Influence of Cell Integrity on Textural Properties of Raw, High Pressure, and Thermally Processed Onions

M.E. Gonzalez, J.A. Jernstedt, D.C. Slaughter, and D.M. Barrett

Abstract: The integrity of onion cells and its impact on tissue texture after high pressure and thermal processing was studied. The contribution of cell membranes and the pectic component of cell walls on the texture properties of onion tissue were analyzed. Neutral red (NR) staining of onion parenchyma cell vacuoles was used for the evaluation of cell membrane integrity and microscopic image analysis was used for its quantification. The content of methanol in tissue as a result of pectin methylesterase activity was used to evaluate the pectin component of the middle lamella and cell walls and the hardening effect on the tissue after 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. In the high pressure treatments, loss of membrane integrity commenced at 200 MPa and total loss of membrane integrity occurred at 300 MPa and above. In the thermal treatments, membrane integrity was lost between 50 and 60 °C. The texture of onions was influenced by the state of the membranes and texture profiles were abruptly modified once membrane integrity was lost. Hardening of the tissue corresponded with pressure and temperature PME activation and occurred after membrane integrity loss.

Keywords: high pressure, image analysis, membrane integrity, neutral red, pectin methylesterase, thermal processing

Practical Application: The texture of vegetables is an important quality attribute that affects consumer preference. Loss of textural integrity also indicates that other biochemical reactions that affect color, flavor, and nutrient content may occur more rapidly. In this study, we analyzed changes in the texture of onions after preservation with heat and high pressure.

Introduction

Fruits and vegetables are important components of the human diet and consumers today are demanding more minimally processed products that have organoleptic characteristics of fresh produce (Garcia and Barrett 2005). There is an increased awareness of quality attributes including color, texture, flavor, and nutrient content (de Belie and others 2000; Waldron and others 2003). Thermal processing has traditionally been used to assure microbial safety of food products and extend their shelf life; but it frequently results in organoleptic and nutritional losses (Ludikhuyze and others 2003). Advanced preservation methods that employ high pressure, electric fields, or other means of microbial destruction are being studied and commercialized due to their ability to deliver a safe product with superior quality (Guerrero-Beltran and others 2005; Barbosa-Cánovas and others 2005).

The loss of cell integrity in plant tissues may lead to undesirable reactions that affect fruit and vegetable quality. In intact fruit or vegetables, enzymes and their substrates may be compartmentalized and reactions may not take place until membranes are ruptured through cutting, bruising, homogenization, or processing (Saltveit 1997; Garcia and Barrett 2005). Aroma generation in onions (Randle and Lancaster 2002), browning (Vámos-Vigyázó 1981), or development of unwanted flavor and aroma compounds (Diaz-Maroto and others 2004) do not occur until there is loss of membrane integrity and loss of cell compartmentalization. In other cases, disruption of cellular compartmentalization may be desired as it may lead to improved bioaccesiblity (Verlinde and others 2008) and extraction yield (Oey and others 2008) of certain nutrients. Enhanced understanding of the role that cellular structure plays at the macroscopic and molecular level (Knorr 1994) as well as quantification of the effects that different methods of preserving fruit and vegetables have on tissue integrity are required to improve food quality and shelf life of products.

The role of cellular structures in determining the mechanical properties of plant tissues (Donald and others 2003; de Escalada Pla and others 2006) and the effect that single cells have on textural properties of tissues are topics of current research interest (Wang and others 2006). Puncture tests, commonly used to analyze the texture of fruits and vegetables, measure the force and deformation required to push a probe into the product and in so doing cause failure to the tissue (Mohsenin 1986; Bourne 2002). Rupture or failure of the material is considered to occur at the maximum force, where a failure in the macrostructure of the specimen is observed (Mohsenin 1986). An intact semipermeable plasma membrane allows for the development of cellular turgor pressure; that together

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with the plant cell wall, imparts rigidity and firmness to plant tissues (Ilker and Szczesniak 1990).

Neutral red (NR) is a lipophilic phenazine dye used for the staining of plant vacuoles (Admon and Jacoby 1980) and has been used for the evaluation of membrane integrity in onion cells (Fincan and Dejmek 2002; Gonzalez and others 2010a). This dye diffuses across cell membranes, protonates within the acidic vacuolar medium, and accumulates in cells of intact tonoplast membranes, showing dark red colored vacuoles (Ehara and others 1996).

In the present study, an anatomical and cytological approach was used to evaluate the integrity of the tonoplast, the vacuole membrane, in onion parenchyma tissue exposed to high pressure and thermal processing. A cell viability NR dye staining method was used to assess membrane integrity of cells and digital image analysis was employed for quantification of cell integrity. The effect that cell membrane integrity has on tissue texture properties was evaluated and the correlation between cell membrane integrity and different textural parameters was studied. The contribution of the pectic component to tissue firmness as a result of PME activation was also analyzed.

Materials and Methods

Raw materials

Spanish yellow onions (*Allium cepa*) cv. "Sabroso" (approximately 8 cm diameter) were provided by Gills Onions (Oxnard, Calif., U.S.A.). The outer papery and the first fleshy scale of the onions were removed. An equatorial 1-cm thick slice was used as a control treatment. Raw untreated, equatorial onion slices that were vacuum packaged (approximately 0 MPa) (3 mil standard barrier nylon/polyethylene vacuum pouches, Ltd., Koch Supplies Inc., Kansas City, Mo., U.S.A.) using a vacuum machine (Easy Pack, Koch, Kansas City, Kans., U.S.A.) were used as a second control.

