship between *Z* and *CI*, experimental results were empirically fitted to the first and second polynomial using SAS, as shown in equation (5, 6). Empirical modeling provided estimated coefficients (β_0, β_1, β_2) and probabilities of each coefficient (Pr value).

$$CI = \beta_0 + \beta_1 \cdot Z \pm \varepsilon \quad (5)$$

$$CI = \beta_0 + \beta_1 \cdot Z + \beta_2 \cdot Z^2 \pm \varepsilon \quad (6)$$

Figure 3 summarized the sequential experimental procedure in the *in situ* electrical conductivity measurement, instrumental texture analysis, and their empirical model fitting.

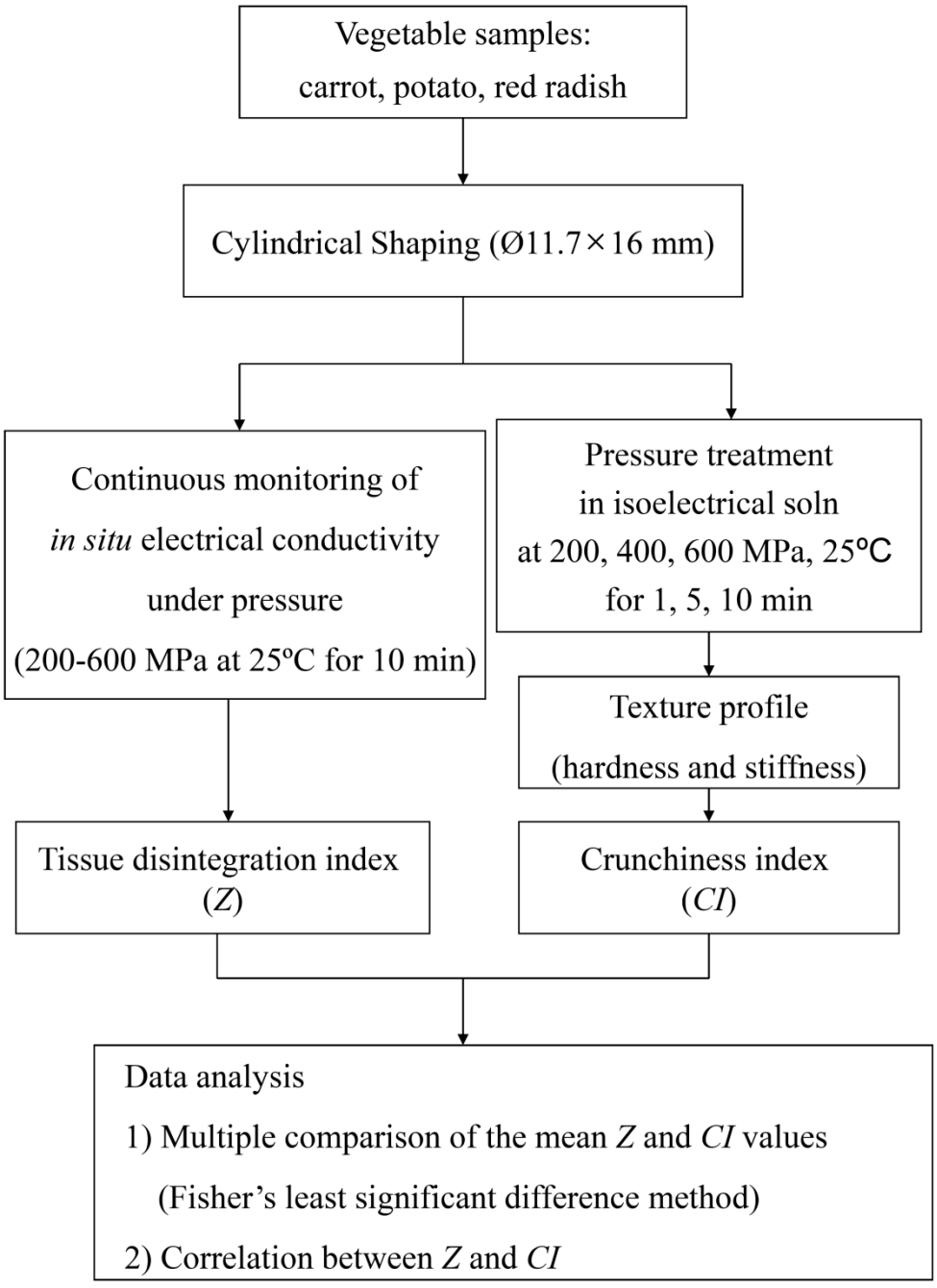


Fig. 3. Flow chart outlining sequential experimental procedure.

