Onion Cells After High Pressure and Thermal Processing: Comparison of Membrane Integrity Changes Using Different Analytical Methods and Impact on Tissue Texture

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Abstract: Two different analytical methods were evaluated for their capacity to provide quantitative information on onion cell membrane permeability and integrity after high pressure and thermal processing and to study the impact of these processing treatments on cell compartmentalization and texture quality. To determine changes in cell membrane permeability and/or integrity the methodologies utilized were: (1) measurement of a biochemical product, pyruvate, formed as a result of membrane permeabilization followed by enzymatic activity and (2) leakage of electrolytes into solution. These results were compared to previously determined methods that quantified cell viability and ^{1}H-NMR T_2 of onions. These methods allowed for the monitoring of changes in the plasma and tonoplast membranes after high pressure or thermal processing. High pressure treatments consisted of 5 min holding times at 50, 100, 200, 300, or 600 MPa. Thermal treatments consisted of 30 min water bath exposure to 40, 50, 60, 70, or 90 °C. There was strong agreement between the methods in the determination of the ranges of high pressure and temperature that induce changes in the integrity of the plasma and tonoplast membranes. Membrane rupture could clearly be identified at 300 MPa and above in high pressure treatments and at 60 °C and above in the thermal treatments. Membrane destabilization effects could already be visualized following the 200 MPa and 50 °C treatments. The texture of onions was influenced by the state of the membranes and was abruptly modified once membrane integrity was lost.

Keywords: high pressure, ion leakage, onions, pyruvate, texture, thermal processing

Practical Application: In this study, we used chemical, biochemical, and histological techniques to obtain information on cell membrane permeability and onion tissue integrity after high pressure and thermal processing. Because there was strong agreement between the various methods used, it is possible to implement something relatively simple, such as ion leakage, into routine quality assurance measurements to determine the severity of preservation methods and the shelf life of processed vegetables.

Introduction

Novel food processing technologies are currently being studied as alternatives to traditional thermal pasteurization methods to satisfy consumer demands for minimally processed products that provide convenience, are free from additives and preservatives (Rastogi and others 2007), remain microbiologically safe, and retain the color, texture, flavor, and nutrient attributes of the fresh product (De Belie and others 2000; Waldron and others 2003). Strategies to meet such demands include modification of existing food processing techniques and/or the adoption of novel technologies that allow for production of higher quality products that are microbiologically secure (Barbosa-Cánovas and others 2005).

In recent years, high pressure processing of biological systems has received the greatest amount of attention (Welti-Chanes and others 2005), compared to other novel technologies. High pressure processing is potentially less detrimental to food flavor and nutrient content than thermal processing because of its limited effect on covalent bonds (San Martín and others 2002; Boonyaratanakornkit and others 2002), especially at moderate temperatures (Oey and others 2008). Most biochemical reactions result in a volume change and are therefore affected by pressure (Patterson 2005). Application of high pressure to food products results in microbial inactivation and may enhance enzyme inactivation (Barbosa-Cánovas and others 2005), though enzymes vary greatly in their ability to withstand pressure (Patterson 2005). As is true for other food processing technologies, microbial death during high pressure treatment is considered to be a result of cell permeabilization, but the mechanisms that microbial membranes undergo under high pressure are still not completely understood (Ulmer and others 2002; Casadei and others 2002).

The presence of membrane-bound compartments or organelles within plant cells allows biochemical reactions to be segregated. Studies show that the plasma membrane that is enclosing the cell cytoplasm differs from other intracellular membranes, for

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example, the tonoplast (surrounding the vacuole) and other organelle membranes in function, structure, and composition (Staehelin and Newcomb 2000). Membranes, which are in a fluid state under physiological conditions, can lose fluidity and undergo phase transitions with thermodynamic parameters such as low temperature and high pressure (Kato and Hayashi 1999), affecting many of their functions. In plants, cell membranes are one of the first targets of plant stresses, and alterations in membrane structure may cause a modification of cellular compartmentalization (Vazquez-Tello and others 1990). As a result of the loss in cell compartmentalization changes, in the tissue architecture (Rastogi and others 2000), as well as chemical reactions such as an increase in browning reactions (Vámos-Vigyázo 1981), oxidation of desirable nutrients, and development of unwanted flavor and aroma compounds may occur (Diaz-Maroto and others 2004). In other cases, disruption of cellular compartmentalization may be desired as it may lead to improved bioaccesability (Verlinde and others 2008) and extraction yield (Oey and others 2008) of certain nutrients.

