Influence of Storage at 4°C on the Stability of High Hydrostatic Pressure Treated Onion

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Abstract

Vázquez-Gutiérrez J.L., Hernández-Carrión M., Quiles A., Hernando I. (2014): **Influence of storage at 4°C on the stability of high hydrostatic pressure treated onion**. Czech J. Food Sci., **32**: 96–101.

The effects of refrigerated storage on the microstructure and physicochemical properties of high hydrostatic pressure (HHP) treated onion were evaluated. Onion was submitted to 100 MPa at 50°C or 400 MPa at 25°C for 5 min, and stored for 28 days at 4°C. Electron microscopy techniques and light microscopy were used for the microstructural study. Total soluble phenolics, soluble protein percentage, and shear force were also studied. HHP treatments affected the cell wall and membrane permeability, favouring the diffusion of soluble material to the apoplast. Storage at 4°C also caused important structural degradation in the HHP-treated samples, which was higher when 400 MPa at 25°C were applied and led to physico-chemical changes during the first week of storage. Interactions between phenolics and solubilised cell wall material or proteins could explain the decrease in soluble phenolics and proteins during storage.

Keywords: *Allium cepa*; postharvest processing; phenolics; protein; shelf life; microstructure

Onion (*Allium cepa* L.) bulbs have been an important food source since ancient times and are rich in flavonoids, a type of phenolics with potent free radical scavenging and antioxidant capacity (Prakash *et al.* 2007).

High hydrostatic pressure (HHP) has an enormous potential in the food industry for the development of value-added food products (Bala *et al.* 2008). HHP has been applied on an industrial scale to many vegetable products (www.hiperbaric.com) and further scientific research is carried out on fruit and vegetable products such as apple (Briones-Labarca *et al.* 2011), strawberry juice (Cao *et al.* 2012), purple sweet potato nectar (Wang *et al.* 2012) or table olives (Pradas *et al.* 2012). According to previous studies, this technology may improve the bioaccessibility and extractability of micronutrients (Roldán-Marín *et al.* 2009; Sánchez-Moreno *et al.* 2009).

Despite the advantages in the preservation of nutritional compounds when compared to thermal treatments, fruit and vegetables may undergo structural and textural changes after HHP processing (Oey *et al.* 2008). These changes can be related to transformations in cell wall polymers due to enzymatic and non-enzymatic reactions (Sila *et al.* 2008), and the compression of the cellular structure following degassing of the tissue (Basak & Ramaswamy 1998). Since endogenous enzymes are incompletely inactivated or even activated after HHP processing (Oey *et al.* 2008), it is necessary to analyse quality changes of HHP-treated products during storage. A few works have studied the microstructural changes in onion after HHP processing (Butz *et al.* 1994; Gonzalez *et al.* 2010). However, further studies regarding microstructural and physicochemical changes of HHP-treated onion during refrigerated storage are necessary.

Supported by the Spanish Government, Grant No. AGL2008-04-798-C02-02, and the Spanish Government and the UPV for two predoctoral grants.

The aim of this study was to evaluate the effects of refrigerated storage on microstructure and physico-chemical properties of HHP-treated onion in order to determine the potential benefits of this treatment to maintain the quality of onions during their commercialization.

MATERIAL AND METHODS

Sample preparation. Onions (*Allium cepa* L., cv. Recas) were harvested in Spain (Cebafruit SAT, Lleida) and stored at 4°C. Outer layers were removed and onions were cut into halves in a longitudinal section. The onion halves were placed in random pairs into 110 mm × 220 mm plastic bags (Doypack type; Amcor, Madrid, Spain). The bags were heat-sealed and placed into a hydrostatic pressure unit of 2350-ml capacity (GEC Alsthom ACB 900 HP, ACIP 665 model; GEC Alsthom, Nantes, France). Water was used as the pressure medium. Two different HHP treatments (100 MPa at 50°C and 400 MPa at 25°C) were applied for 5 minutes. The HHP treatments were chosen according to previous studies (Vázquez-Gutiérrez *et al.* 2010, 2012a). Samples were analysed immediately after the HHP treatment and after 7, 14, 21, and 28 days of storage at 4°C.

Low temperature scanning electron microscopy (Cryo-SEM). The Cryo-SEM observation was carried out according to Vázquez-Gutiérrez *et al.* (2011). Samples (1 mm thick pieces from the second outer layer of the onion halves) were examined at 15 kV under a JSM-5410 SEM microscope (JEOL, Tokyo, Japan).