Preparation of processed samples

High pressure processing treatments. Whole onions were shipped to Kraft Foods Fellow Strategic Research Center (Glenview, Ill., U.S.A.) for high pressure treatment and then the pressure treated onions were shipped overnight to UC Davis under refrigeration on the same day of processing. Sliced vacuum packaged (approximately 0 MPa) onions were exposed to 50, 200, 300, and 600 MPa for a 5-min hold time. The initial high pressure unit temperature (T_i) was approximately 20 °C. The pressure and temperature build-up during pressurization and depressurization were recorded in all experiments. During compression the temperature raised to a maximum of approximately 35 °C at the highest 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. Individually vacuum packed onion slices (approximately 0 MPa) were heated in a water bath at 40, 50, 60, 70, or 90 °C for 30 min. The 30-min hold time was determined in preliminary trials to be the time required for heat penetration to the center of the onion slice at initial temperature approximately 7 °C. The water-bath temperature was set at target temperature so as to not surpass the desired temperature at any point of the tissue, resulting in a relative long processing thermal time. Onions were cooled in ice water for 10 min after processing and kept refrigerated until measurement. Thermal processing treatments were applied at UC Davis.

Cell viability and membrane integrity evaluation

Microscope section preparation and NR staining. Sample preparation and staining was carried as described by (Gonzalez and others 2010a). Briefly, samples pieces (approximately 5 mm × 5 mm) obtained from the 3rd scale at the equatorial region of the onion were used to obtain 400 μm thick sections with a Vibratome 1000 Plus (The Vibratome Co., St Louis, Mo., U.S.A.). Total of 2 onion pieces were taken from the 3rd scale of each onion on opposite sides of the bulb. From each piece, 2 cross sections perpendicular to both epidermises were obtained for staining and microscopic analysis. A 0.5% NR in acetone stock solution was prepared, filtered twice with Whatman paper nr 1, and diluted to 0.04% in 0.2 M mannitol – 0.01 M HEPES (N-[2-hydroxyethyl] piperazine-N'-[2-ethane-sulfonic acid]) buffer, pH 7.8, before tissue staining. Onion sections were transferred to 600 μL wells with dye solution (2 sections per well) for a period of 2 h, after which they were rinsed for 0.5 h in the 0.2 M mannitol – 0.01 M HEPES buffer solution. Sections were observed with a light microscope (Olympus System Microscope, Model BHS, Shinjuku-Ku, Tokyo, Japan) at 4× magnification. A digital color camera (Olympus MicroFire, Olympus, Tokyo, Japan) was attached to the microscope to capture images (Olympus software, Olympus America, Melville, N.Y., U.S.A.). Color photomicrographs (800 × 600 pixel resolution) were captured from 2 selected areas within the parenchyma tissue of each section positioned in between and equidistant from 2 vascular bundles and 4 to 5 cells rows apart from the outer epidermis.

The entire range of processing treatments was replicated on separate days, with the high pressure processes carried out 3 times and the thermal treatments 4 times. Two onions were processed for each high pressure treatment while 1 onion was processed for each thermal treatment at each processing time. For each onion processed, 4 onion specimens were obtained. Two micrographs were taken from each specimen. The total number of micrographs per process treatment was as follows:

$$\begin{aligned}\text{High pressure} &= 3\,\text{replicates} \times 2\,\text{onions/replicate} \\ &\quad \times 4\,\text{specimens/onion} \times 2\,\text{micrographs/specimen} \\ &= 48\,\text{micrographs per high pressure treatment} \\ \text{Thermal} &= 4\,\text{replicates} \times 1\,\text{onion/replicate} \\ &\quad \times 4\,\text{specimens/onion} \times 2\,\text{micrographs/specimen} \\ &= 32\,\text{micrographs per thermal treatment}\end{aligned}$$

A mixed linear model procedure was used to analyze effects of the different treatments using a statistical package (SAS 9.1). The least square significant difference (LSD) test for comparison of means was performed with $P < 0.05$.

Image processing and analysis

Red, green, blue (RGB) color images were transformed into hue, saturation, brightness (HSB) and the saturation image was selected for the determination of the percent area stained of the total photomicrograph. Cell viability and membrane integrity were expressed relative to the raw control treatment (relative stained area). The green component of the RGB images was used to discriminate and quantify extracellular air spaces, which had different refractile properties and were visualized as black regions in the RGB photomicrographs. Image processing software (Image J software) was used for preprocessing and analysis of the photomicrographs. All the procedures used were explained in Gonzalez and others 2010a.

Texture analysis

A puncture test with a 2-mm diameter flat-tipped cylindrical probe was performed with a universal testing machine (model TA.XT2 texture analyzer, Stable Microsystems, Haslemere, England). Eight 1-cm diameter samples were obtained with a cork borer from the same equatorial part of the 3 onion scale as sections for microscopy. The test was performed to a 90% strain ($\epsilon = (L/L_o) * 100$; 90% deformation of the original onion scale thickness, L_o) using a test speed of 1 mm/s. Force–deformation relationships were analyzed and the parameters studied were: initial slope or stiffness (Mohsenin 1986) calculated as the gradient of the line connecting the origin of the curve to 20% maximum force (Nmm^{-1}), maximum force (N) or hardness (Bourne 2002), number of peaks (indicated as a change in slope followed by an increase in the force above 0.15 N), bioyield force (N), stress (or σ = force/area) (Nm^{-2}), percent strain; with L the deformation at bioyield, deformability moduli (D moduli; or $D = (\sigma/\epsilon) * 100$) ($Nm^{-2}$), total area (Nmm), Area 1 (Nmm), which indicates the toughness or work before maximum force (Mohsenin 1986) and was calculated as the area under the curve from the origin of the curve to maximum force and Area 2 (Nmm) that indicates the work after maximum force and was calculated as the area under the curve from maximum force to 90% strain. Correlation coefficients ($r_{x,y}$) between the percent of total area stained, indicator of cell viability and membrane integrity, and the different texture parameters were estimated ($N = 36$). The overall means of the texture parameters were compared using a statistical package (SAS 9.1). The LSD test for comparison of means was performed with $P < 0.05$.

