Microscopic Quantification of Cell Integrity in Raw and Processed Onion Parenchyma Cells

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

Abstract: A cell viability assessment method based computer vision analysis of the uptake of neutral red dye was used to quantify cell membrane integrity in raw and processed parenchyma cells of onion tissues. The presence of stained vacuoles was used as an indicator of tonoplast membrane integrity and photomicrographs were acquired for microscopic image analysis and cell integrity quantification. Two different image analysis methods, involving the analysis of the saturation and green components of RGB (red, green, blue) images, were compared to the conventional cell count method. Use of the saturation component of RGB images allowed for the visualization and quantification of viable and inviable cells as well as extracellular air spaces. The combination of neutral red uptake, as visualization by light field microscopy, and saturation image analysis, allowed for quantitative determination of the effects of high pressure processing on onion cell integrity.

Keywords: image analysis, microscopy, onions, parenchyma cells

Practical Application: Preservation of vegetable tissues may involve heating or other methods that result in the loss of tissue integrity and potentially quality deterioration. In this study, we stained unprocessed and processed onion tissues with neutral red dye and then used a microscope and a computer imaging program to quantify how many cells were intact or ruptured.

Introduction

Fruits and vegetables are plant organs composed of millions of cells with specialized functions and a basic eukaryotic organization which includes a nucleus, cytoplasm, and subcellular organelles, all enclosed by the plasma membrane and a cellulosic cell wall. Mature living plant cells contain a large water and solute filled vacuole that occupies 80% to 90% of the total volume of the cell and is surrounded by the tonoplast membrane (Taiz and Zeiger 2006). Cellular structure determines mechanical properties of plant tissues (Knorr 1994; Donald and others 2003; de Escalada Pla and others 2006), is related to nutrient bioavailability (Verlinde and others 2008), plays a role in chemical and microbial stability, as well as in transport properties and food product engineering (Aguilera 2005).

Food technologists recognize that the microstructure of plant and animal tissues is significant, and plays a role in sensory appeal and shelf life of processed products. Images of food are widely used in the scientific literature to illustrate structural features that compliment information generated by means of other instrumental data, but if quantitative information can also be extracted, the method will open new opportunities to food analysis (Aguilera 2005; Ramirez and others 2009). A multidisciplinary approach involving microscopists, food scientists and food engineers is required to allow the linkage of structure to food properties and to understand the basic mechanisms of physicochemical changes (Aguilera 2005). In the case of plant-based foods, knowledge of the cellular and tissue transformations that result from environmental conditions or processing manipulations is a powerful means to gain a better understanding of biological systems, to optimize processing and product quality (Gomez Galindo and others 2007). Cellular compartmentalization determines that in apple parenchyma tissue, air-drying changes primarily the water content of the cell vacuole, and during freezing the water of the cell vacuole freezes before water associated with other cellular compartments (Hills and Ramigereau 1997).

Quantitative information and numerical data can be extracted by image analysis of photomicrographs (Trejo-Tapia and others 2003; Aguilera and Germain 2007) and simple applications allow for specific measurements such as size, shape, and area fraction (Riva and others 2005). Image analysis has been used for several applications, such as tomato pericarp cell structure quantification (Devaux and others 2008), identification of discoloration caused by bacterial disease (Vizhányó and Felföldi 2000), and quantification of pigments in cell suspensions (Miyanaga and others 2000; Trejo-Tapia and others 2007).

Cell viability methods are based on either physical properties of cells (such as cytoplasmic streaming and membrane integrity or on biochemical characteristics (such as DNA, RNA, or protein synthesis) (Byth and others 2001). The determination of viable cells by membrane integrity assays rely on the uptake and active retention of dyes such as neutral red (Admon and Jacoby 1980; Ehara and others 1996; Repetto and others 2008) and fluorescein (Heslop-Harrison and Heslop-Harrison 1970) in living cells, or the passive staining of the contents of dead cells such as Evans blue

that leaks through ruptured cells (Baker and others 1994). In fleshy fruit tissues of developing grapes (Krasnow and others 2008) and in cucumbers (Sajnin and others 2003) a fluorescent dye, fluorescein diacetate, was used to detect active cellular metabolism. In bacterial cells, carbonfluorescein diacetate and propidium iodide have been used to test for viable cells (Ananta and others 2005). In this study, the viability of onion parenchyma cells was evaluated by using neutral red staining visualized under bright-field microscopy.

Neutral red is a lipophilic phenazine dye used for the staining of plant vacuoles (Admon and Jacoby 1980). The dye is uncharged and unprotonated in alkaline solutions. It diffuses across cell membranes due to its lipophilic nature, and protonates and accumulates in the acidic vacuolar medium, appearing as dark red-colored vacuoles in intact cells (Ehara and others 1996; Fincan and Dejmek 2002). The objective of the present article was to determine the integrity of the tonoplast, which is the vacuole membrane, in onion parenchyma cells and to evaluate image analysis procedures that allow for quantification of viable cells in raw and processed tissue.

Materials and Methods

Raw materials and processing

Spanish yellow onions cv. Sabroso (approx. 8 cm dia) were provided by Gills Onions (Oxnard, Calif., U.S.A.) and used for 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 back to UC Davis under refrigeration.

Raw material. The outer papery scales and the 1st fleshy scale or layer of the onions were removed and an equatorial 1 cm thick slice, packaged without vacuum was used as the control treatment.

