Influence of high hydrostatic pressure on quality parameters and structural properties of aloe vera gel (*Aloe barbadensis* Miller)

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Abstract The aim of this work was to study the effect of high hydrostatic pressure (HHP) on colour, dietary fibre, vitamin C content, polysaccharides content, physico-chemical and structural properties of aloe vera gel at three pressure levels (300, 400 and 500 MPa for 3 min) after 35 days of storage at 4 ± 1 °C. The results showed that HHP exerted a clear influence on most of the quality parameters studied. Moisture, protein and fat contents did not show changes with an increasing pressure. Ash, crude fibre and carbohydrates content increased with increasing pressure. Vitamin C content did not show significant differences after

35 days of storage. The variation of colour in the samples increased at 500 MPa. Total dietary fibre, water holding capacity and firmness increased with pressure. However, all HHP-treated samples presented a decrease in hydration ratio and polysaccharides content; and also minor alterations in the structural properties were produced at HHP of 300–500 MPa, resulting in a high quality gel.

Keywords Aloe vera · High hydrostatic pressure · Polysaccharides · Vitamin C · Firmness · Microstructure · Total dietary fibre

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Introduction

Aloe vera (*Aloe barbadensis* Miller) is a member of the Xanthorrhoeaceae family (U.S.D.A. 2010; I.T.I.S. 2010). It has been used for its medicinal value for several thousand years. Its applications have been recorded in ancient cultures of India, Egypt, Greece, Rome and China (Ahlawat and Khatkar 2011). Aloe vera leaves consist of an outer green leathery margin (skin) and an internal clear gelatinous (gel) matrix, used in health foods as well as for medical and cosmetic purposes. Different properties are ascribed to the inner, colourless, leaf gel and to the exudates produced by the bundle sheath cells of the outer margin of the leaf (Chang et al. 2006; Chang et al. 2011). The tissue of the inner parenchyma contains over 99 % of water. Polysaccharides account for most of the dry matter of the aloe vera parenchyma, with different types of polymers: acemannan, a storage polysaccharide rich in mannose units that is located within the protoplast of the cells, and a wide variety of polysaccharides that form the cell wall matrix (Femenia et al. 2003; Miranda et al. 2010).

Aloe vera is an industrial crop and has been utilized in the food industry for the preparation of healthy food drinks, beverages like tea, milk, ice-cream and confectionery (Pisalkar et al. 2011). The potential use of aloe vera products often involves some type of processing, like heating, and dehydration, where high temperatures (over 90 °C) may cause irreversible modifications to the polysaccharides, affecting their original structure. This may promote important changes in the proposed physiological and pharmacological properties of these active ingredients (Femenia et al. 2003; Chang et al. 2006; García-Segovia et al. 2010). Thus, the choice of a preservation method is a relevant task (Miranda et al. 2010). High hydrostatic pressure (HHP) processing or high pressure processing (HPP) is a non thermal food preservation technique for microbial and enzyme inactivation with reduced effects on nutritional and quality parameters compared to thermal treatments (Tiwari et al. 2009). In addition to food preservation, high pressure treatment can result in food products acquiring novel structure and texture, and hence can be used to develop new products or increase the functionality of certain ingredients (Rastogi et al. 2007). However, studies have shown that high pressure can alter physico-chemical properties of vegetable matrices by inducing changes in their structure and these changes are characterized by an initial texture loss, also called instantaneous pressure softening (IPS), followed by a gradual change during pressure hold (Araya et al. 2007). Despite alterations to the structure of high-molecular-weight molecules such as proteins and carbohydrates, HHP does not affect the smaller molecules associated with the sensory, nutritional and health promoting properties such as volatile compounds, pigments, and vitamins (Keenan et al. 2010).

The aim of this study was to evaluate the effect of high hydrostatic pressure treatment at three pressure levels (300, 400 and 500 MPa) on colour, dietary fibre, vitamin C content, polysaccharides content, physico-chemical and structural properties of aloe vera gel monitored after 35 days of storage at 4 °C.