3. Results and discussion

3.1. Evaluation of ohmic heating and electropermeabilization

Figure 4 describes the changes in the electrical conductivity and temperature of carrot during an electric field application of 0.75 V/cm, at atmospheric pressure (0.1 MPa) and 25ºC, for 10 min. There were no significant changes in the electrical conductivity and temperature of the carrot at the electric field strength of 0.75 V/cm.

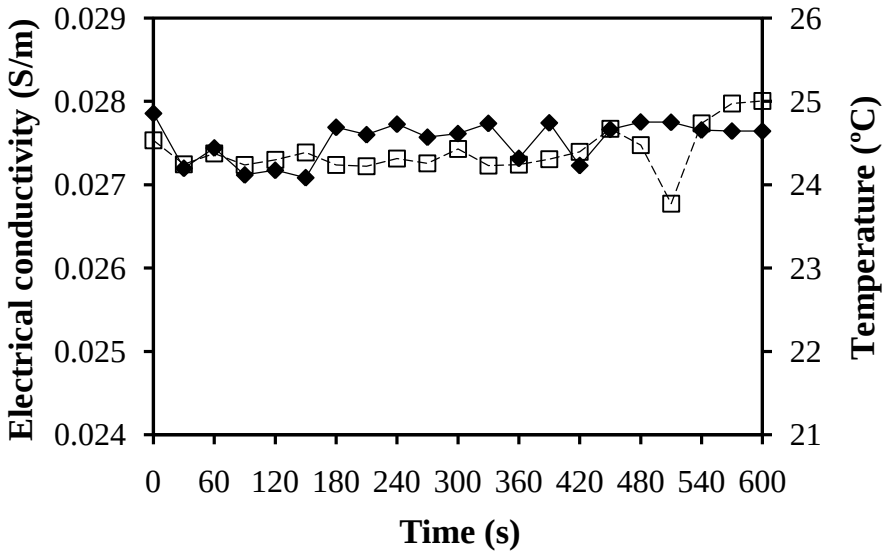


Fig. 4. Verification of ohmic heating and electropermeabilization in carrot at applied electric field strength of 0.75 V/cm (—◆— : temperature, —□—: electrical conductivity).

Application of moderate electric fields (1-1000 V/cm) could induce tissue damage and ohmic heating effects in fruits and vegetables (Bazhal et al., 2003; Kulshrestha and Sastry, 2003; Wang and Sastry, 2002; Weaver and Chizmadzhev, 1996). In this study, 0.75 V/cm of electrical field strength did not induce any electropermeabilization or ohmic heating effect, based on temperature and electrical conductivity measurements. We concluded that 0.75 V/cm of electric

field strength was appropriate for electrical conductivity measurements for the vegetable samples without incurring tissue damage.

3.2. Temperature history of carrot during in situ electrical conductivity measurements under pressure

The temperature history of carrot samples is given in Fig. 5. Temperature of all compressible materials increases during physical compression (heat of compression), depending on their compressibility and specific heat (Patazca et al., 2007; Ting et al., 2002). In the preliminary experiment, compression heating of carrot was determined as approximately 3ºC/100 MPa. Depending on the target pressure (P_p), carrot was precooled in ice water, to compensate for compression heating (CH), elevated pressure and temperature gain (ΔT_H) from surroundings.