Intact plant material of onions contain the odorless cysteine sulphoxides, in particular trans (+)-S-1-propenyl-L-cysteine sulphoxide and (+)-S-methyl-L-cysteine sulphoxide (Randle and Lancaster 2002). When intact cells are disrupted, cysteine sulphoxides are rapidly converted into (alk)enLylsulphenic acid, pyruvate, and ammonia. The reaction is catalyzed by alliinase (EC 4.4.1.4) to form unstable sulphonic acids, which are rapidly converted into either their corresponding thiosulphanates or the lachrymatory factor (Z)-propanethial-oxide, which cause the characteristic smell of fresh onion juice. The primary aroma compounds are relatively unstable and quickly decompose into a variety of strong-smelling volatile sulfur compounds. These derivatives are characteristic of processed onions (Keusgen and others 2002). S-alk(en)yl cysteine sulphoxides are present in the cytoplasm of the cells, while the enzyme alliinase is initially located in the vacuole (Lancaster and Collin 1981). Changes in permeability or rupture of the tonoplast membrane must occur for the enzyme to reach its substrate and the reaction take place. An increase in pyruvate content of the tissue as a result of processing may thus be used as an indicator of a loss in tonoplast integrity.

The quantification of cellular disruption, imparted by the loss of membrane integrity during handling, processing, or storage will allow for a better correlation of plant cell structure changes to the quality of fruit and vegetable products (Knorr 1994; Angersbach and others 1999). This in turn will allow for optimization of food processing for improved cellular integrity and quality retention, and may provide quantitative tools for prediction of shelf life. Plant cell membrane permeability has previously been estimated using a number of methods, including: measurement of the conductivity of leachates and solids lost during soaking and volume exudates (Saltveit 2002), light scattering, fluorescence microscopy and volume-sensitive fluorescent indicators (Stanley 1991; Verkman 2000), electrical impedance (Rastogi and others 1999; Angersbach and others 1999), and nuclear magnetic resonance (Snaar and Van As 1992; Van der Weerd and others 2002).

In this article, an enzymatic method that measures the amount of pyruvate formed in onion tissue (Anthon and Barrett 2006; Schwimmer and Weston 1961) is also proposed for use in determination of changes in cell membrane integrity after high pressure and thermal processing. The results of different membrane permeability and/or integrity methods that allow for the quantification of cell integrity after high pressure and thermal processing, for example, pyruvate analysis, ion leakage, cell viability (Gonzalez and others 2010a), and ^{1}H-NMR T_2 (Gonzalez and others 2010b) are summarized and the impact of membrane integrity on the texture component of quality is determined.

Materials and Methods

Onion treatments

Spanish yellow onions cv. Sabroso (approximately 8 cm diameter) were provided by Gills Onions (Oxnard, Calif., U.S.A.) and used for processing treatments. Whole onions were shipped to the Kraft Foods Fellow Strategic Research Center (Glenview, Ill., U.S.A.) for high pressure treatment and then the pressure-treated onions were shipped back to UC Davis on the same day under refrigeration. Thermal processing treatments were applied at UC Davis. The processing treatments were carried out in triplicate on 3 different days, with 2 subsamples (onions) analyzed per treatment on each day. Pyruvate analysis, ion leakage, and texture measurements were all carried out on the same onions. A mixed linear model procedure was used to analyze effects of the different treatments using a statistical package (SAS 9.1). The overall means were statistically compared using Tukey test ($P < 0.05$).

Raw material. The outer papery scales and the first fleshy scale or layer of the onions were removed and then the onions were cut into 1 cm slices with a single slice obtained from the middle or equator of the bulb for the different cell integrity determinations. Raw untreated equatorial onion slices and packaged without vacuum were used as a first control treatment, and raw untreated onion slices that were vacuum packaged (approximately 0 MPa) (easy Pack, Koch, Kansas City, Kans., U.S.A.) were used as a second control. High pressure and heat treated slices were all vacuum-packaged under the same conditions as the second control.