Materials and methods

Preparation of raw material

Leaves of aloe vera (*Aloe barbadensis* Miller) were provided by the experimental farm of the National Institute for Agricultural Research (INIA) at Intihuasi, Coquimbo, Chile. Homogenous leaves were selected according to size, ripeness, colour, and freshness. Acibar, a yellow-coloured liquid, was extracted by cutting the base of the leaves and allowing them to drain vertically for 1 h. The epidermis was then separated from the gel that was extracted and triturated by a electric blender (Phillips HR1720, Amsterdam, Netherlands). For texture and microstructure analyses,

pieces of aloe vera gel were used, which were manually cut into slabs of 10 ± 1 mm in thickness. All samples were packed in high density polyethylene (HDPE) pouches (water absorption 0.10 %; Curbell Plastics Inc. USA), and kept under chilling conditions in a refrigerated room (4 ± 1 °C) for 4 h until further HHP processing.

High hydrostatic pressure treatment

Packaged samples were placed in a cylindrical loading container and pressure-treated at 300, 400 and 500 MPa for 3 min. Water was employed as pressure-transmitting medium, HHP treatment was conducted at a ramp rate of 17 MPa/s and a decompression time of less than 5 s. A two-litre processing unit (Avure Technologies Incorporated, Kent, WA, USA) within a cylindrical pressure chamber of 700 mm in length and 60 mm in diameter was used to pressurize the aloe vera samples. Pressurization was carried out at ambient temperature (20 ± 1 °C). During pressurization some heat is evolved due to compression, so that initial temperature of the inlet water was set at 10 ± 1 °C in order to keep temperature of pressure-transmitting medium at 20 °C. Pressurized and untreated (control) samples were stored at 4 °C for 35 days. Quality analyses were performed on fresh sample as control sample (F0) and after 35 days of storage on pressurized and untreated (F35) samples. All experiments were done in triplicate.

Proximate analysis

The moisture content was determined by AOAC method no 934.06 (A.O.A.C. 1990) employing a vacuum oven (Gallenkamp, OVL570, Leicester, UK) and an analytical balance with an accuracy of ± 0.0001 g (CHYO, Jex120, Kyoto, Japan). The crude protein content was determined using the Kjeldahl method with a conversion factor of 6.25 (A.O.A.C. no. 960.52). The lipid content was analyzed gravimetrically following Soxhlet extraction (A.O.A.C. no. 960.39). The crude ash content was estimated after incineration in a muffle furnace at 550 °C (A.O.A.C. no. 923.03). The crude fibre was estimated by acid/alkaline hydrolysis of insoluble residues (A.O.A.C. no. 962.09). The available carbohydrate was estimated by difference.

Evaluation of quality parameters

Colour

Colour of the samples was measured using a colorimeter (Hunter Lab, model MiniScan™ XE Plus, Reston, VA, USA). Colour was expressed in CIE L* (whiteness or brightness), a* (redness/greenness) and b* (yellowness/

blueness) coordinates at standard illumination D_{65} and observer angle of 10° . Twenty replicate measurements were performed and results were averaged. In addition, total colour difference (ΔE) was calculated using Eq. (1), where L_0 , a_0 , and b_0 are the control values for the fresh samples.

$$\Delta E = \left[(a^* - a_0)^2 + (b^* - b_0)^2 + (L^* - L_0)^2 \right]^{0.5} \quad (1)$$

Vitamin C content

The determination of vitamin C was performed by certification of NBS (N-Bromosuccinimide) according to Barakat et al. (1955) with some modifications. The oxidizing agent (NBS) was standardized by titrating with the solution of NBS (0.2 g / L) an aliquot of 10 mL of a standard solution of ascorbic acid (0.2 mg/mL), placed in a 250 mL Erlenmeyer flask containing 2 mL of a solution of KI (4 %), 0.8 mL of a solution of acetic acid (10 %), some drops of a solution of starch (1 %) as indicator and 12 mL of distilled water. The end point was reached when a permanent blue colour was observed. For ascorbic acid determination in fresh and treated samples, 100 g of aloe vera gel and 0.5 g oxalic acid were added, crushed, homogenized and filtered. A ten gram solution from these samples was taken and placed in an Erlenmeyer flask containing 5 mL of KI solution, 2 mL of acetic acid solution, some drops of starch solution and 30 mL of distilled water, then titrated with the NBS solution. The content of vitamin C, expressed as mg Vitamin C / 100 g sample was calculated as follows:

$$VITC = \left(\frac{2 \cdot B}{T \cdot M} \right) \times 100 \quad (2)$$

Where T (mL) is the volume of NBS of the standard solution of 2 mg of Vitamin C (AA); B (mL) is the volume of NBS corresponding to the sample and M (g) is the sample mass.

Total polysaccharide content

Polysaccharide content was estimated by a colorimetric analysis. A sample of 1 g of aloe vera gel was extracted first with 80 mL of distilled water in a thermo regulated bath at 100°C for 2 h under constant agitation and then vacuum-filtered. The filtrate was diluted to 100 mL in a beaker according to the methodology suggested by Hu et al. (2003). Two millilitres of the solution and 10 mL of absolute ethanol were added in a plastic tube; sample was centrifuged at 2500 g for 30 min, and the supernatant was removed; the precipitate was dissolved in a final volume of 50 mL water. One millilitre of the filtered solution, 1 mL of phenol at 5 g / 100 mL, and 5 mL of concentrated sulphuric acid (95–97 %, Merck, Darmstadt, Germany) were then added to the tops of the tubes. It was allowed to settle for

30 min. Absorbance was determined at 490 nm (Spectronic® 20 Genesys™, IL, USA). Total polysaccharide content was estimated by comparison with a standard curve generated from D-+-glucose analysis. Measurement was performed in triplicate. All solvents and reagents were purchased from Sigma (Sigma Chemical Co., St. Louis, MO, USA).

Hydration indices

Hydration ratio (HR) and water holding capacity (WHC) were used as hydration indices in order to evaluate the hydration behaviour of aloe vera gel. HR was calculated according to Eq. (3), similar to estimation of rehydration ratio (Miranda et al. 2009) and expressed in g absorbed water / g dry matter. WHC was determined by centrifuging the hydrated samples at 3500 g for 15 min at 20°C in tubes fitted with a centrally placed plastic mesh which allowed water to drain freely from the sample during centrifugation. WHC was calculated as the amount of water retained with respect to initial mass of water absorbed according to Eq. (4).

$$HR = \frac{W_{hyd} \cdot X_{hyd} - W_{hhp} \cdot X_{hhp}}{W_{hhp}(1 - X_{hhp})} \quad (3)$$

$$WHC = \frac{W_{hyd} \cdot X_{hyd} - W_l}{W_{hyd} \cdot X_{hyd}} \cdot 100 \quad (4)$$

Where W_{hyd} is the weight of the sample after the hydration process, X_{hyd} is the corresponding moisture content on a wet basis, W_{hhp} is the weight of the sample after HHP process, X_{hhp} is the corresponding moisture content on a wet matter basis and W_l is the weight of the drained liquid after centrifugation.

Total dietary fibre

The total dietary fibre (TDF) was determined by the gravimetric-enzymatic method (A.O.A.C. no. 985.29) suggested by the Official Method of Analysis (A.O.A.C. 1990) using a Total Dietary Fibre Assay Kit (TDF100A; Sigma-Aldrich).

Gel firmness

The gel firmness, a physical property defined as the maximum force required to puncture the aloe vera tissue, was measured as an indicator of texture. Firmness of samples was measured using a Texture Analyzer (Model TA-XT Plus, Surrey, UK). The puncture diameter was 3 mm, with a travel distance of 15 mm at a test speed of 1.5 mm / s. The

maximum force was measured by making one puncture in each sample, altogether 10 slabs were tested per treatment. The mean value of firmness for each treatment was then calculated and the results were expressed as N / mm.