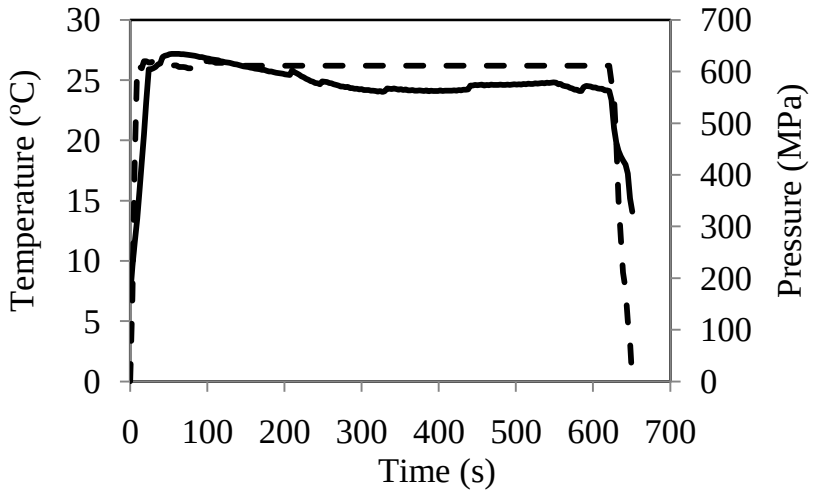


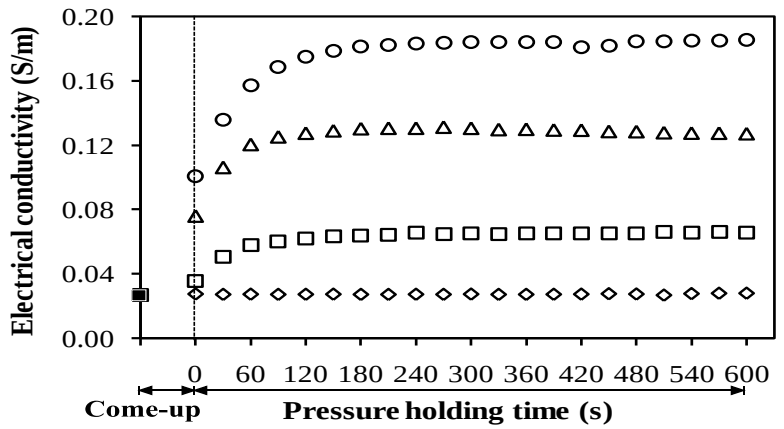
Fig. 5. Temperature history of carrot during electric conductivity measurement at 600 MPa (——: temperature, – – – -: pressure).

Although an effort was made to match the target temperature of 25ºC after pressure equilibration, there was a temperature deviation (25±2ºC) at the initial stage of pressure

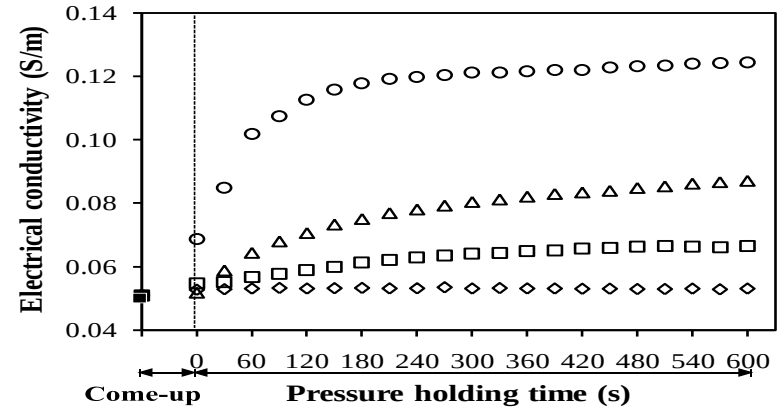
equilibration. Temperature is influential on the electrical conductivity of biological tissues. The electrical conductivity of vegetable and fruit linearly increases at elevated temperatures (Palaniappan and Sastry, 1991; Sarang et al., 2008; Tulsiyan et al., 2008; Wang and Sastry, 1997). Palaniappan and Sastry (1991) reported that the electrical conductivity of carrot increases from 0.033 S/m at 30ºC to 0.034 S/m at 32ºC based on a linear model of electrical conductivity. We concluded that a temperature deviation of 25±2ºC would be a minor effect on *in situ* electrical conductivity in comparison to that of elevated pressure. More detailed information is provided on this topic in a later section.

3.3. In situ electrical conductivity of the vegetable samples under pressure

Figure 6 shows the influence of elevated pressure and pressure holding time on the *in situ* electrical conductivity of each vegetable sample. All of the vegetable samples demonstrated increasing *in situ* electrical conductivity at elevated pressure and prolonged holding time. 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±0.003 S/m, at 0.1 MPa and 25ºC, and then reached up to 0.185±0.023 S/m, at 600 MPa and 10 min holding time. Wang and Sastry (1997) reported the electrical conductivity of raw carrot as 0.033 S/m, at 0.1 MPa and 30ºC. Freeze/thawed carrots had the highest electrical conductivity of 0.581±0.054 S/m, indicating complete tissue damage. 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.



(a)



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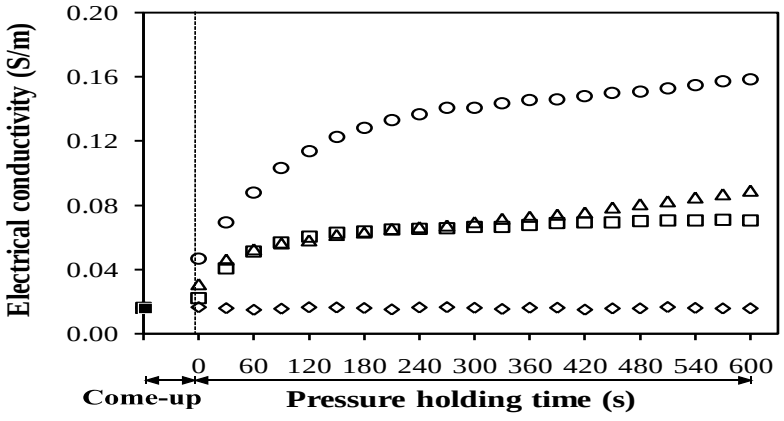


(c)

Fig. 6. *In situ* electrical conductivity of the vegetable samples during pressure treatment : (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 approximate 20 MPa/s.