High pressure processing treatments. sliced vacuum-packed onions were exposed to 50, 200, 300, and 600 MPa for a 5-min hold time and initial processing unit temperature (T_i) approximately 20 °C. The pressure build-up time and temperature were recorded in all experiments. During compression, the temperature raised to a maximum of approximately 35 °C dependant on the pressure level. The high pressure unit had a 6 L vessel and an 800 MPa maximum pressure level (Stansted Fluid Power HP Iso-Lab System, Stansted, Essex, U.K.). The pressure transmitting medium was 1 part propylene glycol and 2 parts of water.

Thermal processing treatments. Onion slices (1 cm thick) were individually vacuum-packed (approximately 0 MPa) and heated in a water bath at either 40, 50, 60, 70, or 90 °C for 30 min, which was the time determined in preliminary trials to be required for heat penetration to the center of the onion slice. Slices were kept in refrigerator before initiating the experiment. Onions were cooled in ice water for 10 min after processing and held at 4 °C until measurement.

Pyruvate analysis

The amount of pyruvate generated enzymatically was used as a measure of the loss of cell integrity and the subsequent action of the alliinase enzyme in onion tissue. Pyruvate was assayed using 2, 4-dinitrophenylhydrazine (DNPH) following the procedure of Anthon and Barrett (2003), with the reactant volumes adjusted for the purpose of the test. The processed onion samples and controls were allowed to stand at room temperature for a period of 2 h to allow the breakdown of S-alk-(en)yl-L-cysteine sulphoxides and the formation of pyruvate.

Samples were prepared in 2 different ways to meet different objectives. To determine the extent to which the processing

treatment inactivated alliinase, residual activity of alliinase remaining in the onion tissue after processing was measured by completely homogenizing tissue and measuring the amount of pyruvate formed. The samples were prepared by homogenizing approximately 1 g of onion tissue with water (1 : 1), 15 min were allowed for alliinase to react with the cysteine sulphoxides, after which 2 parts 1 M trichloracetic acid (TCA) were added. The homogenate was centrifuged for 5 min at 16.1 relative centrifuge force (Centrifuge 5415D, Eppendorf, Hamburg, Germany). The reaction was started by mixing in a culture tube 150 uL of the clarified homogenate, 1.5 N NaOH to neutralize the acid in the onion homogenate, and 800 uL of distilled water to reach a final 1 mL volume. Then, 1 mL of 0.025% DNPH in 1 N HCL was added, to the samples and they were placed in a water bath, heated at 37 °C for 20 min. The reaction was ended by adding 1 mL of 1.5 N NaOH. Absorbance values were measured at 515 nm. A blank and standards were prepared by adding 0 to 50 uL of 5 mM pyruvate and water volumes were adjusted appropriately.

To measure the impact of the food process (high pressure or thermal) on cell integrity, the amount of pyruvate formed as a result of the processing treatment was determined by inactivation of alliinase before tissue homogenization. Control and processed onion samples were homogenized with 1 M TCA and water in the ratio of 1 : 2 : 1. The homogenate was centrifuged and the procedure was followed as described previously mentioned, but the sample volume for the reaction was doubled due to the lower pyruvate content and the volumes of water and aliquot of 1.5 N NaOH were adjusted, respectively. The amount of endogenous pyruvate initially present was measured in the raw control and the additional amount of pyruvate formed was used as an indicator of cell membrane damage result of processing.

Ion leakage

The 3rd scale of each onion and equatorial region were used to obtain 1 cm diameter onion disks with a cork borer. After excision onion disks were rinsed twice in deionized water for about 1 min each time, surface blotted with a paper towel and transferred to petri-dishes, then covered with damp tissue paper to avoid moisture loss (Saltveit 2002) and kept in the refrigerator for 3 h. Samples were equilibrated to room temperature for 1 h and bathed in 20 mL of a previously determined isotonic solution (0.2 M mannitol). Conductivity measurements were obtained at different time periods, 0 min, 15 min, 30 min, 1 h, 2 h, 3 h, and 4 h. The ion leakage of excised onion disks was calculated as the percent of total conductivity of the solution (% total conductivity = conductivity measured at a time interval × 100/total conductivity). The total conductivity was obtained after a double cycle of a freeze (−20 °C) thaw (ambient temperature) procedure to cause complete rupture of cells (Palta and others 1977a, 1977b). Conductivity measurements were obtained with a conductivity meter (Accumet portable AP65, Fischer Scientific, Singapore). The data were fitted to the models: $y = ax/(b + x)$ and to the more frequently used $y = C_o.\ (1 - \exp^{-k.x})$, (Murray and others 1989; Saltveit 2002) where y is the % of total conductivity of the solution and x the time interval for both equations. In the first model, the parameter a is the asymptote of the curve that represents the limiting % total conductivity reached and parameter b is the time to reach half asymptote (1/2 time). In the second model, C_o is the y-intercept of a linear equation fitted to the linear portion of the data at long incubation times, and k the rate of electrolyte leakage to the medium (Saltveit 2002). The experimental data were fitted with a nonlinear procedure (SAS 9.1). The best model was selected by minimizing the standard errors of the model and the effect of the treatment on the model parameters was analyzed using Tukey test ($P < 0.05$).