Methanol content in tissue

The amount of methanol present in onion tissue as a result of pectin methyl esterase (PME) activity was assayed by a spectrophotometric method following the procedure of Anthon and Barrett (2004). For this assay, a different set of onions was used and the hardness (maximum force) (Bourne 2002) of the tissue was measured. The methanol was enzymatically oxidized to formaldehyde with alcohol oxidase and was colorimetrically determined with Purpald (4-amino-3-hydrazinio-5-mercapto-1, 2, 4 triazole). The sample to be measured for methanol was prepared by homogenizing 1 part of sample (approximately 1 g) with 50% trichloracetic acid (TCA) and H_20 in a ratio 1 : 2 : 1. The homogenate was centrifuged and the reaction was started by adding 25 μL of the supernatant to 90 μL of 100 mM phosphate buffer (pH 7.5), 10 μL of alcohol oxidase at 0.01 U/μL, 25 μL of homogenate, and H_20 to a finale volume of 200 μL. The samples were incubated in a water bath at 30 °C for 10 min after which 200 μL of 5 mg/mL Purpald in 0.5N NaOH were added. Samples were incubated 30 additional minutes after which 600 μL of H_20 were added. Absorbance was measured at 550 nm. The experiment was triplicated with 2 subsamples (onions) analyzed per treatment on each time. 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 compared using the LSD test ($P < 0.05$).

Results and Discussion

Cell viability and membrane integrity evaluation

NR has been widely used for cell viability determination (Guttenberger 2000; Repetto and others 2008). In this study, staining with NR dye allowed for the differentiation of viable from inviable cells and image analysis allowed for quantification of each cell type and of the impact of high pressure and thermal treatments on tissue integrity. Viable cells were identified where the NR dye was up taken and retained within the vacuole, therefore, imparting an intense red color and the whole cell had a smooth appearance. Inviable cells were identified as those cells where no intense red dye was visually present, either as a result of mechanical damage due to cutting or processing, or where cells had a granulated texture with pink coloration, indicating only the presence of cell walls and cell debris (Gonzalez and others 2010a) and loss of vacuole membrane integrity. Examples of photomicrographs for control, vacuum packaged, high pressure, and thermally treated onions are shown in Figure 1 and 2.

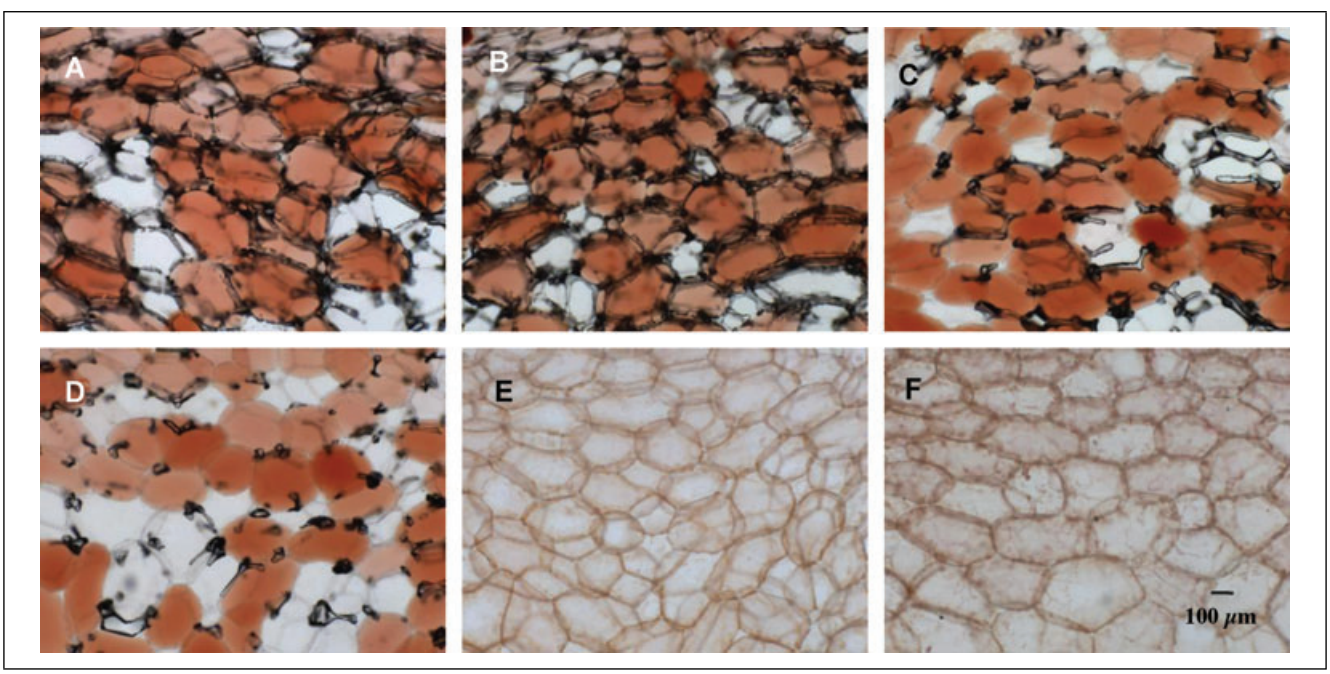

Figure 1–Photomicrographs of high pressure treated onions: (A) control, (B) vacuum packaged, (C) 50 MPa, (D) 200 MPa, (E) 300 MPa, and (F) 600 MPa.

According to the cell viability determination and image quantification (Figure 3 and 4), high pressure and thermal treatments could be classified into groups that were statistically different in terms of their effects on percentage of viable cells as follows:

(control, vacuum packaged, 50 MPa) > (200 MPa)
≫ (300 and 600 MPa)
(control, vacuum packaged, 40 and 50 °C)
≫ (60 and 70 °C) > (90 °C)

The results on cell viability agree with the results of ^{1}H-NMR T_2 (Gonzalez and others 2010b), electrolyte leakage, and pyruvate analysis (Gonzalez and others 2010c), methods used to determine cell integrity after high pressure and thermal processing. Butz and others (1994) also observed microscopic damage of the tonoplast of onion epidermal cells that were associated with 300 MPa treatments and detected changes in enzymatic activity associated with membrane damage above 100 MPa.