Pressure treatment induces the transport of solutes from inside to outside the cell (and vice versa) with changed cell permeability (Préstamo and Arroyo, 1998). 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. Additionally, a more compact cellular structure and removal of air pockets would also increase electrical conductivity under pressure (Eshtiaghi and Knorr, 1993). Increasing *in situ* electrical conductivity was stabilized after approximately 3 min holding time at each pressure level. The stabilized electrical conductivity suggests that there is a certain threshold of pressure holding time to minimize further tissue damage in vegetables.

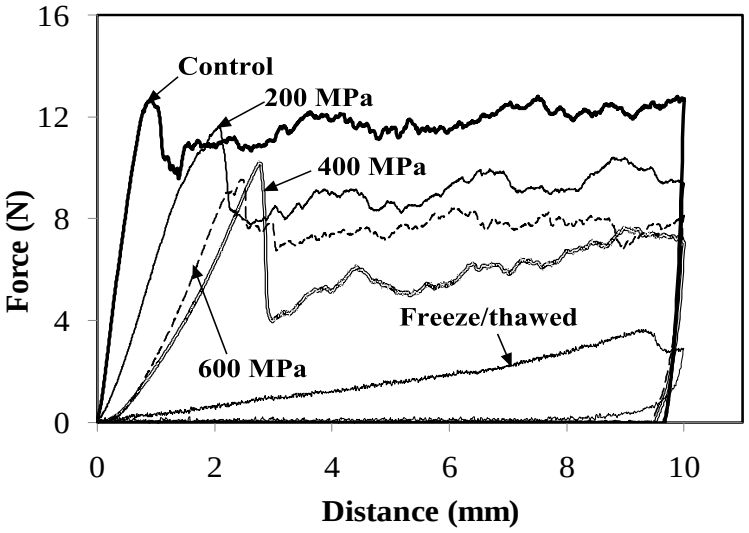
Potato samples showed a similar trend in *in situ* electrical conductivity under pressure to that of carrot. Initially, the electrical conductivity of raw potato was 0.051 ± 0.007 S/m, and then it reached 0.118 ± 0.001 S/m at 600 MPa and 10 min holding time. Angersbach et al. (2002) reported the electrical 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 freeze/thawed potato, indicating complete tissue damage. Elevated pressure and prolonged holding time increased *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 *in situ* observation of the diamond anvil cell. The degree of starch gelatinization 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 under pressure.

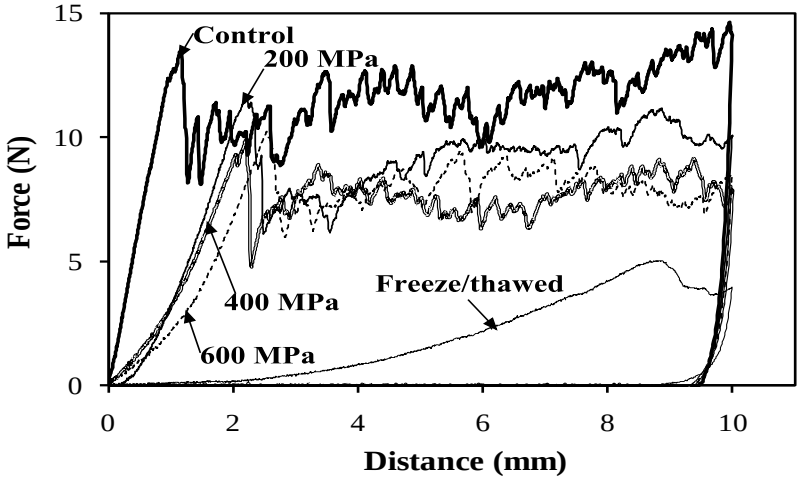
Raw red radish electrical conductivity was 0.016±0.002 S/m, and then increased up to 0.173±0.110 S/m at 600 MPa and 10 min holding time. Freeze/thawed radish had the maximum electrical conductivity of 0.279±0.002 S/m. Previous researchers reported an increase in electrical conductivity of Japanese white radish after a pressure treatment of 400 MPa at 25℃ 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.

3.4. Influence of pressure and holding time on texture quality: puncture tests

The force-deformation curve of the puncture test is given in Fig. 7. Puncture tests were conducted after pressure treatments at 200, 400, and 600 MPa for 1, 5, and 10 min (25ºC). Figure 7 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. 400 MPa and 600 MPa treatments resulted in less hardness and stiffness in the carrot tissue.