Cryo-SEM observations

Sample microstructure was observed by Cryo-SEM in a JEOL JSM-5410 microscope (Jeol, Tokyo, Japan). Lyophilized aloe vera samples were rehydrated for 10 h at room temperature. Square rehydrated samples of more or less 4 mm×1.5 mm×5 mm were cryo-fixed by immersion in slush nitrogen (−210 °C), fractured, etched at −90 °C, 10^{−5} Torr for 15 min, gold-coated, and viewed on the SEM cold-stage. The fractured surface was viewed directly while it was maintained at −150 °C or lower. The micrographs were taken at a magnification of 350 in order to observe changes in cell structure.

Statistical analysis

Determinations in triplicate were applied for almost all analysis, except for colour (20 replicates) and gel firmness (10 replicates). All data were expressed as mean±standard deviation (SD). The effect of pressure on each quality parameter was analyzed using Statgraphics® Plus 5 (Statistical Graphics Corp., Herndon, VA, USA) applying an analysis of variance (ANOVA). Differences among the media were analyzed using the least significant difference (LSD) test with a significance level of $\alpha=0.05$ and a confidence interval of 95 % ($p<0.05$). In addition, the multiple range test (MRT) included in the statistical program was used to demonstrate the existence of homogeneous groups within each parameter.

Results and discussion

Proximate analysis

HHP effects on proximate composition was compared with the fresh sample (F0) and the evolution of the components was also monitored after a period of 35 days of storage at 4 °C. Table 1 shows the mean values and standard deviations of the composition for fresh aloe vera gel (F0) as well as for pressurized samples and non-pressurized samples (F35), stored for 35 days. The moisture contents of the fresh and the stored samples of aloe vera gel were practically the same (F0=98.9±0.05 %; F35=99.1±0.03 %), although statistically different at 95 % confidence interval. Compared to control sample (F0), moisture content of HHP-treated samples showed a slight decrease. However, among the pressurized samples, significant differences was not observed ($p>0.05$). Protein was

Table 1 Effect of high hydrostatic pressure treatment on physical and chemical properties of aloe vera gel

Samples	Proximate analysis			Physical and chemical quality characteristics					
	Moisture %	Ash %	CF %	CH (b. d.) %	PS mg/100 g d. m.	HR g a. w. / g d. m.	WHC g r. w. /100 g w.	TDF g /100 g fw.	Firmness N/mm
F0	98.9±0.05 ^a	0.20±0.00 ^a	0.13±0.02 ^a	0.83±0.06 ^a	61560±247 ^a	16.1±3.2 ^a	71.0±2.2 ^{ac}	0.511±0.009 ^a	3.44±1.13 ^a
F35	99.1±0.03 ^b	0.20±0.01 ^a	0.11±0.01 ^b	0.71±0.09 ^b	29480±479 ^b	32.5±2.2 ^b	35.4±2.3 ^b	0.597±0.006 ^{bc}	4.65±1.06 ^b
300 MPa	98.7±0.08 ^c	0.23±0.04 ^b	0.15±0.02 ^{ac}	1.03±0.10 ^c	38205±392 ^c	17.2±2.8 ^{ac}	67.6±3.4 ^{ac}	0.608±0.013 ^c	5.69±1.52 ^c
400 MPa	98.8±0.03 ^c	0.18±0.03 ^a	0.12±0.03 ^{ab}	0.95±0.08 ^c	33496±358 ^d	14.6±0.9 ^a	67.1±5.8 ^a	0.564±0.012 ^b	5.35±1.13 ^{bc}
500 MPa	98.7±0.01 ^c	0.24±0.00 ^b	0.16±0.03 ^c	0.98±0.11 ^c	37218±363 ^e	20.5±1.6 ^c	74.2±4.4 ^c	0.561±0.012 ^b	5.15±0.90 ^{bc}

Values are mean±standard deviation, $n=3$ for all parameters, except for firmness $n=10$. Protein content was 0.03±0.01 and fat content was 0.01±0.01 in all the samples.

Different letters in same column indicate significant differences ($p<0.05$).