Onion samples pressure treated at or above 300 MPa or thermally treated at or above 60 °C showed a complete absence of viable cells by NR staining (Figure 1 and 2) yet there was some indication of a small number of stained areas in the image analysis (Figure 3 and 4), especially at 60 and 70 °C. These stained areas that did not correspond to viable cells (for example, 4% in 300 and 600 MPa treated tissues; 28%, 21%, and 6% in 60, 70, and 90 °C treated tissues) were due to binding of the NR dye to cellular structures such as the cell wall, membranes, and nucleus. Interaction of NR with lipophilic structures and cell walls has been previously reported (Lillie 1977; Guttenberger 2000). Faint nucleus staining could be observed in some cells after the 60 and 70 °C treatments and intense nucleus staining was observed in cells that had been exposed to 90 °C. Wang and others (2003) reported that NR interactions changed from intercalating between DNA pairs at temperatures between 5 and 25 °C, to aggregating along the double helix at 40 °C. The intensely stained nuclei observed at 90 °C may have resulted from a combination of different DNA-NR binding interactions and a DNA change in conformation occurring after exposure to high temperature.

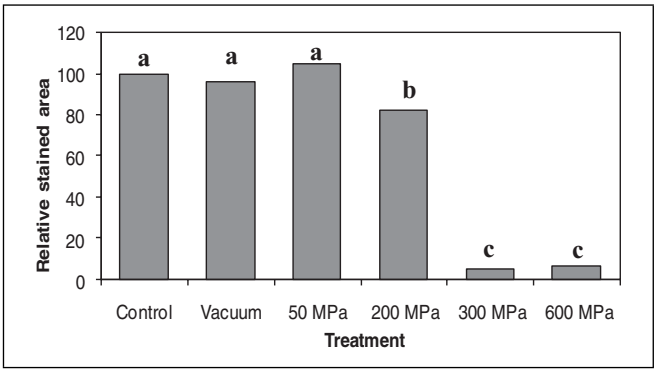

Figure 3–Relative stained area in control, vacuum packaged, 50, 200, 300, and 600 MPa treatments ($P < 0.05$) based on the analysis of the saturation component of HSB images.

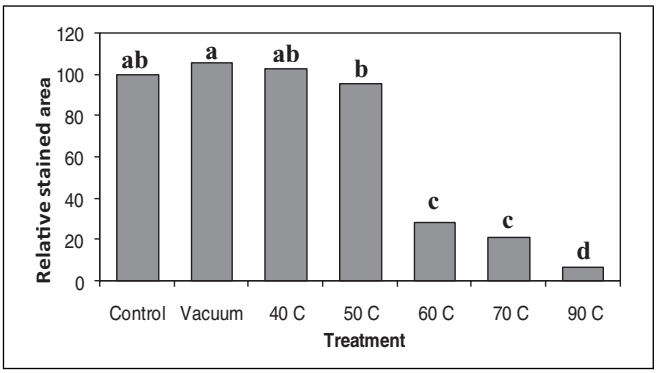

Figure 4–Relative stained area in the control, vacuum packaged, 40, 50, 60, 70, and 90 °C treatments ($P < 0.05$) based on the analysis of the saturation component of HSB images.

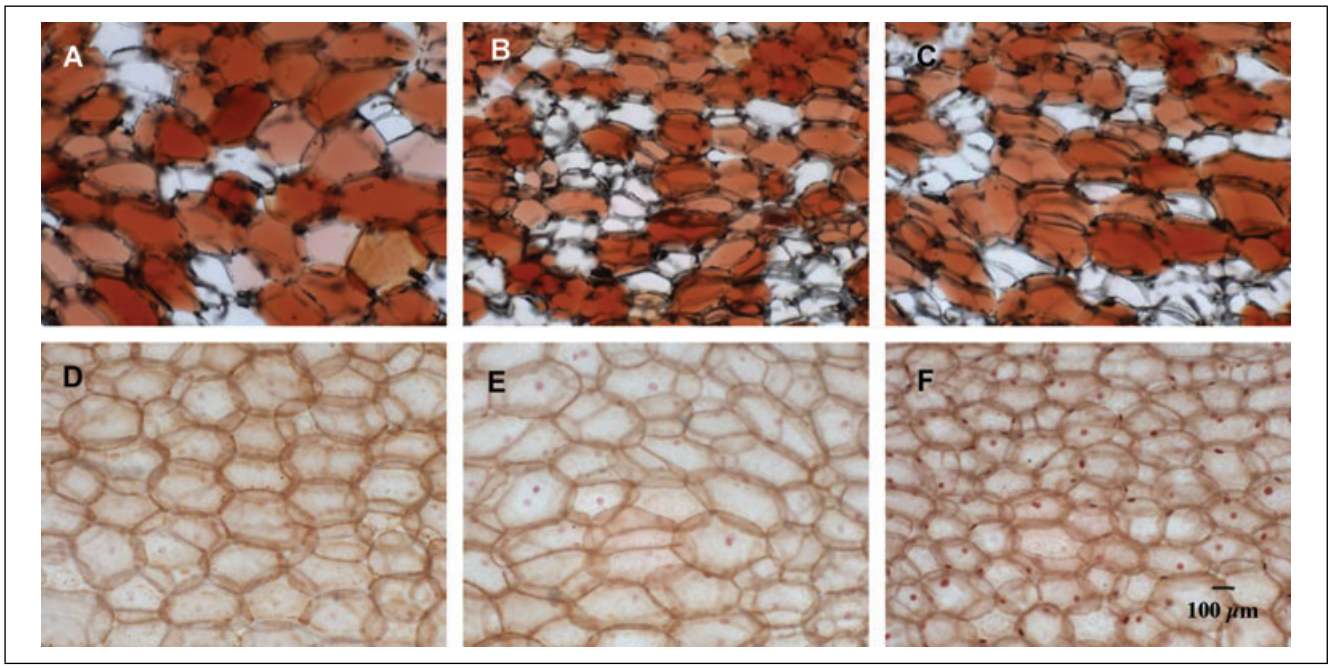

Figure 2–Photomicrographs of thermally treated onions: (A) control, (B) 40 °C, (C) 50 °C, (D) 60 °C, (E) 70 °C, and (F) 90 °C.