High pressure processing treatments. Sliced onions were vacuum packaged using a vacuum machine (Easy Pack, Koch, Kansas City, Mo., U.S.A.) and exposed to 200 and 600 MPa for a 5-min hold time. The initial high pressure unit temperature (T_i) was approximately 20 °C. 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.

Light microscopy

Microscope section preparation. Sample pieces (approximately 5 × 5 mm) taken from the 3rd scale at the equatorial region of the onion, were mounted on a sample holder with instant glue and allowed to dry for 5 min with the upper surface of the onion piece covered with Parafilm M® (American Natl. Can, Menasha, Wis., U.S.A.) to minimize desiccation. Section specimens approximately 400 μm were obtained using a Vibratome 1000 Plus (The Vibratome Co., St. Louis, Mo., U.S.A.).

Toluidine blue O (TBO) staining. TBO stains cell walls and therefore was used to visualize cells for identification and determination of cell sizes (O'Brien and others 1964). Sections 400 μm in thickness were transferred to a microscope slide with a drop of deionized water. A drop of 0.025% TBO in 1% aqueous sodium tetraborate ($Na_2 B_4 O_7.10 H_2O$) was added over the tissue; it was let stand 1 min and then 2 drops of deionized water were added. The section was covered with a cover slip and the excess water was removed with paper tissue. Cross-sections, radial longitudinal, and tangential longitudinal sections were obtained to observe cell dimensions.

Neutral red (NR) staining. Two onion pieces were taken from the 3rd scale of each onion on opposite sides of the bulb. From each piece, two 400 μm thick cross-sections perpendicular to both epidermises were obtained for staining and microscopic analysis. An NR stock solution was prepared and a fresh dilution was made each time before bathing the samples. To prepare the stock solution, 0.5% NR was dissolved for 15 min in acetone and then filtered twice using Whatman paper nr 1 (Whatman Intl. Ltd., Springfield Mill, Maidstone, Kent, U.K.). The filtered stock solution was 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, and used as the dyeing solution.

After Vibratome sectioning, samples were quickly rinsed in deionized water to remove debris and cell contents released by the mechanical damage of sectioning. Onion sections were transferred to porcelain wells containing the dye solution for a period of 2 h, after which they were rinsed again for half an hour in the 0.2 M mannitol–0.01 M HEPES buffer solution. Sections were mounted on a microscope slide with a drop of deionized water, covered with a coverslip and immediately 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 MicroFire software, Olympus America, Melville, N.Y., U.S.A.). Color photomicrographs (800 × 600 pixel resolution, white balance corrected) were captured from 2 selected areas within the parenchyma tissue of each specimen. The selected areas were positioned in between and equidistant from 2 vascular bundles and 4 to 5 cells rows apart from the outer epidermis (Figure 1). Two onions were used per treatment and from each onion 4 onion specimens were obtained. Two micrographs were taken from each specimen. The total number of micrographs per treatment was as follows:

2 onions × 4 specimens/onion × 2 micrographs/specimen

= 16 micrographs per treatment

To verify vacuole cell staining and to differentiate it from the whole cell, plasmolysis was induced in the peeled inner epidermal tissue by immersing it in different concentrations (0.1 to 0.4 M) of mannitol solutions.

Image processing and analysis

Image processing software (Image J software, NIH, Bethesda, Md., U.S.A.) was used for preprocessing and analysis of the photomicrographs. To correct for uneven illumination (Aguilera and Germain 2007; Macbiophotonics 2008), photomicrographs were flat field corrected. The flat field image, which consisted of averaging 15 independent views of the microscope slide and cover slip with a drop of the rinsing buffer solution, was obtained at
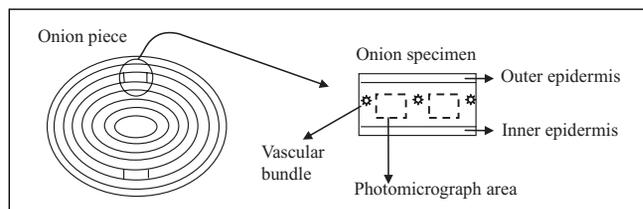

Figure 1–Onion slice indicating the positions within an onion scale from where onion pieces were obtained for light microscopy analysis (left). Selected areas within the parenchyma tissue of an onion specimen were used to obtain photomicrographs for digital image analysis (right).

the same illumination intensity as the sample photomicrographs. Quantification methodologies were developed for the analysis of the photomicrographs. Two different methods were compared to the conventional cell count method.

1. *Cell count method*. This method consisted of counting the number of stained and unstained cells in the whole area of the photomicrograph; results were expressed as a percentage of total number of cells. The method of de Vos (2008) was used to record the cell count. Red to pink stained cells were counted as viable cells, while cells that either retained no dye and/or presented a rough appearance that was not smooth were counted as inviable cells.

2. *Analysis of the saturation component of HSB images*. RGB (red, green, blue) color images were transformed into HSB (hue, saturation, brightness) color space and the saturation image (which had the highest contrast between viable and inviable cells) was selected for further analysis. Saturation component images were processed using different filters and the processed image was used to calculate percentage of the total photomicrograph area stained and unstained, indicators of cell viability.