(a)



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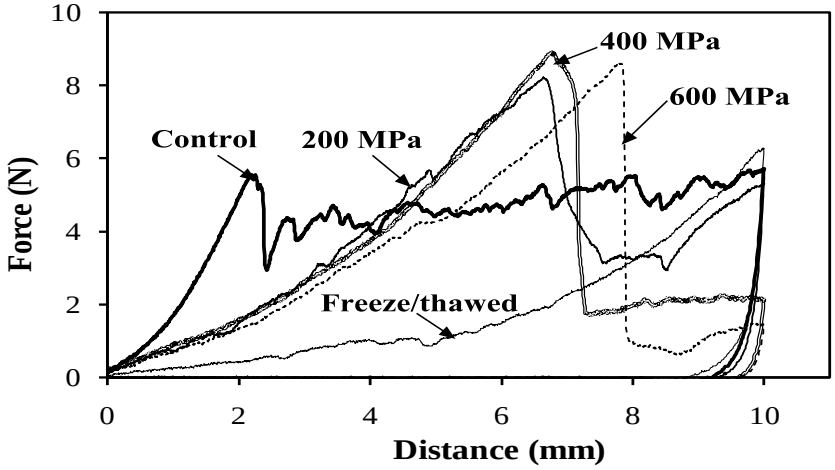


(c)

Fig. 7. Sample force-deformation curves for (a) carrot, (b) potato and (c) red radish.

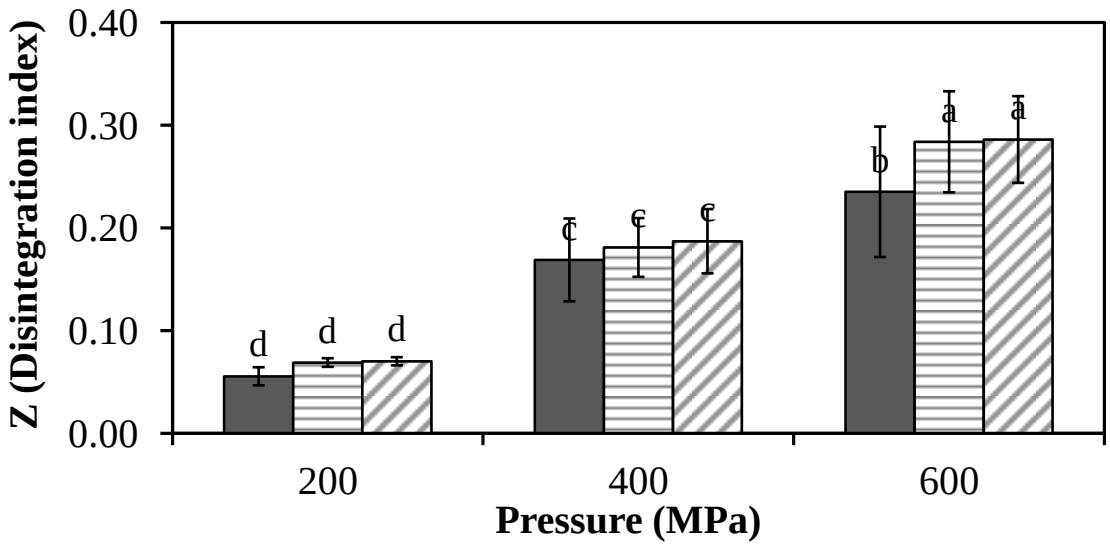
As expected, freeze/thawed samples had the greatest textural failure. Freezing and thawing plant tissues induces a gradual breakdown in the organization of the protoplasmic structure. In most cases, it results in the rupture of the plasmalemma with the subsequent loss of turgor pressure in cells (Delgado 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 freeze/thawed samples, in decreasing order. Whereas, red radish had different trends in the force-deformation curve to those of carrot and potato. Pressure-treated red radishes had higher puncture forces in the force deformation curve than those of controls. 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.

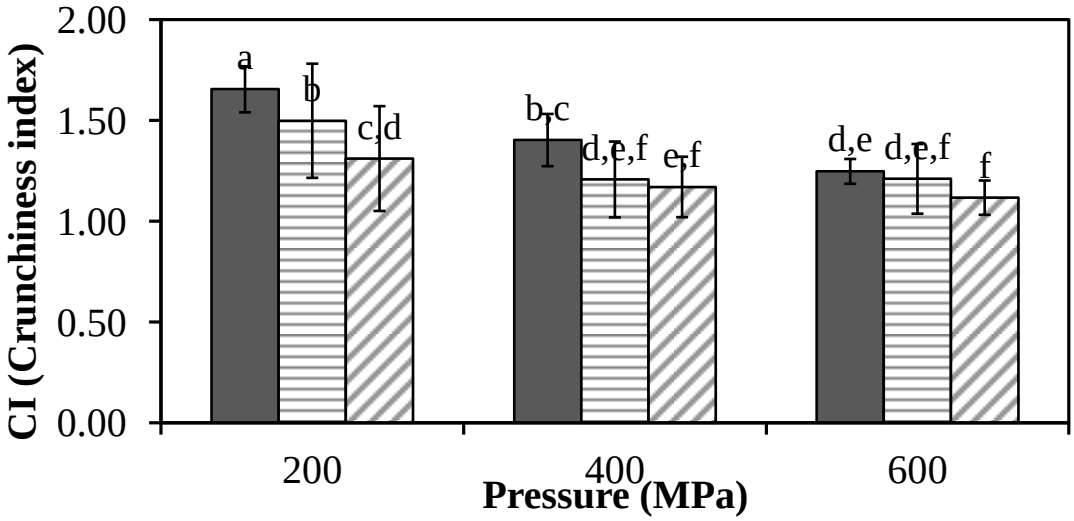
3.5. Influence of pressure and holding time on tissue disintegration index (Z) and cruchiness index (CI)