Refraction of air spaces differs from liquid-filled plant vacuoles and appear as black regions in the RGB photomicrographs (Figure 1 and 2). The percent of air spaces within the tissue was calculated and is shown in Figure 5 and 6. The vacuum packaging step (approximately 0 MPa) appeared to be mild enough that no differences with respect to the control could be observed. In high pressure treatments, the amount of air contained within the tissue decreased with increasing levels of pressure. At 50 and 200 MPa, there was a decrease in the air spaces present with respect to the control while no air was present after the 300 and 600 MPa treatments. In the thermal treatments, following the 60, 70, and 90 °C treatments, there was no air present within the samples. Air spaces with differences in size, orientation, and distribution have been reported to make an important contribution to the textural properties of tissues (Khan and Vincent 1993). Values determined by image analysis in raw tissue corresponded with ranges described for other fruits and vegetables, for example, 1% air fraction in potato tissue and 7% to 25% in apple tissue (Lin and Pitt 1986; Dražeta and others 2004).

Effects of high pressure and temperature on texture

Force-deformation curves obtained for high pressure and thermally treated onions were able to be distinguished into two types of profiles differentiating raw and mild treatments from more severe ones, for both types of processing (Figure 7 and 8). The force-deformation curves of raw and mild processing treatments were characterized by a rapid initial slope, primarily exhibiting an elastic response until the bioyield was reached. After the bioyield and failure, several additional peaks were present. On the other hand, severely treated onions (300 and 600 MPa or 60, 70, and 90 °C) had a highly viscoelastic initial response, and the bioyield

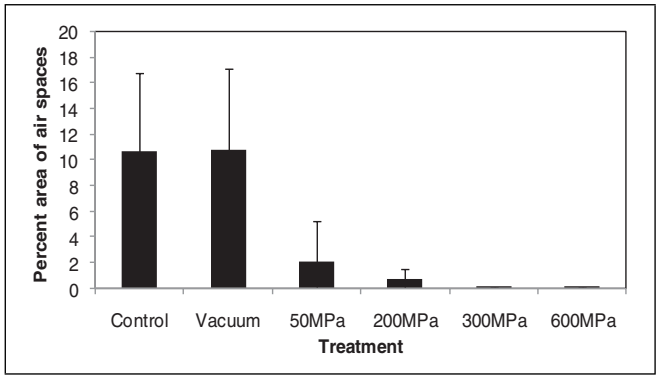

Figure 5–Percent of total area occupied by air spaces for high pressure treatments: control, vacuum packaged, 50, 200, 300, and 600 MPa.

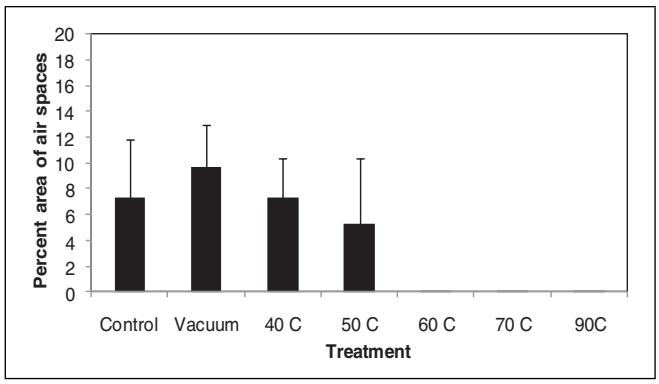

Figure 6–Percent of total area occupied by air spaces for thermal treatments: control, vacuum packaged, 40, 50, 60, 70, and 90 °C.