3. *Analysis of the green component of RGB images*. The green component of the RGB images was selected because it also had optimal contrast between viable and inviable cells. A threshold level was applied to select for red to pink pixels of viable cells. The green component of the RGB images discriminated extracellular air spaces, which had different refractile properties and were visualized as black regions in the RGB images.

Results and Discussion

Onion cell characteristics

Toluidine blue O staining of onion tissue showed that in the parenchyma, cell length ranged between 200 and 370 μm in the radial longitudinal axis parallel to the epidermis (Figure 2A), and between 250 and 500 μm in the cross-sectional transverse axis perpendicular to the epidermis (Figure 2B). Based on these results, a 400 μm section thickness was selected for further study, to obtain an intact specimen of one cell layer thickness that would allow NR uptake observation and acceptable microscopic resolution. Tissue sectioning caused mechanical damage, as illustrated by the Toluidine blue O staining. Stained cell walls and the middle lamella appeared purple (O'Brien and others 1964). Void cells with unstained areas were visible when part of the cell wall was removed during sectioning (Figure 2B, 2C).

Cell viability

Neutral red dye has been widely used for the estimation of cell viability since penetration of the dye into the tissue depends on the integrity of the cell membrane and the capacity to maintain pH gradients (Guttenberger 2000a; Repetto and others 2008), and because the dye is relatively non toxic (Dubrovsky and others 2006). In this study, the staining of cell vacuoles allowed for the differentiation of viable from inviable cells in onion parenchyma tissue (Figure 3) and image analysis allowed for quantification of the impact of high pressure processing on cell integrity.

Viable cells were identified as those where the NR dye was up taken and retained within the vacuole; these cells had a red color and smooth appearance. Different intensities of red were observed among stained cells of untreated and treated samples (Figure 3A and 3B). Variations in cell color after staining have been attributed to differences in cell vacuolar pH or differences in the composition of the vacuolar sap, where the composition could influence the amount of complexing compounds present, resulting in differential trapping of the dye (Timmers and others 1995; Guttenberger 2000a). Air spaces within the tissue appeared as black regions in
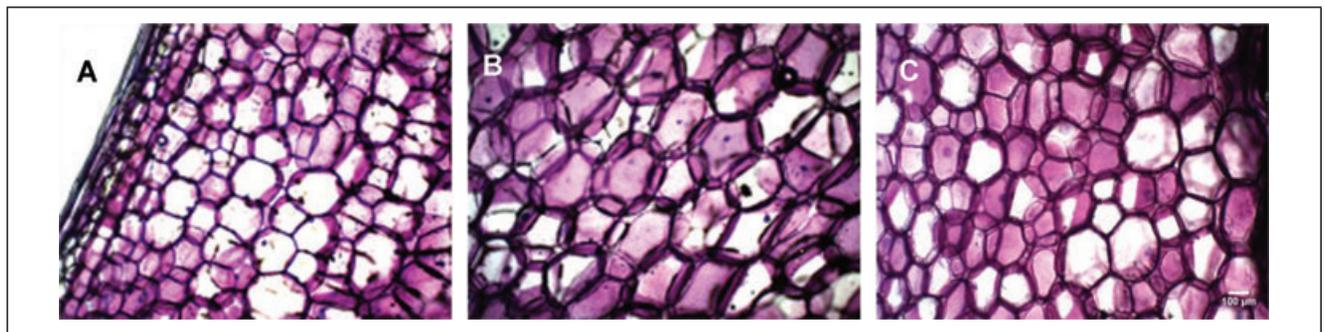

Figure 2–Onion tissue stained with 0.025% TBO-sodium tetraborate. Radial longitudinal (A) and cross-sections (B) of raw onion sections and cross-section of 600 MPa high pressure treated onion (C).
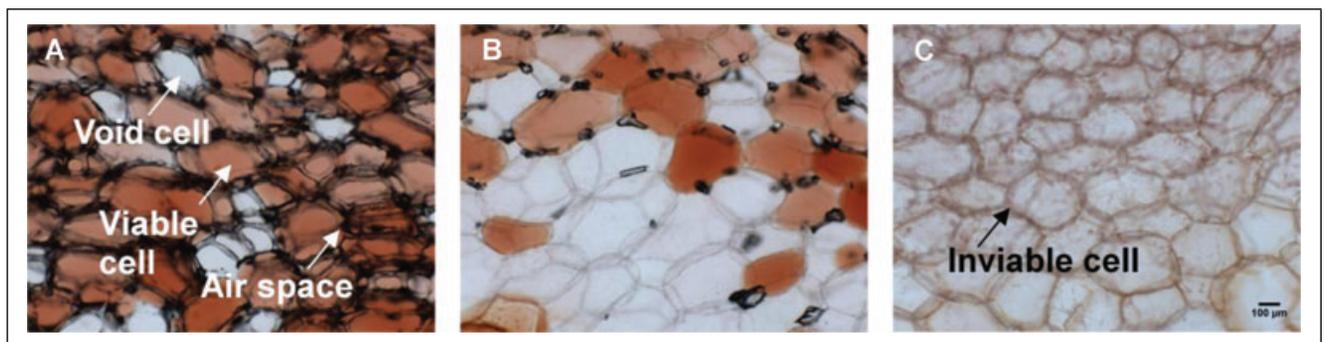