CF Crude fibre; CH (b. d.) Carbohydrate (by difference); PS Polysaccharides; HR Hydration ratio; WHC Water holding capacity; TDF Total dietary fibre; w. water; fw. fresh weight; a. w. absorbed water; r. w. retained water

present only in a very small fraction, which remained constant at 0.03 % in all samples (fresh and HHP-treated) and no significant differences in protein content were observed ($p>0.05$). The fat content was the smallest fraction in all the samples and did not show any significant difference ($p>0.05$). The ash content of the aloe vera gel remained constant in fresh (F0) and stored (F35) samples, whereas in HHP-treated samples ash content varied from 0.18 to 0.24 %, with samples showing significant differences ($p<0.05$). Similar increase in ash content of pressurized fresh aloe vera gel has been observed (Vega-Gálvez et al. 2011a). However, the difference is very small and may be attributed to variation of ash content in different samples. The crude fibre plays an important role in the composition of aloe vera gel due principally to presence of pectic substances, cellulose and hemicellulose, which stimulate intestinal transition (Femenia et al. 1999; Femenia et al. 2003). In pressure treated samples a slight but significant increase in fibre content was observed ($p<0.05$). This increase may be compared to similar increase in content of non-starch polysaccharides reported during extrusion cooking of oat meal and potato peels (Dhingra et al. 2012). In proximal composition of aloe vera gel, carbohydrates are the second main component following moisture. This was also observed in this study. Moreover, carbohydrates was shown to increase from 0.71 % (F35) to values higher than 0.95 % in the HHP-treated samples ($p<0.05$). This may also be due to increase in content of non-starch polysaccharides (Dhingra et al. 2012).

Colour

Total colour variation (ΔE) is the more interpretable factor when examining colour attributes (Hartyáni et al. 2011). In this study, ΔE for fresh aloe vera gel and treated samples were 6.89 for F35 and 7.41, 8.93 and 18.83 at 300, 400 and 500 MPa, respectively. ΔE values are a good indicator of progressive changes in colour of HHP-treated aloe vera gel. According to the results observed, ΔE values increased significantly ($p<0.05$) at 500 MPa showing deviation from original colour in the samples, which may be attributed to enzymatic browning reactions (Landl et al. 2010). Guerrero-Beltran et al. (2005) observed enzymatic browning in HHP-treated mango purée at 379–586 MPa. According to MRT results, there were three homogenous groups (F35–300; 300–400 and 500 MPa) with significant differences among them ($p<0.05$). Similar results were shown by Landl et al. (2010) working with HHP-treated apple purée at 400 and 600 MPa for 5 minutes at 20 °C and stored in refrigeration for 3 weeks at 5 ± 1 °C, which showed colour deterioration in the purée sample between day 7 and 14 of cold storage. Krebbers et al. (2002) working on colour of green beans obtained a ΔE value of 42.6 at 500 MPa after an storage period of 1 month at 6 °C. It is well known that application of high pressures (100–800 MPa) causes permeabilization

of plant and microbial cells (Dornenburg and Knorr 1993; Prestamo and Arroyo 1999). This will cause damage to the chloroplasts, resulting in leakage of chlorophyll into the intercellular space. This phenomenon is probably the cause of the initially more intense bright green colour on the surface of the HHP-treated beans. Van Loey et al. (1998) studied the effects of pressure and temperature on chlorophyll degradation in a broccoli-extract. They also found significant reduction of chlorophyll when pressure was combined with temperatures higher than 50 °C. The colour loss is due to conversion of labile chlorophylls a and b to yellow-olive-coloured pheophytin. Storage significantly affected the HHP-treated beans. Negative effects on the colour after HHP treatment were probably caused by residual activity of enzymes such as lipoxygenase, peroxidase or chlorophyllase. Effects of storage on green colour after pressurization have been studied, but only in purées and extracts of guacamole (Palou et al. 2000; Sohn and Lee 1998; Van Loey et al. 1998). They have also observed decreases in greenness during storage. Since some colour change was observed in pressure-treated aloe vera gel after a storage time of 35 days at 4 °C, it may be possible that the applied pressure treatment was not sufficient to inactivate the enzymes responsible for browning of the gel.