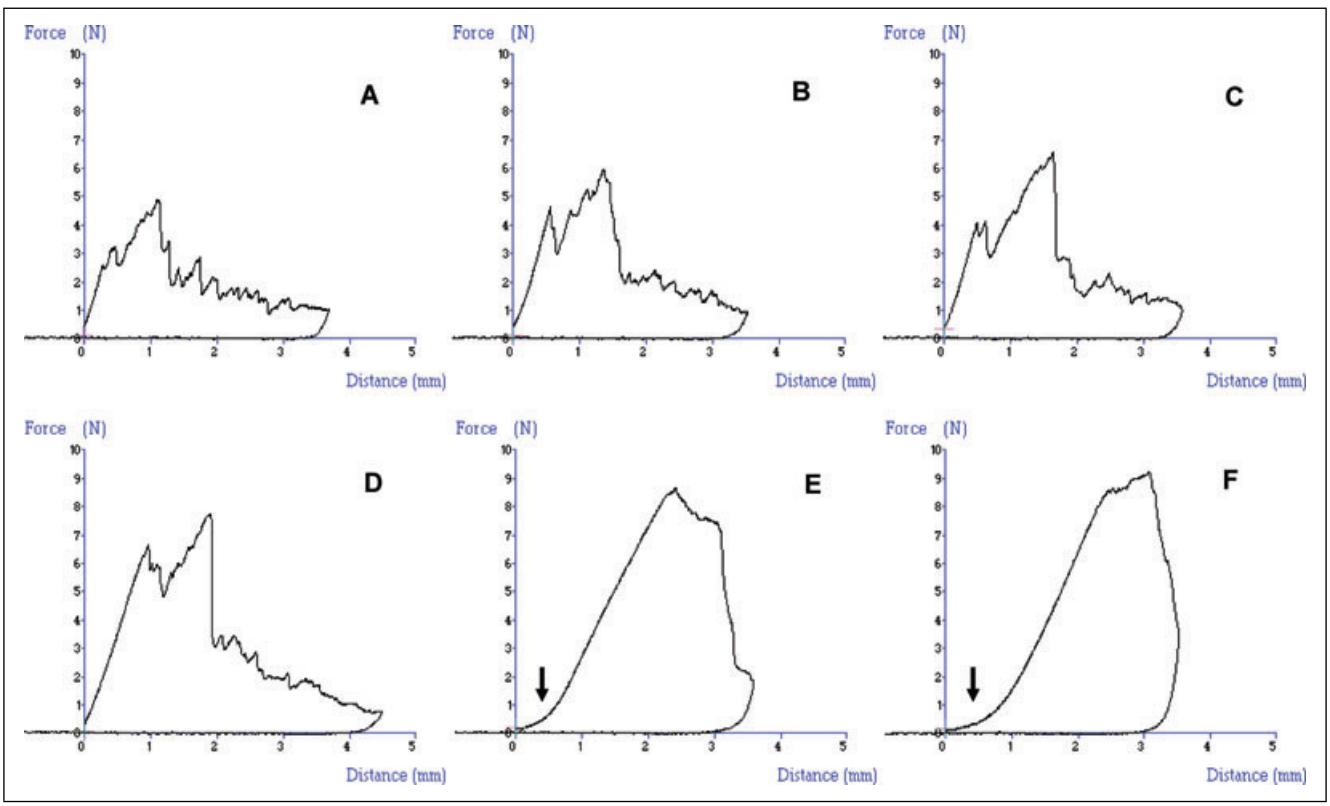

Figure 7–Force-deformation curves of controls and high pressure treated onions: (A) control, (B) vacuum packaged, (C) 50 MPa, (D) 200 MPa, (E) 300 MPa, and (F) 600 MPa. Arrow indicates pseudoplastic deformation.

coincided with failure or rupture and the presence of multiple peaks was reduced. Initial viscoelastic deformation coincided with treatments that led to membrane rupture and PME activation.

Jackman and Stanley (1992) explained that the initial pseudoplastic deformation observed when applying a puncture test to tomato pericarp as a stretching or plastic deformation of the middle lamellar pectin allowing the rearrangement of cells, and a compaction of interstitial air spaces that provided little resistance to the applied load, with a concomitant amount of shear and stress required before the bioyield was initiated. The mechanisms proposed by Jackman and Stanley (1992) may hold true for severely treated onions, where increased pectin cross-linking at high levels of pressure (300 and 600 MPa) or temperature (60 and 70 °C) may have occurred after pectin demethylation and interstitial air spaces were no longer present in the tissue (Anthon and Barrett 2006).

Texture parameters were analyzed for high pressure (Figure 9) and thermal (Figure 10) processing treatments. The initial slopes of the force–deformation curves did not differ statistically between control, vacuum packaged, and 50 MPa treatments. The initial slope of the 200 MPa treatment was 18% less than that of the control, but did not differ statistically from the vacuum packaged or 50 MPa treatments. The 300 and 600 MPa treatments had the lowest initial slope (72% less than the control). The maximum force increased with pressure levels of 300 and 600 MPa. The 200 MPa treatment had fewer peaks as compared to the control, vacuum packaged, and 50 MPa treatments, while 300 and 600 MPa treatments had the least number of peaks and did not differ statistically from each other.

Figure 10 shows the results of selected texture parameters for thermal conditions. The initial slopes were similar for the raw, vacuum packaged, 40 and 50 °C treatments and decreased with treatments of 60 °C and above (78%, 72%, and 85% less than control for 60, 70, and 90 °C treatments, respectively). Maximum force increased at 70 °C and was reduced at 90 °C. The number of peaks present was significantly reduced with 60, 70, and 90 °C treatments as compared to the rest of the treatments.

Correlation coefficients between percent area stained and texture parameters were estimated (Table 1). The strong positive correlations observed (above 0.80) between percent area stained and initial slope, number of peaks and D moduli (Table 1) indicate that these texture parameters relate to the viability of the cells and the state of the membranes. Initial slope and D moduli have been related to the stiffness and deformability of the material under the load (Bourne 2002). Therefore tissues with higher numbers of cells with intact membranes are stiffer and less ductile. The high

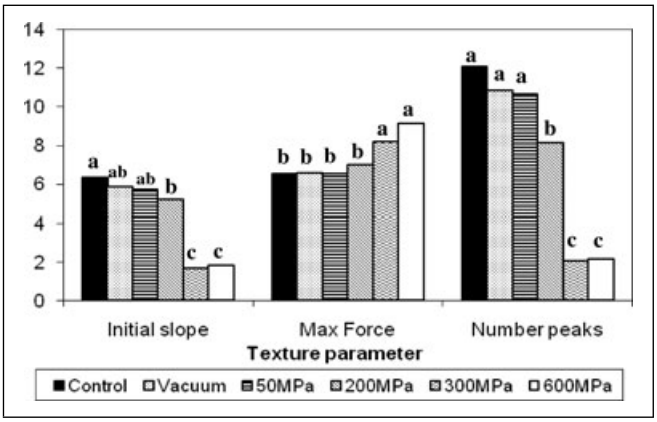

Figure 9–Texture parameters (initial slope [Nmm^{-1}], maximum force [N], and number of peaks) for control, vacuum packaged, and high pressure treated (50, 200, 300, and 600 MPa) onions.