Figure 3–Cross-section specimens stained with neutral red dye of control (raw) with presence of viable and void cells (A), 200 MPa (B), and 600 MPa treatment indicating inviable cells.

the photomicrograph. Inviable cells were assumed where no dye was visually present as a result of mechanical damage (Figure 3A) or where a granulated texture with faint pink color indicated the presence of cell walls and cell debris (Figure 3C). The granulated texture and pink color was concentrated primarily at the edges of inviable cells in contrast to the stained viable cells, which had the dye clearly distributed within the vacuole, visually occupying the entire volume of the cell. Nonacidotropic accumulation of neutral red dye has been reported and in *Allium porrum*. Guttenberger (2000b) observed that neutral red stained vascular

bundles and exodermises of roots and root tip regions (mainly meristems) where no vacuoles were present. These authors also found heavy nonacidotropic staining in cell walls of specialized cells, called idioblasts, of freeze–thawed root tips. Addition of a rinsing step in higher pH buffer solution, as applied here, or use of a higher ionic strength solution helped visually reduce this background interference (Guttenberger 2000a). Staining of lipophilic structures such as suberin and lipids, (Lillie 1977), phenolic substances in the cell sap (Ehara and others 1996; Guttenberger 2000a), as well as interaction of the protonated dye
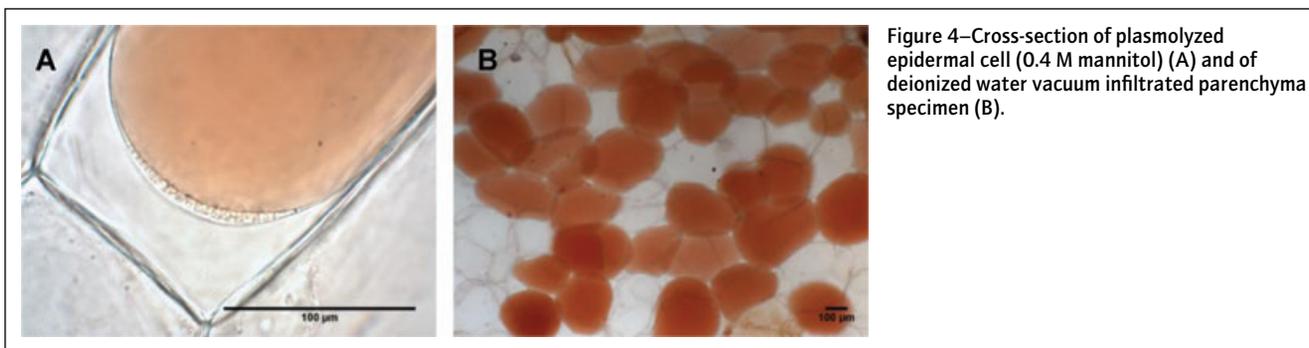

Figure 4–Cross-section of plasmolyzed epidermal cell (0.4 M mannitol) (A) and of deionized water vacuum infiltrated parenchyma specimen (B).
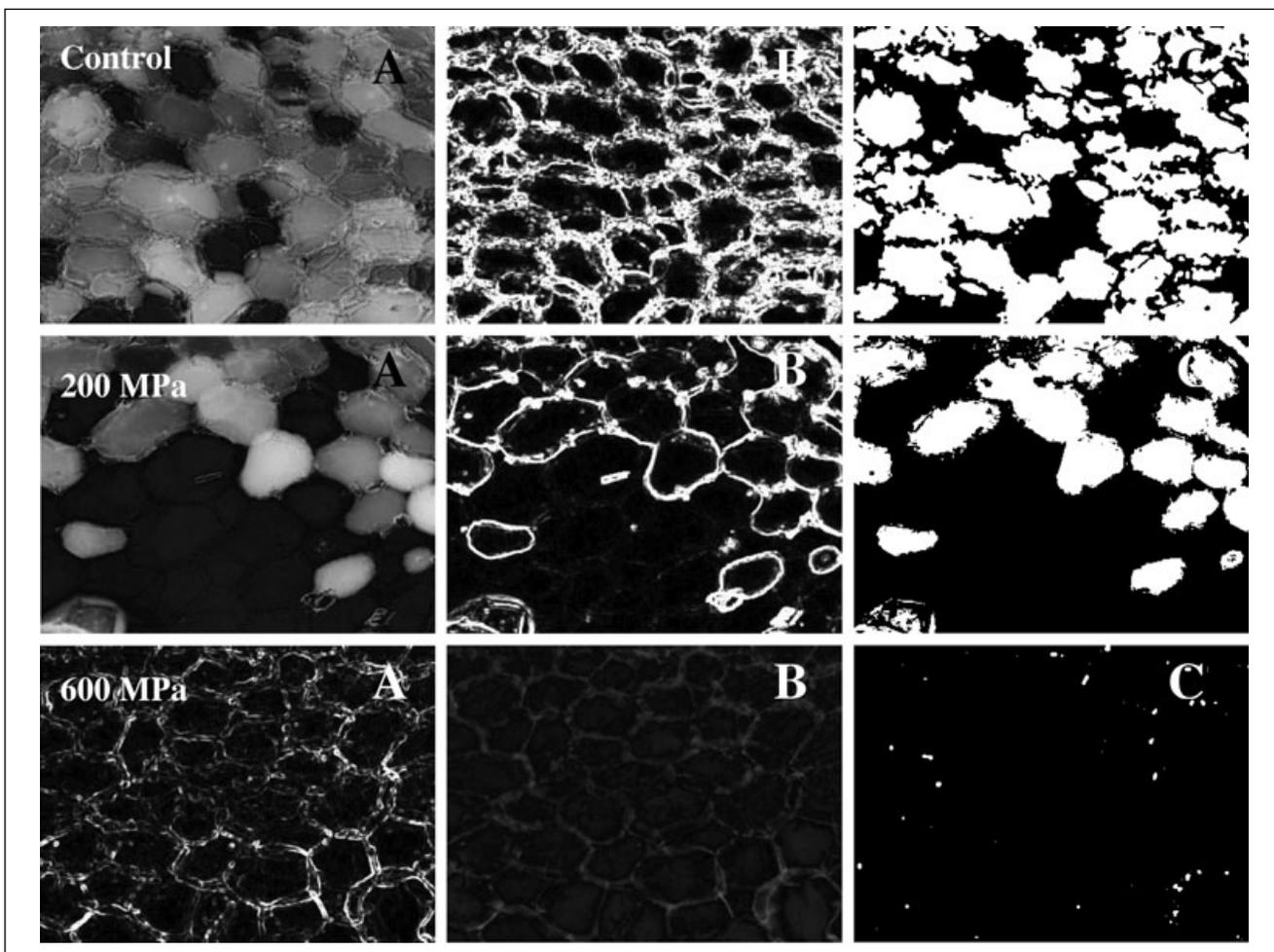
Figure 5–Image processing steps for the quantification of the percentage of total area stained based on the saturation component of HSB images. Rows pertain to treatments control, 200 MPa, and 600 MPa. Columns represent the images result of the processing steps: saturation component image after transformation from RGB image to HSB image (A); variance filter applied to the saturation component image (B); final image result of the processing steps and threshold level applied used for image analysis and quantification (C).