Changes in the tissue disintegration index (*Z*) and cruchiness index (*CI*) of each vegetable sample are shown in Fig. 8-10. In carrot and potato samples, there were overall trends of increasing *Z* and decreasing *CI* values, with elevated pressure and prolonged holding time.

Freeze/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. 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 ($P<0.05$). No significance of holding time was attributable to stabilized *in situ* electrical conductivity within approximately 3 min holding time at each pressure level (Fig. 6). Major textural changes in carrot are expected to occur during the initial pressure holding time (3 min) based on the calculated *Z* values. These textural changes are characterized by an initial texture loss, instantaneous pressure softening, followed by a gradual change in the vegetable tissue during pressure hold (Basak and Ramaswamy, 1998). The authors states that an instantaneous pressure softening results primarily from the application of a pressure pulse (first stage) which is generally followed by a time-dependent first order rate increase or decrease in firmness (second stage). 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 at each holding time of 1 min ($P<0.05$); whereas, it had a minor effect at 5 and 10 min holding times. The effects of holding time were only statistically significant effect only 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).



(a)



(b)

Fig. 8. Changes in *Z* (a) and *CI* (b) at elevated pressure and pressure holding time: Carrot. Values with different letter are significantly different ($P<0.05$). Z and CI of freeze/thawed carrot were 1±0.101 and 0.192±0.033, respectively. ■ 1 min; ▤ 5 min; ▨ 10 min.