Texture

A puncture test (2 mm diameter flat-tipped cylindrical probe) was performed with a texture analyzer (TA.XT2, Stable Micro System, Haslemere, England). Eight 1 cm diameter pieces were obtained from the equatorial region of the 3rd scale on each onion. The test was performed to 90% deformation of the original onion scale thickness and the test speed was set to 1 mm/s. The initial slope (gradient from initial point to 20% maximum force) (Nmm^{-1}) was analyzed.

Integration of cell membrane integrity evaluation methods and texture analysis

The different methods described previously were used to evaluate onion cell membrane integrity and were compared to the previously obtained cell viability (Gonzalez 2010a) and ^{1}H–NMR T_2 (Gonzalez 2010b) results. The relationship between membrane integrity and texture quality was evaluated.

Results and Discussion

Pyruvate analysis

Pyruvate formed in onion tissue as the result of the hydrolysis of S-alk(en)yl cysteine sulphoxides (located in the cytoplasm) by

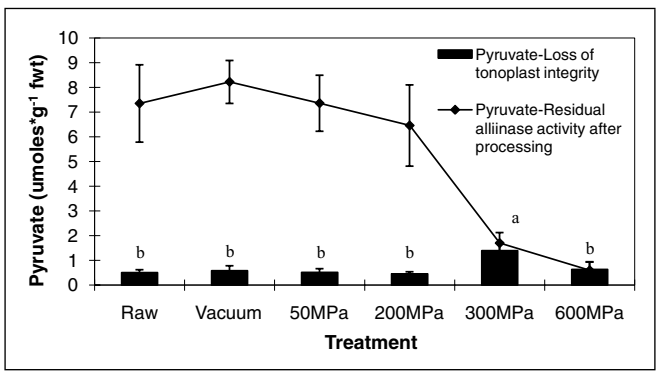

Figure 1–Pyruvate formed in onion tissue during high pressure processing (indicator of integrity of the tonoplast membrane) and residual alliinase activity (indicator of alliinase inactivation due to high pressure processing). (fwt = fresh weight).

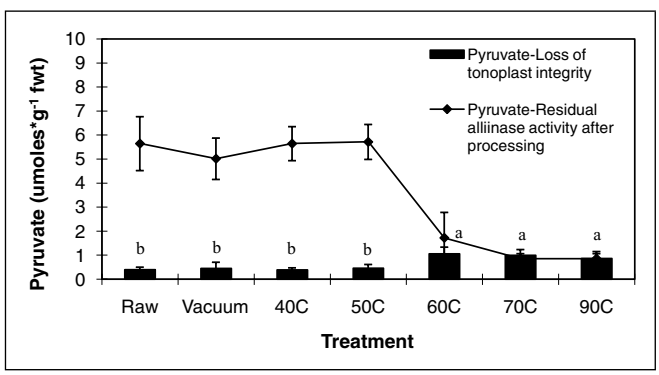

Figure 2–Pyruvate formed in onion tissue during thermal processing (indicator of integrity of the tonoplast membrane) and residual alliinase activity (indicator of alliinase inactivation due to thermal processing) (fwt = fresh weight).

the action of the vacuole-located enzyme alliinase (Lancaster and Collin 1981; Randle and Lancaster 2002) was used as indicator of loss of tonoplast membrane integrity caused by the processing treatments. Significant increases in pyruvate content indicated that changes at the tonoplast membrane level occurred following high pressure treatments at 300 MPa or higher (Figure 1). At higher pressure levels (for example, 300 and 600 MPa), alliinase was also inactivated by high pressure, as indicated by the significantly lower amount of pyruvate formed by the residual alliinase activity after the 600 MPa pressure treatment.