with negative charges in cell walls (Stadelmann and Kinzel 1972) and interaction with DNA (Wang and others 2003) have been reported and may account for the retention of stain in inviable cells. To verify vacuole cell staining and differentiate it from the whole cell, plasmolysis was induced in the inner epidermal tissue by using different concentrations (0.1 to 0.4 M) of mannitol solutions. In plasmolyzed cells, red staining of vacuoles and yellow staining of cytoplasm was observed (Figure 4A). A vacuum infiltration in deionized water treatment was included to verify that when extracellular air spaces were occupied by the solution, black regions would disappear in the photomicrograph (Figure 4B).

Image analysis method selection

To determine the best method for quantifying viable cells, saturation component images and green component images of photomicrographs were processed and analyzed and compared to the cell count method. The fact that inviable cells in processed tissues retained some stain required some additional processing steps during image analysis of onion photomicrographs.

Image processing of the saturation component of HSB color space images. Examples of the saturation component of HSB color space images and processing steps to obtain the fi-

nal processed images (of the exemplified images of Figure 3), are shown in Figure 5. Onion tissue that had been submitted to high levels of high pressure and in which cells were observed as inviable (no intense red stain retained within the cell), there was
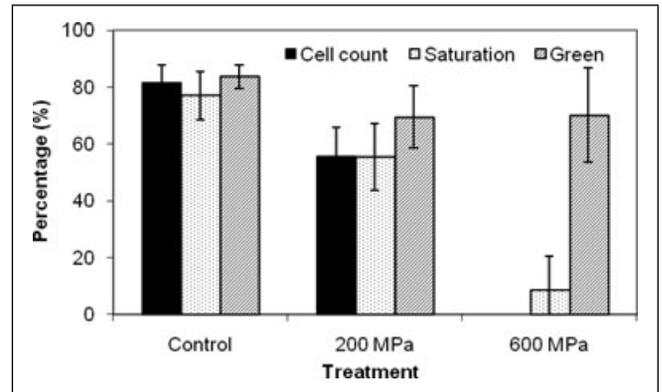

