

High pressure processing: novel technology for preserving avocado slices

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Abstract

The objective of this research was to study the effect of high pressure processing (HPP) on the quality, physiology and microstructure of avocado slices. Avocado slices were subjected to HPP (High Pressure Processing) treatment with pressures of 200 to 600 MPa and durations of 3 to 10 min. The samples were examined immediately after treatment to evaluate changes in colour profile, polyphenoloxidase (PPO) activity, and changes in microstructure, respiration rate and ethylene production measured immediately after treatment. Flesh colour was affected to some extent, with L (lightness) and C (chroma) values decreasing after HPP treatment. However, these changes were not evident to the eye. HPP dramatically reduced respiration rate and ethylene production 1 hour after treatment, and, after 17 hours at 20°C, almost complete elimination was observed after treatment at higher pressures. HPP was found to increase PPO activity and activity increased with higher pressure exposure. Microscope observation (Cryo-SEM) showed changes in the structure of the cell wall, disruption of the cellular network and coalescence of oil vesicles at 600 MPa.

In conclusion, the results of this study have shown that HPP has dramatic and potentially beneficial effects in terms of reducing respiration rate and ethylene production of avocado slices. Effects on colour, while measurable, were not readily apparent to the eye. Further work is required to examine the effects on shelf life of avocado slices.

El objetivo de esta investigación fue el estudio de el uso de altas presiones de procesamiento (HPP) en la calidad y la microestructura de tajadas de aguacate. Muestras de tajadas de aguacate fueron sometidas a altas presiones de procesamiento de 200 a 600 Mpa por períodos de 3 a 10 minutos. Las muestras fueron examinadas inmediatamente despues del procesamineto para evaluar los cambios de color , cambios en la actividad de polifenol oxidasa (PPO), y cambios en la microestructura, tasa de respiración y producción de etileno. El colour de la pulpa fue afectado y hubo una disminución en los valores L (lightness) y C (croma) a pesar de que estos cambios no fueron evidentes a simple vista. El HPP redujo la tasa de respiración y de producción de etileno dramaticamente, medida una hora despues del procesamiento, y, despues de 17 horas a 20C hubo una eliminación completa de estos valores en las muestras procesadas a las presiones mas altas. El HPP incremento la actividad de la enzima PPO y este incremento fue mayor a mayores presiones. Hubieron cambios en la microestructura (Cryo-SEM) de la pared celular, red celular y tambien coalescencia de las vesiculas de aceite a 600 MPa. En conclusion los resultados de esta investigación han demostrado que el HPP puede causar cambios dramáticos y potencialmente beneficiosos en terminos de la reducción en la tasa de respiración y en la producción de etileno de las tajadas de aguacate.

Los efectos en el color fueron medidos pero no aparentes a simple vista. Investigaciones futuras examinaran los efectos en la vida de anaquel de las tajadas de aguacate.

1. Introduction

Recently, there has been an increasing consumer demand worldwide for high quality, minimally processed fruit products that are not only convenient, but also have fresh appearance, texture, and are free from artificial food additives (Palou et al. 2000). For industrial applications, High Pressure Processing (HPP) involves applying extremely high pressures (up to 600 MPa; \cong 87,000 psi) to product held in sealed pouches. It causes minimal changes in the 'fresh' characteristics of foods by eliminating degradation found with other more conventional food processing methods, such as thermal treatment (Ramaswamy et al. 2011).

Studies have shown multiple benefits of HPP on a wide variety of food products, from fruit juice to guacamole, that lead to better retention of antioxidant content and colour, as well as microbial inactivation (Ludikhuyze et al. 2002). The development of safe and reliable HPP methods is crucial to ensure that the shelf life of food products such as horticulture produce can be extended without compromising their nutritional qualities. While there is published work on avocado pulp and guacamole, there is little information on avocado slices). Thus, the purpose of this study was to evaluate the response of avocado slices to HPP immediately after treatment.

2. Methodology

2.1 Fruit sourcing and ripening

Avocado fruit were sourced from three local growers in the North Island of New Zealand and fruit were stored at 4°C for 3 weeks before the experiment. Fruit were ripened for 24 hours at 20.2°C using ethylene ($>100 \mu\text{L L}^{-1}$). Before processing, firmness was measured using an Aweta unit and fruit selected to be within the range of 20-26, which is a minimally ripe firmness (White et al. 2009).