Light microscopy (LM) and transmission electron microscopy (TEM). Samples were fixed, dehydrated, contrasted and resin embedded according to Vázquez-Gutiérrez *et al.* (2012b). Semi-thin sections (1.5 μ) were stained with 2 g/l toluidine blue and examined under a Nikon Eclipse E800 light microscope (Nikon, Tokyo, Japan). Ultrathin sections (0.05 μ) were stained with 40 g/l lead citrate and observed under a Philips EM400 transmission electron microscope (Philips, Eindhoven, the Netherlands) at 80 kV.

Total soluble phenolics. The amount of total soluble phenolics (TSP) was determined using the Folin-Ciocalteu reagent (Singleton & Rossi 1965). 8 g of onion juice were added to 25 ml of 80% methanol and homogeniaed with an IKA-ultraturrax (T25 basic). The homogenate was filtered and brought to 50 ml with distilled water. One ml of this sample solution, 6 ml of distilled water and 0.5 ml of the Folin-Ciocalteu reagent were mixed and vortexed. After 3 min, 1 ml of 20% Na_2CO_3 was added. The tubes were vortexed and absorbance was measured at 760 nm after 90 min at room temperature. Results were expressed in % of fresh weight (FW) with gallic acid as a standard. Three different onion juices per sample were analysed. Each juice was prepared by blending 60 g of onion from three different onion halves.

Soluble protein percentage. Water-soluble nitrogen was extracted from lyophilised onion juice according to the official method AOAC 932.08 (AOAC 2000). Both crude and water-soluble proteins were quantified by N-Kjeldahl using a mixture of K_2SO_4, $CuSO_4$, and Se (10:1:0.1) as catalyser and a conversion factor of 6.25. Three different onion juices per sample were analysed and the results were expressed as soluble protein percentage (SPP) from the total crude protein.

Shear force. Shear force was determined at room temperature with a TA.XTplus Texture Analyser (Stable Micro Systems, Surrey, UK) as the load in newtons (N) needed to cut all the layers of a 15 mm wide onion segment at its equatorial zone with a knife blade at 1 mm/s test speed as shown in Figure 1. Values were an average of eight measurements (four different onion halves, two measurements per half).

Statistical analyses. Multi-factor categorical designs with two non-quantitative factors (treatment and storage time) were used for the physicochemical properties. The designs were analysed using a multifactor analysis of variance and the least significant difference (*LSD*) procedure was applied to evaluate statistically significant differences between means at $P < 0.05$ (Statgraphics Centurion XVI; Manugistics, Inc., Rockville, USA).

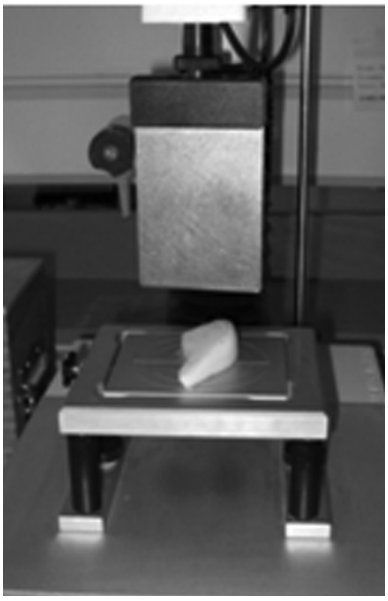

Figure 1. Detail of the knife blade used and the position of onion samples for the shear force tests

RESULTS AND DISCUSSION

Microstructural study. The parenchymatous tissue of untreated onion (control) is compact with rounded cells (Figure 2A). The inside of the cell is almost completely occupied by a large vacuole full of soluble solids, since a dense eutectic artifact is observed in the Cryo-SEM micrographs (Neri *et al.* 2011). When these samples are examined by LM, cells appear homogeneously stained with toluidine blue (Figure 2D). When observed in greater detail by TEM, cell walls show a proper and homogeneous packing of the cellulose fibres. The plasmalemma (cell membrane) and the tonoplast (vacuole membrane) are closely attached to the cell wall (Figure 2G).

When onion is treated with 100 MPa at 50°C, the intercellular spaces, which were filled with air in the raw sample, become flooded with soluble material (Figure 2B). Cells show slight distortion due to compression (Figure 2E). Cell walls still present compact cellulose fibres, but the tonoplast is slightly retracted towards the centre of the cell (Figure 2H).

When processing conditions of 400 MPa at 25°C are applied to onion, the tissue degradation is greater (Figure 2C). Solubilisation of the cell wall material can be observed in some areas and cells are distorted (Figure 2F). Cell walls present irregular thickness, the bonds between cellulose fibres start to weaken and the plasmalemma has been pulled away from the cell wall (Figure 2I).