Figure 7–Quantification of intact cells expressed as a percentage of total cells in the cell count method and as a percentage of total area stained in the saturation component method.
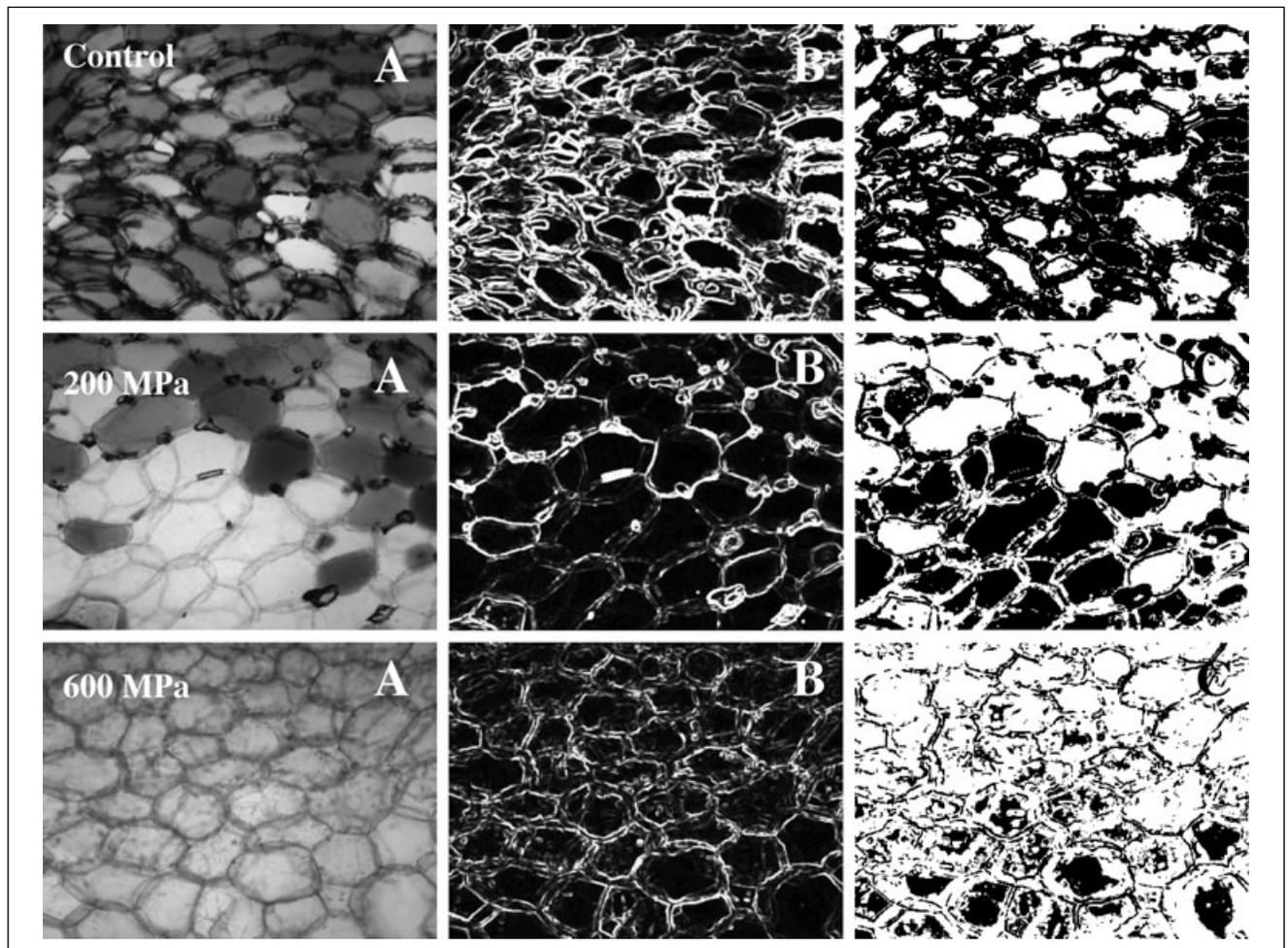

Figure 6–Image processing steps toward the quantification of the percentage of total area stained based on the green component of RGB images. Rows pertain to treatments control, 200 MPa, and 600 MPa. Columns represent the images result of the processing steps: green component of RGB image (A); variance filter applied to the green component image (B); final image result of the processing steps and threshold level applied used for image analysis and quantification (C).
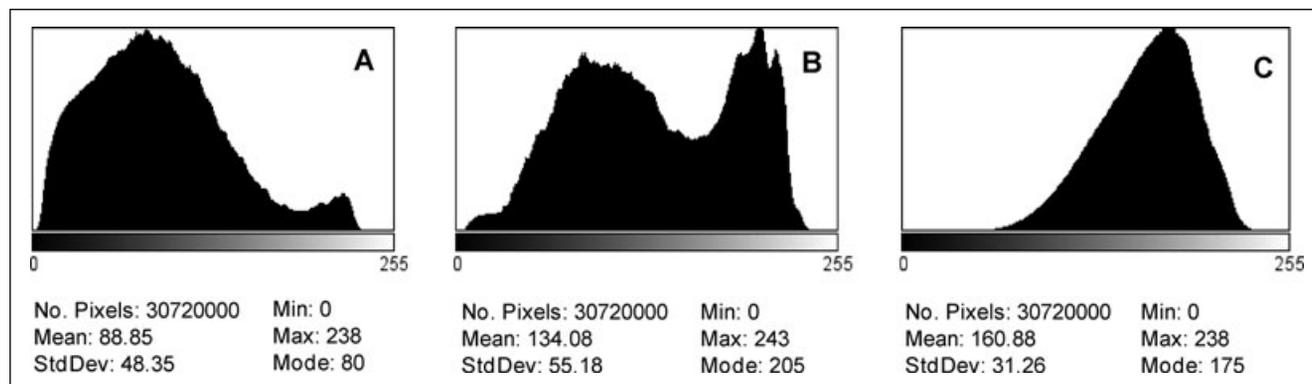
Figure 8–Average histograms of the green component of RGB images of control (A), 200 MPa (B), and 600 MPa (C) treated onions. (Mean, Std Dev, Min, and Max refer to pixel value.)

concentrated dye on the edges of the cells. To remove these dyed edges a variance filter that highlighted edges in the image (Figure 5B) by replacement of each pixel with the neighborhood variance (radius = 1 pixel) was applied, and subtracted from the original saturation image. A threshold was applied to the resulting image based on a threshold cutoff point selected from the pixel values of the stained regions. The threshold procedure converted the grayscale image into a binary image. To recover incorrectly removed edges, a binary dilation procedure, that causes an increase in bright object sizes by adding pixels to the edges of objects (Devaux and others 2008), was performed to recover part of the area incorrectly removed from the intact cells. To reduce noise, a median filter was applied. Examples of the final processed images are shown in Figure 5C. The total area stained was automatically calculated from the processed images. A minimum area (7854 μm^2) threshold was set, which accounted for a approximately 50 μm radius minimum cell size, and objects with areas below the minimum were not quantified.