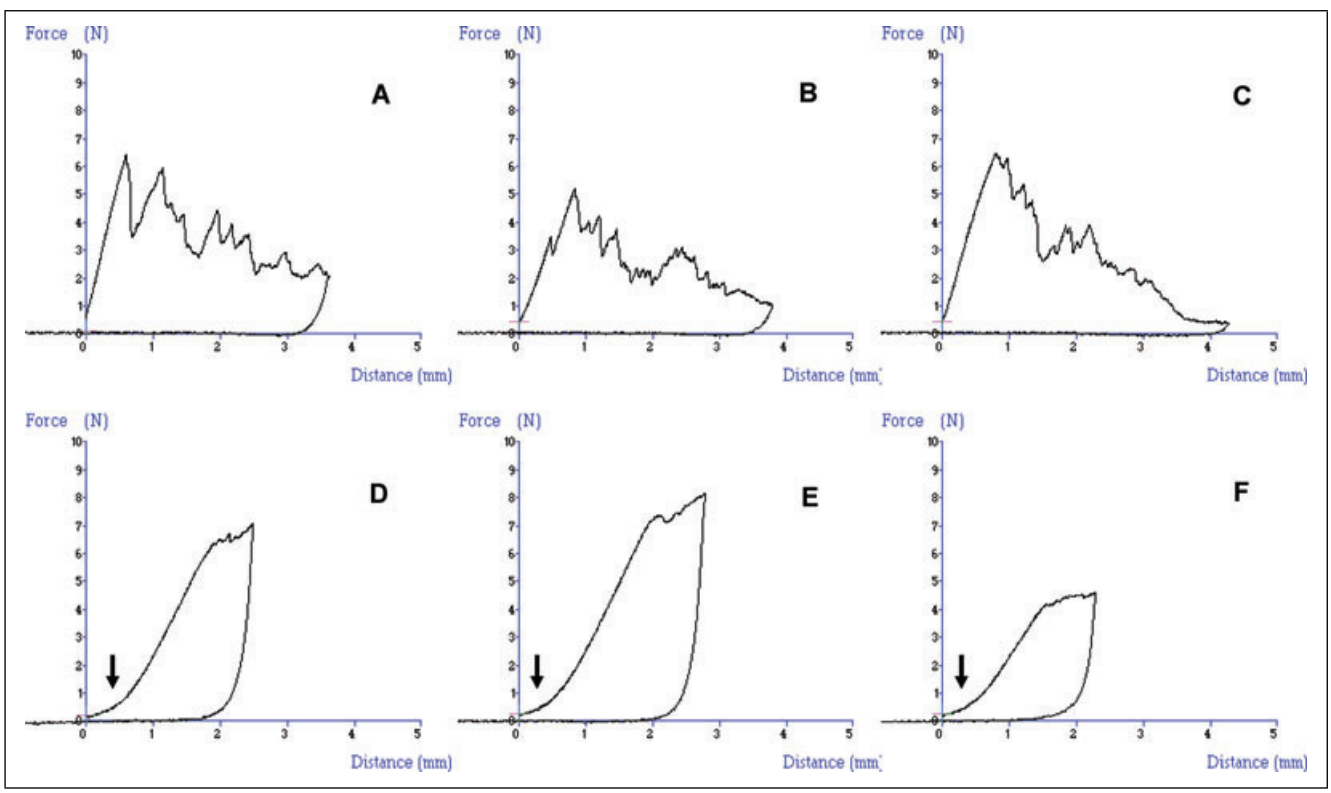

Figure 8–Force-deformation curves of control and thermally treated onions: (A) control, (B) 40 °C, (C) 50 °C, (D) 60 °C, (E) 70 °C, and (F) 90 °C. Arrow indicates pseudoplastic deformation.

positive correlation between percent area stained and number of peaks suggests that multiple peaks arise from the intactness of the different cell layers. The negative correlation between percent strain and the percent area stained (−0.88 for high pressure and −0.77 for thermal treatments, respectively) indicates that with a greater number of intact membranes there was less deformation of the tissue material at bioyield. The results confirm the importance of the maintenance of cell membrane integrity for tissue texture. Intact semipermeable membranes are necessary to develop and maintain the pressure of liquid cell contents on the membranes, or turgor within plant cells, and turgor is an important component of the texture of fruits and vegetables (Shackel and others 1991; Sajnin and others 2003).

Methanol content and hardening of onion tissue

Methanol formed in plant tissue is primarily a result of PME catalyzed demethylation of pectins in the middle lamella and cell walls and determination of this volatile content is one way of nondestructively determining PME activity in whole tissue pieces (Anthon and Barrett 2006). PME catalyzes the removal of the methyl groups from the polyglacturonic acid chain, leaving an increased number of carboxyl groups that can cross-link calcium ions and increase the firmness of the tissue (Grant and others 1973; Van Buren 1979; Greve and others 1994). The amount of methanol produced as a result of the 300 and 600 MPa treatments was more than 2-fold that of the control (Figure 11). In the thermal treatments, there was a 2- and 3-fold increase in methanol content after 60 and 70 °C treatments, respectively, relative to the control, while the amount of methanol produced decreased at 90 °C indicating PME inactivation above 70 °C (Figure 12). The hardness of the tissue, measured as the maximum force (Bourne 2002) on same onion samples, is also shown in Figure 11 and 12. The changes in maximum force paralleled the changes in methanol content for both the high pressure and thermally processed onions, suggesting that the increase in hardness was the result of a decrease in pectin methylesterification caused by the thermal or high pressure activation of PME. This concept has long been used for texture preservation, with PME activation during low temperature (60 to 70 °C) blanching regimes of fruits and vegetables (Stanley and others 1995; Anthon and Barrett 2004; Anthon and others 2005), and this has been observed by other investigators with high pressure treatments (Sila and others 2007a, 2007b).