After 14 days of storage, the porosity of cell walls and membranes increases in samples treated with 100 MPa at 50°C (Figure 3A). The tonoplast retracts towards the centre of the cell as a consequence of the loss of the cell content. The degradation of the adhesion bonds between some cells causes their separation and consequently the intercellular spaces are bigger at this stage of the storage (Figure 3C). The cell walls still preserve their integrity and the plasmalemmas are still attached to them (Figure 3E).

Cells of the samples treated with 400 MPa at 25°C have lost their turgor and present a higher level of distortion after 14 days of storage (Figure 3B). At this point, greater cell wall degradation can be observed in these samples (Figure 3D). The cell walls have widened during the two weeks of storage and present more irregular borders (Figure 3F).

After 28 days of storage, the tonoplast still maintains its integrity in the samples treated with 100 MPa at 50°C, but it retracts towards the centre of the cell (Figure 4A). Cells show a high level of distortion and widespread degradation of cell wall components (Figure 4C). These effects come as a result of progressive migration of the cell content towards the apoplast throughout the storage time. A marked separation of the cellulose fibres is observed, which explains the widening of the cell walls compared to earlier stages of storage (Figure 4E).

At the end of storage, the cell separation and distortion have increased in the samples treated with 400 MPa at

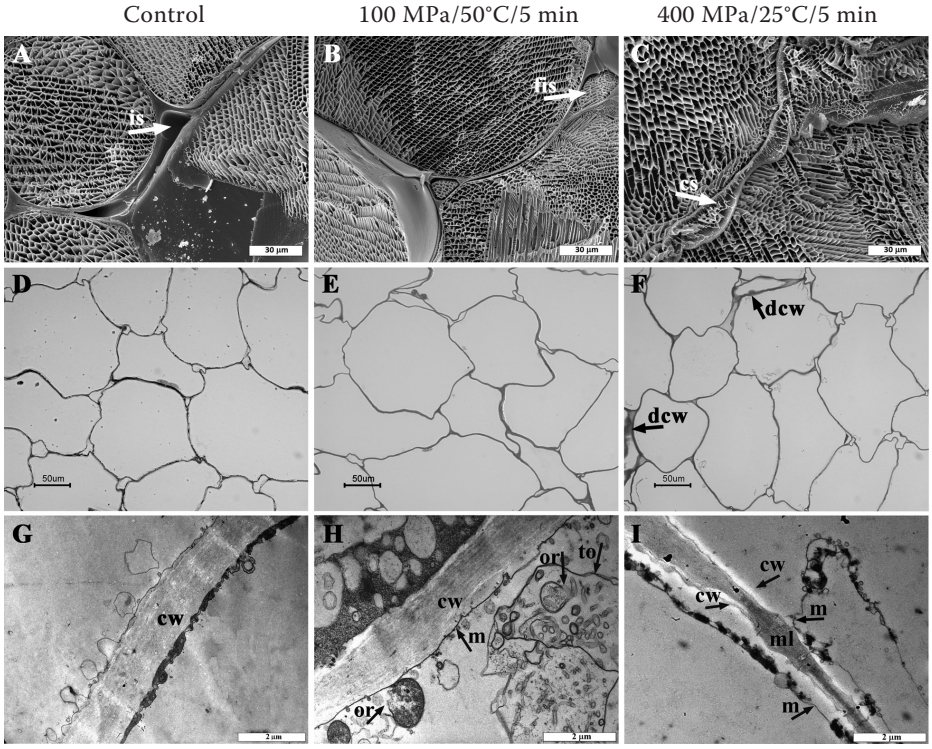

Figure 2. Microstructure of untreated and HHP-treated onion by Cryo-SEM (A, B, C), LM (D, E, F) and TEM (G, H, I)

is – intercellular space; fis – flooded intercellular space; cs – cell separation; dcw – degraded cell wall; cw – cell wall; m – membrane; to – tonoplast; ml – middle lamella