Image processing of the green component of RGB color space images. A similar image processing procedure to that applied with the saturation component images was developed for the green image component and is shown in Figure 6. A variance filter was first applied followed by a thresholding step to facilitate the selection of stained areas.

The different image analysis and quantification procedures (cell count, saturation, and green analysis methods) were compared (Figure 7). Raw control onions did not show 100% viable cells due to cell sectioning damage as illustrated by the TBO staining. The saturation image analysis method, where percent stained and unstained areas were used as indicators of percent of viable and inviable cells, respectively, showed comparable results to the cell count method for the control and 200 MPa treatments. Saturation analysis of images themselves or in combination with other color parameters has been applied successfully in previous food and agricultural applications (Choi and others 1995; Karcher and Richardson 2003; Zheng and others 2006). The 600 MPa treatment showed, however, that 9% of the total photomicrograph area was stained, which was higher than the 0% viable cells indicted by the cell count method.

In this study, analysis of the green component of the RGB images did not distinguish clearly between the different types of cells. The histogram analysis of the green images helped explain the results (Figure 8) where the green intensity distributions of 2 populations of cells could be identified with considerable overlap. Discriminant analysis (SAS 9.1 2003) of the green intensity

distributions correctly identified control photomicrographs (with a high percent of living cells) with 77% accuracy (Figure 8A) and 600 MPa photomicrographs with 83% accuracy (Figure 8C) while the 200 MPa treatment (Figure 8B), allowed only 8% accuracy. Fincan and Dejmek (2002) used an *in situ* image visualization method to quantify cell permeabilization by NR staining during and after pulsed electric field treatments on onion epidermis tissue by taking serial images and analyzing the green component of RGB images. Other successful methods used for quantification of neutral red uptake have included the use of spectrophotometric methods after extracted dye accumulated within the cells (Ehara and others 1996; Repetto and others 2008). The results of the current study indicate that analysis of the saturation component of the color images provides better characterization of NR staining on onion parenchyma cells than the green color component.

Conclusions

The image analysis method comparison indicated that analysis of the saturation image had a high correspondence to the cell count method and therefore determined an accurate method for quantification of viable cells in raw and processed tissue. It has been verified that neutral red staining is a valid method to determine membrane integrity of plant tissues and in combination with image analysis can be used to quantify cell structure changes after processing. Further work has been carried by the authors to relate cell viability results with other chemical and biochemical techniques that indicate physical changes at the cell membrane level.

Acknowledgments

References

Admon A, Jacoby B. 1980. Assessment of cytoplasmic contaminations in isolated vacuole preparations. Plant Physiol 65:85–7.
Aguilera JM. 2005. Why food microstructure? J Food Engr 67:3–11.
Aguilera JM, Germain JC. 2007. Advances in image analysis for the study of food microstructure. In: McClements Julian, editor. Understanding and controlling the miscrostructure of complex foods. Abington Cambridge, England & Boca Raton, Fla.: Woodhead Publishing Limited and CRC Press LLC. Chapter 10, p 261–87.

Ananta E, Volkert M, Knorr D. 2005. Cellular injuries and storage stability of spray-dried *Lactobacillus rhamnosus* GG. Int Dairy J 15:399–409.

Baker CN, Banerjee SN, Tenover FC. 1994. Evaluation of alamar colorimetric MIC method for antimicrobial susceptibility testing of gram-negative bacteria. J Clin Microbiol 32:1261–7.

Byth H, Mchunu B, Dubery I, Bornman L. 2001. Assessment of a simple, non-toxic Alamar Blue cell survival assay to monitor tomato cell viability. Phytochem Anal 12:340–6.

Choi K, Lee G, Han YJ, Bunn JM. 1995. Tomato maturity evaluation using color image-analysis. T ASAE 38:171–6.

de Escalada Pla M, Rojas AM, Gerschenson LN. 2006. Effect of immersion and turgor pressure change on mechanical properties of pumpkin (*Cucumis moschata*, Duch.). J Sci Food Agric 86:2628–37.

de Vos K. 2008. Cell counter plugin. Available from: http://rsb.info.nih.gov/ij/plugins/cell-counter.html. Accessed June 2008.

Devaux MF, Bouchette B, Legand D, Guillon F, Lahaye M. 2008. Macro-vision and grey level granulometry for quantification of tomato pericarp structure. Postharvest Biol Technol 47:199–209.

Donald AM, Baker FS, Smith AC, Waldron KW. 2003. Fracture of plant tissues and walls as visualized by environmental scanning electron microscopy. Ann Bot 92:73–7.

Dubrovsky J, Guttenberger M, Saralegui A, Naapsucialy-Mendivil S, Voight B, Baluška F, Menzel D. 2006. Neutral red as a probe for confocal laser scanning microscopy studies of plant roots. Ann Bot 97:1127–38.

Ehara M, Noguchi T, Ueda K. 1996. Uptake of neutral red by the vacuoles of green alga, Micrasterias pinnatifida. Plant Cell Biol 37:734–41.