2.2 Slice preparation for HPP treatment

Avocado fruit from the three different avocado growers were treated on each of three separate days (i.e. three replications). Avocado were sliced and vacuum-packed using a commercial high oxygen barrier pouch (69 μm thick) with an oxygen transmission rate of less than 10 cc and held on ice until treated. Within 3 h, the slices were subjected to pressure of 200 to 600 MPa for durations of 3, 6 or 10 min (at ambient temperatures; Figure 1, a 2-L Avure HPP equipment). Slices were then transported on ice back to the laboratory and assessed within 3 hours for colour change (L^* , C , h°), respiration rate (CO_2 production), ethylene production, and tissue samples taken for measurement of polyphenol oxidase (PPO) activity and cell microstructure by Cryo-SEM. Control samples were sealed in bags and transported together with the HPP-treated product in an identical manner.



Figure 1. Avure small-scale experimental High Pressure Processing unit and sample chamber.

2.3 Colour measurement

L^* , C , and h° colour values (Minolta CR-300 colorimeter; Konica Minolta Sensing, Inc., Osaka, Japan) were used to evaluate the extent of browning and loss of greenness in the avocado

slices before and after HPP treatment. Values were measured in triplicate. The L^* value defines the lightness, the C value the chroma (intensity) and the h° angle (actual colour).

2.4 Measurement of respiration rate and ethylene production

Avocado slices from each treatment were removed from the HPP pouch, weighed into known-volume respiration jars, and sealed for 1 h at 20°C. The amounts of ethylene and carbon dioxide produced by the slices were measured in triplicate using gas chromatography after 1 h, followed by a further sample monitored after 17 h at 20°C.

2.5 Polyphenol oxidase activity

Polyphenol oxidase activity of the avocado slices were carried out in triplicate using a spectrophotometer following the methods adapted from Lopez-Malo et al. (1998), and Jacobo-Velasquez et al. (2010). Results are expressed as change of optical density (410 nm as a function of reaction time ($\Delta OD/\text{min}/\text{mg}$ protein)).

2.6 Microstructure analyses

Cryo Scanning Electron Microscopy (Cryo-SEM) analysis was performed using a Polaron PP2000 Cryo Transfer system (Quorum Technologies, Ringmer UK) attached to an FEI Quanta250 Scanning Electron Microscope (SEM; FEI Hillsboro OR). Blocks of tissue about 4 x 6 x 2 mm excised from control or treated fruit were placed in aluminium sample holders, frozen in liquid nitrogen slush, transferred to the PP2000 preparation stage and fractured using a cold metal probe. The sample was then sublimed at -90°C for between 3 and 7 minutes, sputter coated with gold/palladium and observed with the SEM under conditions described by Hallett et al. (1992).

3. Results and Discussion

3.1 Effect of HPP on the colour profile of avocado slices

High pressure processing resulted in subtle changes to the appearance of avocado slices (Figure 2). Based on the objective measurement using a Chromameter, increasing pressure (200 - 600 MPa) and exposure time decreased the lightness (L^*) of the slices and the slices became slightly darker (57.47 - 52.45 for the outside (i.e. dark green layer) of slices, and 69.54 - 65.56 for the side of slices). Hue angle (h°) for the side of the slices increased slightly with HPP treatment up to 600 MPa (from 101.4 - 105.07). We also observed a similar trend for chroma (C) to that of hue angle, where the side and outer edge of the avocado slices also decreased marginally, from 32.64 - 30.68 and 28.05 - 26.27, respectively. However, the effect of exposure time with increasing pressure to HPP on colour changes was not conclusive.



Figure 2. Avocado slices in high oxygen barrier pouch before (left) and after (right) high Pressure Processing (HPP) treatment at 600 MPa for 6 min.

3.2 Effect of HPP on carbon dioxide and ethylene production

High pressure processing had a dramatic effect on the respiration rate and ethylene production of the avocado slices. HPP treatment at 200 MPa resulted in some reduction in respiration rate (to \cong 30% of control after 1 h), whereas the most dramatic effect was observed on samples subjected to the highest pressure treatment (600 MPa, duration of 10 min). This resulted in reduction of carbon dioxide (CO₂) and ethylene (C₂H₄) production by as much as 75% and 78% respectively after only 1 h after treatment.

Ethylene Production of was more sensitive to HPP treatment than that of CO₂, and, 17 h after HPP treatment, ethylene production could only be measured in the 200 MPa treatment (reduced further after the first point of measurement (1 h after treatment) by 20%). However, treatments at 400 and 600 MPa resulted in nearly complete elimination of CO₂ production as measured 17 h after treatment. We propose that the amount of CO₂ production detected immediately after treatment was the remaining CO₂ diffusing out of the tissue, and thus it does not represent respiratory activity *per se*.

3.3 Effect of HPP on polyphenol oxidase activity

HPP treatment resulted in an increase in PPO activity by \cong 2, 3 and 6-fold for 200, 400 and 600 MPa, respectively compared to that of the control. The increase in activity was probably due to increase in extractability and the colour data support these findings.