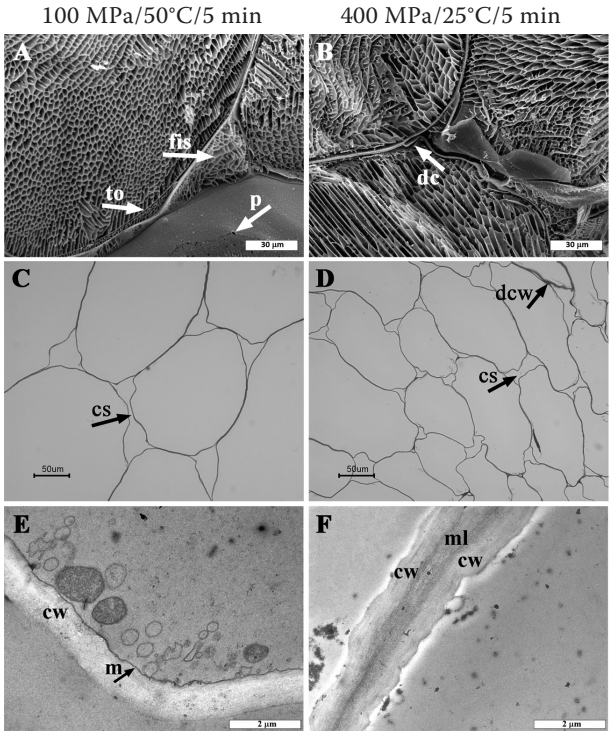

Figure 3. Microstructure of HHP-treated onion by Cryo-SEM (A, B), LM (C, D), and TEM (E, F) after 14 days of storage

fis – flooded intercellular space; p – pitting; to – tonoplast; dc – distorted cell; cs – cell separation; dcw – degraded cell wall; m – membrane; cw – cell wall; ml – middle lamella

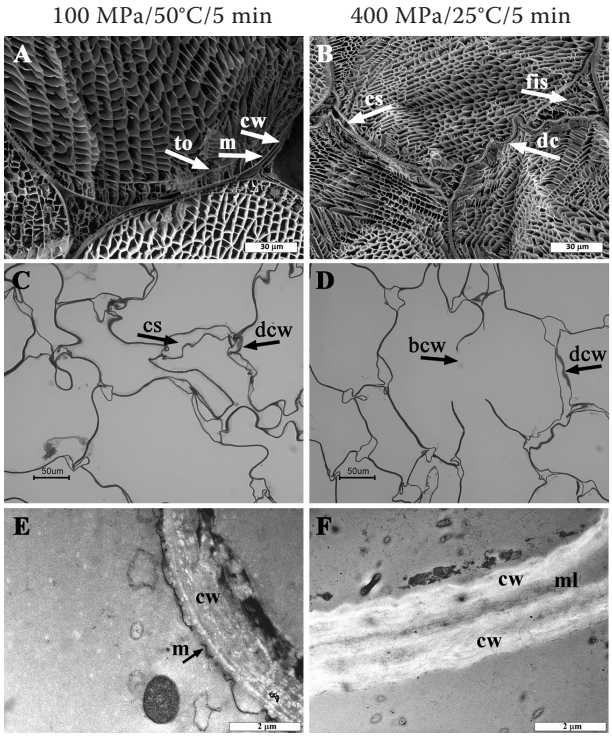

Figure 4. Microstructure of HHP-treated onion by Cryo-SEM (A, B), LM (C, D), and TEM (E, F) after 28 days of storage

to – tonoplast; m – membrane; cw – cell wall; cs – cell separation; fis – flooded intercellular space; dc – distorted cell; dcw – degraded cell wall; bcw – broken cell wall; ml – middle lamella

25°C (Figure 4B). Moreover, unlike the samples treated with 100 MPa, the tonoplast is no longer visible after applying 400 MPa, which would explain the greater and faster loss of turgor in these samples. The level of degradation and distortion of the cells is greater than in the other HHP-treated samples and some broken cell walls can be observed (Figure 4D). The cell walls look very pale by TEM, indicating a high level of solubilization of the cell wall material (Figure 4F).

Total soluble phenolics. The TSP content of the untreated onion (C) increases after 7 days of storage (Figure 5A). According to the microstructural study, the intercellular spaces of the control sample are progressively filled with the cell content as the storage time increases (Figure 3A and 4A). In this way, the tissue degradation throughout the storage time favours the extractability of phenolic compounds.

At the beginning of storage, the HHP-treated samples show higher TSP content than the control ones, being this statistically significant ($P < 0.05$) in the samples treated with 100 MPa at 50°C (T1), which also show the highest values of TSP until the 14th day of storage. The differences between the control and the HHP-treated samples decrease as the storage time increases. The application of HHP favours a rapid diffusion of soluble compounds, as it has been observed in the microstructural study, which would explain the initial differences in TSP between the control and the HHP-treated samples. As the untreated tissue begins to degrade with the storage time, the soluble compounds also spread and therefore the differences from the HHP-treated samples decrease. The fact that the samples treated with 400 MPa (T2) have lower TSP values than the untreated ones as the storage time increases could be due to partial degradation or insolubilization of phenolic compounds in the former ones during storage.