Fincan M, Dejmek P. 2002. In situ visualization of the effect of a pulsed electric field on plant tissue. J Food Engr 55:223–30.

Gomez Galindo F, Rasmusson AG, Widell S, Kaack K, Sjöholm I. 2007. Plant stress physiology: opportunities and challenges for the food industry. Crit Rev Food Sci 46:749–63.

Guttenberger M. 2000a. A rapid staining procedure for arbuscules of living arbuscular mycorrhizas using neutral red as acidotropic dye. Plant Soil 226:211–8.

Guttenberger M. 2000b. Arbuscules of vesicular-arbuscular mycorrihzal fungi inhabit an acidic compartment within plant roots. Planta 211:299–304.

Heslop-Harrison J, Heslop-Harrison Y. 1970. Evaluation of pollen viability by enzymatically induce florescence: intracellular hydrolysis of fluorescein diacetate. Stain Technol 45:115–20.

Hills B, Remigereau B. 1997. NMR studies of changes in subcellular water compartmention in parenchyma apple tissue during drying and freezing. Int J Food Sci Technol 32:51–61.

Image J software. 2008. National Institutes of Health, Bethesda, Maryland, USA. Available from: http://rsb.info.nih.gov/ij/. Accessed May 2008.

Karcher D, Richardson MD. 2003. Quantifying turfgrass color using digital image analysis. Crop Sci 43:943–51.

Knorr D. 1994. Plant-cell and tissue-cultures as model systems for monitoring the impact of unit operations on plant foods. Trends Food Sci Technol 5:328–31.

Krasnow M, Matthews M, Shackel K. 2008. Evidence for substantial maintenance of membrane integrity and cell viability in normally developing grape (Vitis vinifera L.) berries throughout development. J Exp Bot 59:849–59.

Lillie RD. 1977. HJ Conn's Biological stains a handbook on the nature and uses of the dyes employed in the biological laboratory. 9th ed. Baltimore, Md.: Williams and Wilkins. 692 p.

Macbiophotonics. 2008. Image J for Microscopy. Available from: http://www.macbiophotonics.ca/imagej/index.htm/. Accessed June 2008.

Miyanaga K, Seki M, Furusaki S. 2000. Analysis of pigment accumulation heterogeneity in plant cell population by image-processing system. Biotechnol Bioengr 67:493–7.

O'Brien TP, Feder N, McCully ME. 1964 Polychromatic staining of plant cell walls by Toluidine blue O. Protoplasma 59:367–73.

Ramirez C, Germain JC, Aguilera JM. 2009. Image analysis of representative food structures: application of the bootstrap method. J Food Sci 74:R65–72.

Repetto G, del Peso A, Zurita JL. 2008. Neutral red uptake assay for the estimation of cell viability/cytotoxicty. Nat Protoc 3:1125–31.

Riva M, Campolongo S, Leva AA, Maestrelli A, Torreggiani D. 2005. Structure-property relationships in osmo-air dehydrated apricot cubes. Food Res Int 38:533–42.

Sajnin C, Gamba G, Gerschenson, Rojas AM. 2003. Textural, histological and biochemical changes in cucmber (*Cucumis sativus* L) due to immersion and variations in turgor pressure. J Sci Food Agric 83:731–40.

SAS 9.1. 2003. SAS Inst. Inc., Cary, N.C., U.S.A.

Stadelmann EJ, Kinzel H. 1972. Vital staining of plant cells. Methods Cell Biol 5:325–72.

Taiz L, Zeiger E. 2006. Plant physiology, 4th ed. Sunderland, Mass.: Sinauer Associates, Inc.

Timmers ACJ, Tirlapu UK, Schel JHN. 1995. Vacuolar accumulation of acridine orange and neutral red in zygotic and somatic embryos of carrot (*Daucus carota* L.). Protoplasma 188:236–4.

Trejo-Tapia G, Hernández-Trujillo R, Trejo-Espino JL, Jiménez-Aparicio A, Rodríguez-Monroy M. 2003. Analysis of morphological characteristics of Solanum chrysotrichum cell suspension cultures. World J Microbiol Biotechnol 19:929–32.

Trejo-Tapia G, Cuevas-Celis J, Salcedo-Morales G, Trejo-Espino JL, Arenas-Ocampo ML, Jiménez-Aparicio A. 2007. *Beta vulgaris* L. suspension cultures permeabilized with Triton X-100 retain cell viability and betacyinines production ability: a digital image analysis study. Biotechnol Prog 23:359–63.

Verlinde P, Oey I, Hendrickx M, Van Loey A. 2008. High-pressure treatments induce folate polyglutamate profile changes in intact broccoli (Brassica oleraceae L. cv. Italica) tissue. Food Chem 111:220–9.

Vizhányó T, Felföldi J. 2000. Enhancing colour differences in images of diseased mushrooms. Comput Electron Agric 26:187–98.

Wang Z, Zhang Z, Liu D, Dong S. 2003. A temperature-dependant interaction of neutral red with calf thymus DNA. Spectrochim Acta A 59:949–56.

Zheng C, Sun DW, Zheng L. 2006. Correlating colour to moisture content of large cooked beef joints by computer vision. J Food Engr 77:858–63.

E: Food Engineering & Physical Properties