This finding is contradictory to what has been reported in other work (Palou et al. 2000; (Lopez-Malo et al. 1998), where the PPO residual activity of an avocado paste reduced to approximately 24 - 36%. This is probably because in the latter study, the pH of the avocado paste was acidified, a technique commonly used to decrease PPO activity (Jacobo-Velasquez et al, 2010). However, further studies are needed to confirm these results.

3.4 Effect of HPP on microstructure of avocado slices

Analysis of the microstructure of the treated and untreated avocado tissue was carried out using Cryo-SEM (Cryo Scanning Electron Microscopy), showed major effects on cell integrity (Figure 3). The oil vesicles inside the parenchyma cells are obvious in the control tissue, and with increasing pressure a coalescing of the oil droplets is observed. In addition, exposure to low pressure (200 MPa for 10 min) resulted in the cell wall becoming more diffuse and less easy to distinguish, while exposure to the highest pressure resulted in complete loss of definition of the cell wall and loss of cellular compartmentalisation.

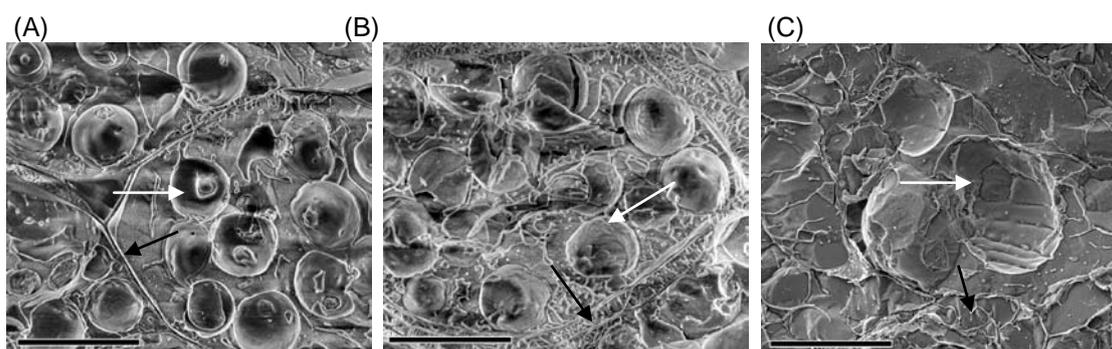


Figure 3. Cryo Scanning Electron Microscopy image of control untreated slices (A), treated slices (200MPa, 10 min; B) and treated slices (600 MPa, 10 min; C) of avocado tissue. White arrows indicate the coalescing of oil vesicles and the black arrows, the cell walls. The black bar indicates 20 μ m.

4. Conclusion

This study shows that HPP has beneficial effects on reducing respiration rate and ethylene production of avocado slices. It implies that HPP is a promising novel technique to preserve fresh

avocado slices with no additives. However, further work is required to evaluate the effects of HPP on the quality of avocado slices during storage.

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References:

1. Palou, E, Hernandez-Salgado, C, Lopez-Malo, A, Barbosa-Canovas, G, V, Swanson B, G, Welti-Chanes, J 2000, 'High pressure processed guacamole', *Innovative Food Science and Emerging Technologies*, Vol. 1, Issue. 1, pp. 69-75
2. Ramaswamy, R, Balasubramaniam, V, M, Kaletunc, G, S 2011, *High pressure processing: fact sheet for food processor*. Retrieved: July 11, 2011, from <http://ohioline.osu.edu/fse-fact/pdf/0001.pdf>.
3. Ludikhuyze, L, Loey, A, Indrawati, O, Hendrick, M 2002, *High pressure processing of fruit and vegetables*, Fruit and vegetable processing: improving quality, New York, pp. 346-362
4. White, A, Woolf, A, B, Hofman, P, J, and Arpaia, M, L 2009, *The International Avocado Quality Manual*, Plant and Food Research Ltd. ISBN 0-478-06845-X. pp70.
5. López-Malo, A, Palou, E, Barbosa-Cánovas, G, V, Welti-Chanes, J, Swanson, B, G 1998, 'Polyphenoloxidase activity and colour changes during storage of high hydrostatic pressure treated avocado puree', *Food Research International*, Vol .31, No.8, pp. 549-556
6. Jacobo-Velasquez, D, A, Hernandez-Brenes, C 2010, 'Biochemical changes during storage of high hydrostatic pressure processed avocado paste', *Journal of Food Science*, Vol. 75, No. 6, pp. S264-270
7. Hallett, I, C, MacRae, E, A, Wegrzyn, T, 1992, 'Changes in kiwifruit cell wall ultrastructure and cell packing during ripening', *International Journal of Plant Sciences*, Vol. 153, No.1 , pp. 49-60