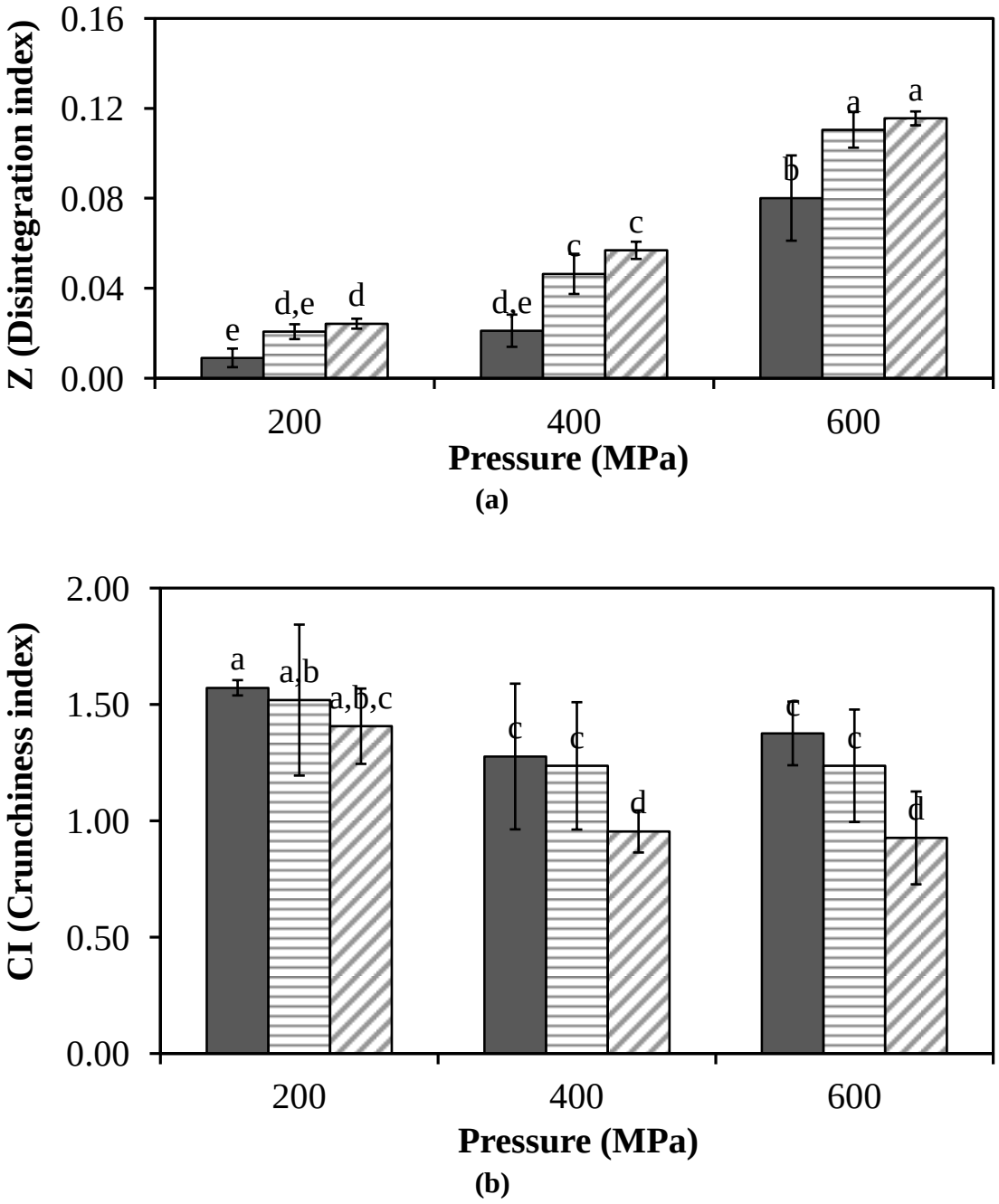


Fig. 9. Changes in *Z* (a) and *CI* (b) at elevated pressure and pressure holding time: Potato. Values with different letter are significantly different (P<0.05). Z and CI of freeze/thawed potato were 1±0.048 and 0.266±0.039, respectively. ■ 1 min; ▤ 5 min; ▨ 10 min.