In the thermally treated onions, changes at the tonoplast membrane level occurred as a result of the 60, 70, and 90 °C thermal treatments and alliinase inactivation was also observed with these higher temperature treatments (Figure 2). These results indicate that the pyruvate content in tissue formed as a result of either high pressure or thermal processing is of limited value by itself in assessing tonoplast rupture, because at the temperature and pressure levels required for membrane rupture, substantial inactivation of alliinase also occurred. The reduced amount of pyruvate present in processed samples as a consequence of alliinase inactivation was in agreement with the changes in organoleptic characteristics reported by Butz and others (1994) in high pressure treated onions. The use of pyruvate as an indicator of membrane integrity would be useful in processes that do not lead to enzyme inactivation.

Ion leakage

A common feature accompanying membrane alterations is an increase in the leakage of ions into solution, with electrolyte leakage long being used as a measurement of the intactness and permeability of cell membranes (Murray and others 1989; Vasquez-Tello and others 1990). Membrane rupture and ion leakage into plant cell apoplasts leads to undesirable changes that make the appearance of plant tissue more translucent. Since the leakage is from a high concentration inside the cell, to a low concentration outside it, the efflux may be considered to be passive diffusion, whereas influx must be due to active transport. Increased net ion leakage may result from either an increased efflux due to damage to the semipermeability of the plasma membrane, or a decreased influx due to damage to the active transport system (Palta and others 1977a).

Both high pressure and thermal treatment of onions resulted in significant increases in ion leakage from the tissue. The relationship between conductivity (the electrolyte concentration in the solution in which the onion disks were bathed) and time followed an asymptotic curve (Figure 3 and 4). The equation $y = ax/(b+x)$ best described the ion leakage behavior. Table 1 indicates the parameters of the fitted model for the different processing treatments. The higher the level of pressure or temperature applied, the higher the limiting% total conductivity reached. High pressure treatments of 300 and 600 MPa and thermal treatments at 60, 70, and 90 °C reached 100% of total conductivity within the 4 h incubation period in 0.2 M mannitol. A complete loss of membrane integrity was achieved, in which case the tonoplast and plasma membranes no longer acted as cell barriers. Ion leakage resulting from milder treatments indicated more subtle changes in membrane permeability, as observed in the significantly lower limiting total conductivity values of the 200 MPa and 50 °C treatments (Table 1). While half time values required to reach the limiting % total conductivity appeared to vary, no statistical differences were observed between the different high pressure or thermal treatments, indicating no additional information described by this parameter.

Texture and cell membrane integrity analysis

Tissue stiffness was determined from the initial slope of a force/deformation curve in a puncture test of the onion disks following either high pressure or thermal treatments (Mohsenin 1986; Bourne 2002). Figure 5 illustrates a comparison of the changes in cell membrane integrity as determined by the different methods we evaluated in this article and in 2 others, for example, pyruvate

Table 1–Comparison of the limiting percent of total conductivity and the half times obtained for the controls, high pressure, and thermally treated onions.

Treatment	Limiting total conductivity (%)	Half time (min)
High pressure		
Raw	9^{c}	19^{a}
Vacuum	11^{c}	16^{a}
50 MPa	11^{c}	16^{a}
200 MPa	27^{b}	16^{a}
300 MPa	108^{a}	21^{a}
600 MPa	108^{a}	23^{a}
Thermal		
Raw	8^{d}	19^{a}
Vacuum	9^{cd}	21^{a}
40 °C	11^{bc}	26^{a}
50 °C	14^{b}	29^{a}
60 °C	104^{a}	49^{a}
70 °C	110^{a}	53^{a}
90 °C	114^{a}	55^{a}

Values with a common letter do not differ significantly ($P < 0.05$).