Sila and others (2007a) reported PME to be heat labile, with thermal inactivation of extracted carrot PME occurring at about 50 °C, while pressure resistance occurred up to 600 MPa, especially at temperatures below 40 °C. In intact plant tissues, heat penetration rates and temperature distribution in various locations as well as the food matrix itself may influence the catalytic activity of PME (de Roeck and others 2008) inactivation of PME at higher temperatures in onion tissue (above 70 °C). By following the molar mass distribution of pectin polysaccharides in carrots in brine treated at high temperature and atmospheric pressure (80 °C, 0.1 MPa) or at a high pressure sterilization treatment (80 °C, 600 MPa), de Roeck and others (2008) showed that the solubilization of cell wall components occurred as a result of high-temperature processing only, whereas the combination of high-temperature and high pressure processing did not solubilize the cell wall. Therefore,

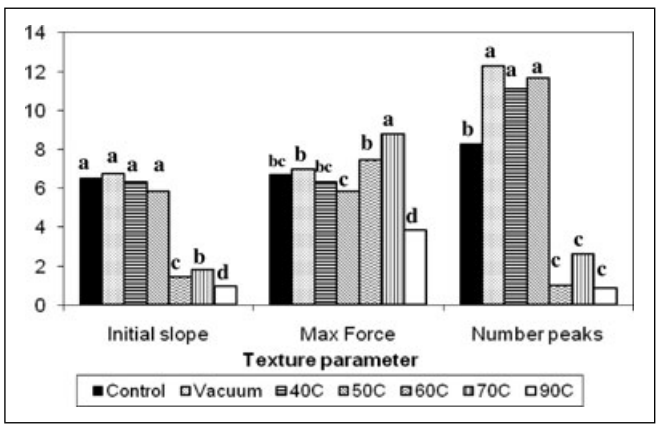

Figure 10–Texture parameters (initial slope [Nmm^{-1}], maximum force [N], and number of peaks) for control and thermally treated (40, 50, 60, 70, and 90 °C) onions.

Table 1– Correlation coefficients ($r_{x,y}$) between percent area stained (indicator of cell viability and membrane integrity) and the different texture parameters.

Texture parameter	HPP	Thermal
Initial slope	0.85[a]	0.89[a]
N peaks	0.90[a]	0.81[a]
€ (%strain)	−0.88[a]	−0.77[a]
Max force	−0.63[a]	0.13 ns
Bioyield	−0.68[a]	0.01 ns
Stress	−0.68[a]	0.01 ns
D moduli	0.83[a]	0.83[a]
Total area	−0.40[a]	0.14 ns
Area 1	−0.83[a]	−0.45[a]
Area 2	0.76[a]	0.67[a]

[a]Correlation coefficient (rx,y) is significant, $P < 0.05$.
ns = correlation coefficient (rx,y) is not significant, $P > 0.05$.

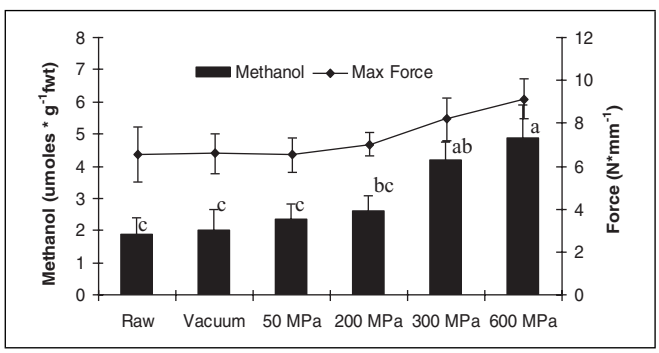

Figure 11–Methanol content and hardness of controls and high pressure treated onions. Values with a common letter do not differ significantly ($P < 0.05$); fwt = fresh weight.

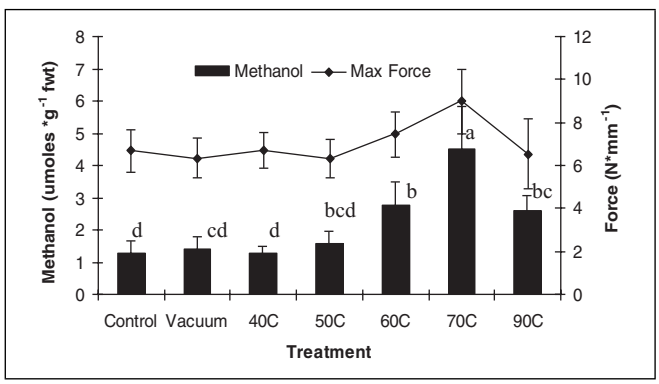

Figure 12–Methanol content and hardness of controls and heat-treated onions. Values with a common letter do not differ significantly ($P < 0.05$); fwt = fresh weight.

in high pressure treatments, the firming effect occurs as a result of PME activation without solubilization of cell walls. A 3rd contribution to tissue hardness may come from a high pressure-induced compaction effect measured as a decrease in the onion scale thickness at 300 and 600 MPa, as compared to the control (data not shown).

The negative correlation between maximum force and percent area stained observed for high pressure treatments (−0.63, Table 1) may indicate that tissues may harden even though cell membrane rupture occurs. Anthon and Barrett (2006) have already suggested that some PME activation may result as a consequence of membrane rupture and leakage between compartments, changing the environment of the enzyme and its access to pectin substrates. PME inactivation at temperatures of 90 °C may explain the lack of correlation between percent area stained and maximum force in the thermal treatments.

Conclusions

Information extracted from the present study allowed for quantification of viable cells in onion parenchyma tissue and provided information on onion cell integrity and the effects of 2 different processing methods. Once membrane integrity was lost, several texture parameters were affected (for example, initial slope, number of peaks, D moduli), which changed the texture profile of the onion tissue. Viable cells with intact membranes were a requisite for maintenance of the raw tissue texture characteristics. Hardening of the tissue corresponded with an increase in methanol content, indicating PME activation and changes occurring at the pectin level of the cell wall. In future research, it is important to understand changes that occur at the molecular level of the cell membranes, which will affect membrane permeability, cell compartmentalization, and tissue texture. Further understanding of the effects of processing on plant cell integrity will allow for implementation of processing methods and perhaps pretreatments, in such a way that textural properties are preserved.

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