Soluble protein percentage. As shown in Figure 5B, the SPP of the untreated onion decreases significantly ($P < 0.05$) from the 7th day of storage. The HHP-treated samples have a significantly higher ($P < 0.05$) SPP than the control immediately after the treatment. HHP can cause deprotonation of charged groups and disruption of salt bridges and hydrophobic bonds, resulting in conformational changes and protein denaturation (US FDA 2000) which could affect their solubility. This increase in SPP after the HHP treatments could also be due to physical damage of the structure that may have af-

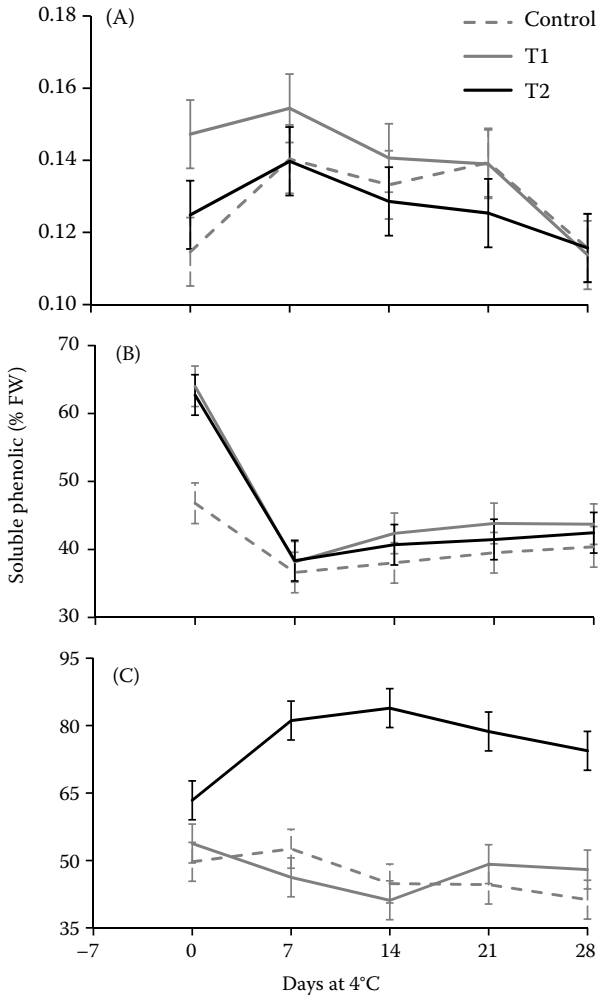

Figure 5. Treatment-storage time interaction plot for total soluble phenolics (A), soluble protein (B), and shear force (C) of untreated and HHP-treated onion with *LSD* intervals

C – control; T1 – 100 MPa/50°C/5 min; T2 – 400 MPa/50°C/5 min; FW – fresh weight

fected the interactions of the proteins at the cellular level or to an increased activity of some hydrolytic enzymes on the cell walls. However, SPP decreases in both HHP-treated samples after 7 days of storage. As it has been observed in the microstructural study, pressure and later storage affect the permeability of membranes and it can even cause the breakage of both plasmalemmas and cell walls (Figures 2–4). The widespread decrease in SPP during the storage time could be due to variations of pH or electrostatic interactions between the membrane proteins and pectins from the cell walls that would affect the protein solubility. The absence of differences between the HHP-treated and the control samples after 7 days of storage could be attributed to the fact that the tissue degradation throughout the storage time in all the samples could reduce the influence of the HHP treatments, as it happened with the TSP values (Figure 5A).

Shear force. Overall, the samples treated with 400 MPa at 25°C have significantly higher ($P < 0.05$) shear force values than the other samples throughout the storage period (Figure 5C). These samples have shown to have the highest cell wall degradation (Figures 2–4), which would favour a better contact between the pectic compounds and the enzyme pectin methylesterase. This would lead to the formation of calcium pectates, thus to the strengthening of the structure (Basak & Ramaswamy 1998). The microstructural changes undergone in the samples treated with 100 MPa at 50°C do not seem to have a great influence on the texture during 28 days of storage compared with the untreated samples.

CONCLUSIONS

Storage at 4°C causes important structural degradation in HHP-treated onion. Physico-chemical changes take place in the HHP-treated samples due to variations in the permeability of membranes and cell walls, which causes the progressive migration of the cell content towards the apoplast. Although HHP treatments increase the TSP and SPP contents, the differences between the control and the HHP-treated samples in terms of these parameters decrease progressively during storage. This could be attributed to interactions between phenolics and other compounds such as solubilised cell wall material or proteins.

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Received for publication November 19, 2012
Accepted after corrections June 6, 2013

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