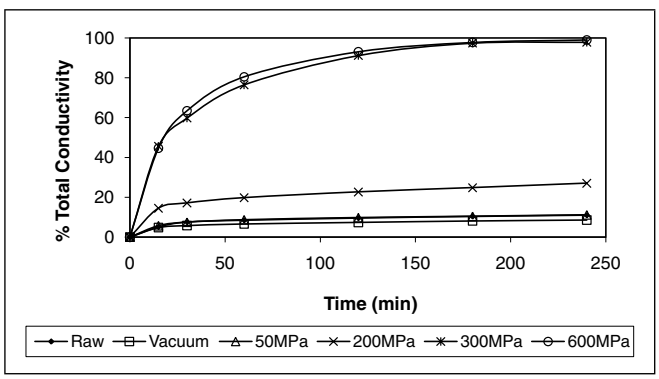

Figure 3–Cumulative increase in ion leakage as a percent of total conductivity in onion discs of controls and high pressure treated onions.

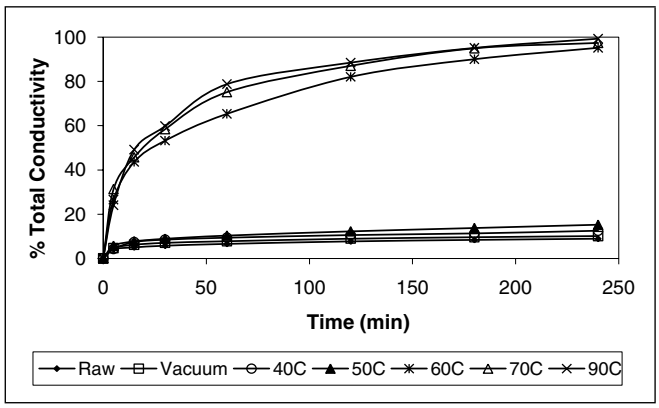

Figure 4–Cumulative increase in ion leakage as a percent of total conductivity in onion discs of controls and thermally treated onions.