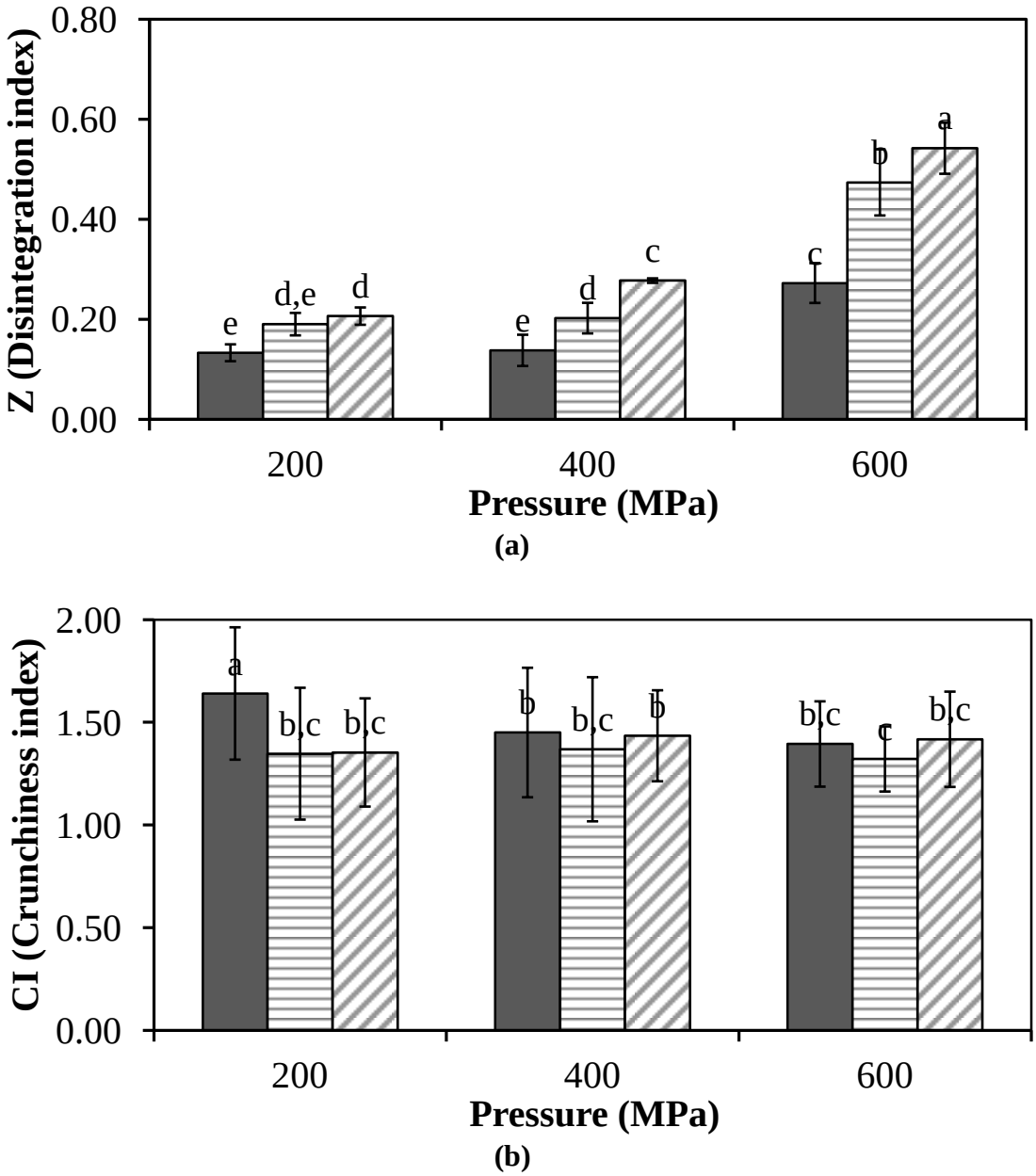


Fig. 10. Changes in *Z* (a) and *CI* (b) at elevated pressure and pressure holding time: Red radish. Values with different letter are significantly different (P<0.05). Z and CI of freeze/thawed radish were 1±0.007 and 1.300±0.198, respectively. ■ 1 min; ▤ 5 min; ▨ 10 min.

In the potato samples, elevated pressure significantly increased *Z* values ($P<0.05$). 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 suggest a threshold of pressure holding time for 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.

The effect of elevated pressure on *Z* values in red radish samples were not clear below 600 MPa. 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 this study, prolonged holding time significantly 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 continuously occur at elevated pressure. These were not in accordance with the findings in pressure-treated carrot and potato samples. In Fig. 7, pressure-treated red radish shows higher puncture force (hardness) than control samples. 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 25ºC using instrumental texture analysis. The authors proposed that high pressure significantly delays softening rates in radish samples, 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 freeze/thawed red radish was 1.300, which was considerably higher than those of freeze/thawed carrot and potato. In the force-deformation curve of freeze/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).

3.6. Empirical model fitting

The relationship between *Z* and *CI* were fitted to the first and second order polynomial (5, 6), and then tabulated in Table 1. In the first order polynomial, all of the vegetable samples showed the negative linear coefficient (β_1) indicating an inverse relationship between *CI* and *Z*. The highest coefficient of determination (R^2) was calculated as 0.866, in the carrot sample, followed by the potato and radish sample, in decreasing order. Carrot and potato samples had significant linear coefficient (β_1) in the probability test ($Pr<0.05$). 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 1. Estimated coefficients and probability test of the fitted first-order polynomial parameters between *Z* and *CI* ($CI=\beta_0+\beta_1 \cdot Z \pm \varepsilon$)

	Carrot		Potato		Radish	
	Coefficients	Pr > |t|	Coefficients	Pr > |t|	Coefficients	Pr > |t|
β_0	1.622*	0.0001	1.412*	0.0001	1.572*	0.0001
β_1	-1.511*	0.0001	-1.232*	0.0025	-0.369	0.1164
R^2 values	0.866		0.656		0.251	
SSEY (ε)	0.171		0.272		0.184	

* Significant at 5% level

Table 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 (β_1). Although quadratic coefficient β_2 indicated a positive coefficient, no significance was found in the probability test (Pr>0.05). Neither first nor second order empirical models demonstrated a close relationship between *Z* and *CI* in red radish samples.

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

	Carrot		Potato		Radish	
	Coefficients	Pr > |t|	Coefficients	Pr > |t|	Coefficients	Pr > |t|
β_0	1.759*	0.0001	1.661*	0.0001	1.753*	0.0001
β_1	-2.701*	0.0044	-6.874*	0.0035	-1.586*	0.0368
β_2	1.144	0.1122	5.479	0.0097	1.197	0.0798
R^2 values	0.904		0.858		0.502	
SSEY (ε)	0.153		0.185		0.159	

* Significant at 5% level

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). In our study, pressure-induced textural changes were evaluated with two different methods of *in situ* electrical conductivity and instrumental texture analysis, to provide a better understanding of vegetable tissue under pressure.

4. Conclusion

In situ electrical conductivity measurement and its cell 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 at elevated pressures and holding times, and then stabilized at specific holding times of constant pressure. 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 between Z and CI. *In situ* electrical conductivity measurement is an effective tool for understanding the pressure-induced textural changes of vegetable tissue in comparison to instrumental texture analysis.

Nomenclature

CH compression heating (ºC/MPa)

CI crunchiness index

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

Grad slope of the force deformation curve

I current (A)

k cell constant (m^{-1})

P pressure (MPa)

T temperature (ºC)

V voltage (V)

v volume (m^3)

Z tissue disintegration index

β parameters in the empirical model

σ electrical conductivity (S/m)

Subscripts

atm atmospheric condition (0.1 MPa)

ft freeze/thawed sample

H heat transfer from the surroundings

i initial condition

p pressurized condition

raw raw sample

tret treated sample

0,1,2,3 parameters (intercept & slope) in the empirical model fitting

20% slope at 20% of the maximum puncture force

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