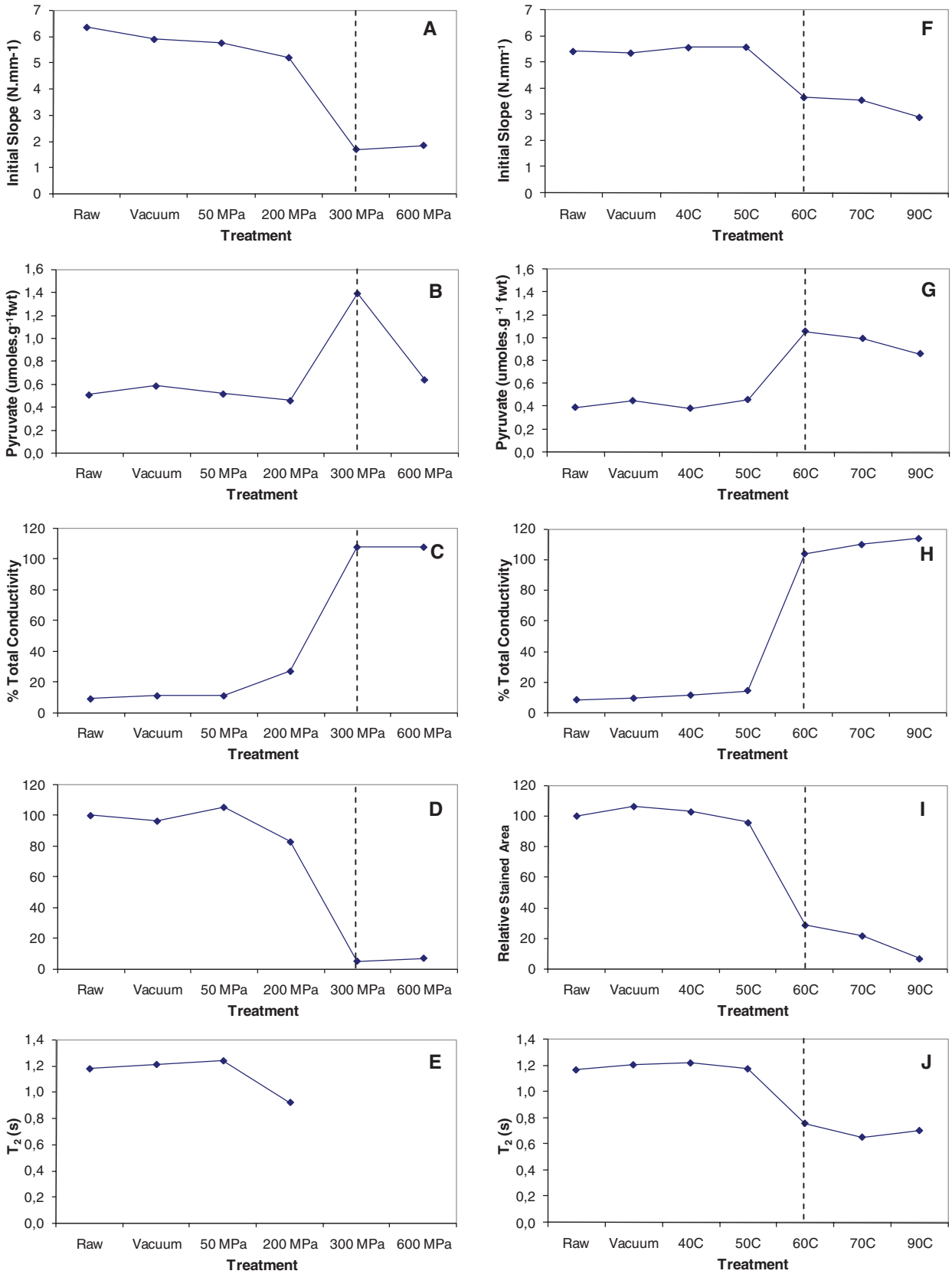

Figure 5–Relationship between tissue stiffness (A,F) and cell membrane integrity as determined by various methods: pyruvate content (B,G); ion leakage (C,H); cell viability (D,I); and ^{1}H-NMR T_2 (E,J) for high pressure (left) and thermally processed (right) onion samples. (*) Ion leakage measured as % total conductivity; cell viability measured as relative stained area. (**) Dash line indicates membrane rupture; fwt = fresh weight.

content, ion leakage (% of total conductivity), cell viability, or % of relative stained area (Gonzalez 2010a) and ^{1}H-NMR T_2 (Gonzalez 2010b).

Both thermal and high pressure treatments reduced tissue stiffness with the most significant changes taking place at pressures above 200 MPa and temperatures above 50 °C (Figure 5A and 5F). In the high pressure processed onions, the 200 MPa treatment resulted in a small loss of tissue stiffness (Figure 5A) compared to the raw control. This change in stiffness at 200 MPa coincided with the initial loss in membrane integrity detected after the 200 MPa treatment by the ion leakage (Figure 5C), cell viability (Figure 5D), and ^{1}H-NMR T_2 (Figure 5E) methods. The noticeable change in tissue stiffness after the 300 and 600 MPa treatments coincided with the complete loss of membrane integrity as determined by ion leakage, cell viability, and pyruvate content in tissue (^{1}H-NMR T_2 was not measured above 200 MPa in Gonzalez 2010b). Similarly, the most significant decrease in stiffness in the thermally processed onions occurred between 50 and 60 °C, and coincided with the greatest changes in pyruvate content, ion leakage, cell viability, and T_2 determinations (Figure 5G to 5J). These results indicate that after onion processing, once membrane integrity was lost, the tissue stiffness declined dramatically. This is a consequence of the loss of turgor pressure within the onion cells (Greve and others 1994; Araya and others 2007), emphasizing the importance of cell turgor as a component of the texture of fruits and vegetables (Shackel and others 1991).

These findings in onion cells correspond with previous studies in microorganisms, which have found that the damage to cellular membranes as a result of various food processes is the underlying mechanism responsible for cell death (Ananta and others 2005). The causes of increased membrane permeabilization and rupture in plant cells during processing, as lipid phase transitions and/or denaturation of membrane bound proteins as suggested for microorganisms (Ulmer and others 2002; Casadei and others 2002) are topics of research that can yield insight into processing optimization to maintain cell structure and texture attributes.

Conclusions

Several approaches, chemical, biochemical, and histological, were used to obtain information on cell membrane permeability and integrity after high pressure and thermal processing. There was strong agreement found between the methods employed for determining the range of high pressure or temperature treatments that result in changes in membrane integrity. The studies emphasize the validity of using these methods and the ability to quantify changes in plant tissue integrity, that allow for a more valid comparison of novel technologies to traditional thermal processing methods. Knowledge of cell structure changes that occur as a result of processing will allow for improvement of shelf life monitoring and prediction and optimization of the texture quality of minimally processed vegetables, to maintain "fresh-like" characteristics.

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