Vitamin C content

In general, results of the vitamin C content after 35 days of storage did not show any significant difference ($p>0.05$) between HHP-treated and untreated gel samples. An average of 131.17 ± 12.71 mg vitamin C / 100 g dry matter was determined in the samples. Butz et al. (2003) evaluated the influence of applying 600–800 MPa of pressure on vitamin C contents of different kinds of fruit juices, fruits and vegetables and they concluded that in most cases, high pressure did not induce any loss of vitamin C content in the fruit and vegetable matrices. Fernández-García et al. (2001) found that orange juice and mixed juice of orange, lemon and carrot processed at 500 and 800 MPa for 5 min showed none or only insignificant reductions of vitamin C compared to unprocessed juice. Hartyáni et al. (2011) working with pulsed electric field and HHP-treated citrus juices showed an increase in the vitamin C content in orange and tangerine juices. Krebbers et al. (2002) observed that ascorbic acid retention in green beans was 92 % after high pressure treatment at room temperature for 1 min at 500 MPa.

Polysaccharides content

The total polysaccharides content in aloe vera gel is shown in Table 1. For the fresh sample (F0), a content of 61560 ± 247 mg / 100 g dry matter was determined. According to

ANOVA, there was significant differences among all analyzed samples ($p<0.05$), and a decrease in polysaccharides content was observed after 35 days storage. For the untreated sample after storage (F35), there was a loss of 52 % in polysaccharides content compared to the fresh product (F0). However, HHP-treated samples (300, 400 and 500 MPa) showed a minor loss of polysaccharides with respect to the fresh product (F0); all of them showed retention of polysaccharides higher than 55 %. Yang et al. (2009) working with longan fruits pericarp treated at high pressure of 300–500 MPa, reported a polysaccharides content of 18.3 mg / g and observed a major loss at a pressure of 500 MPa (6.4 mg / g) which might be due to the structural modification of water-soluble polysaccharides. Chang et al. (2006), working with different temperatures in aloe vera gel juice, reported that a decrease of the polysaccharides content was observed at lower temperatures during the process, mainly due to the enzymatic action that led to hydrolysis of long-chain polysaccharides molecules to others of smaller size.

Measurement of hydration indices

Table 1 shows the changes occurring in the hydration properties of aloe vera gel as a result of HHP treatment. When analyzing the hydration indices, WHC and HR, an increase in HR from 16.07 ± 3.22 to 32.55 ± 2.20 g absorbed water / g dry matter was observed after 35 days of storage of the fresh gel. However, hydration capacity of HHP-treated samples decreased from 32.55 ± 2.20 to 17.27 ± 2.79 , 14.62 ± 0.96 and 20.50 ± 1.61 g absorbed water / g dry matter at 300, 400 and 500 MPa, respectively. From ANOVA, least significant differences were found ($p<0.05$) among the samples, and according to MRT, there were three homogenous groups, namely F0-300-400 MPa; F0-300-500 MPa and F35. In the same Table 1 behaviour of WHC index, an estimate of the ability of the food matrix to hold water, can be seen. WHC values increased as pressure increased. According to ANOVA, there is a least significant difference ($p<0.05$) among the samples. MRT also revealed three homogenous groups: F35, F0-300-400 MPa and F0-300-500 MPa. The maximum value for WHC occurred at 500 MPa (74.17 ± 4.38 g retained water / 100 g water). WHC behaviour is influenced by physico-chemical interactions and on the setting of the product on a microstructure (Aguilera and Stanley 1999; Miranda et al. 2009). Moreover, it is generally accepted that the degree of hydration is dependent on the degree of cellular and structural disruption (Guizani et al. 2008). Some authors have reported that high-pressure treatment helps to reduce water loss, probably because of disaggregation and unfolding of protein and pH of the mixture (Cheftel and Culioli 1997; Butz et al. 2003; Tabilo-Munizaga and Barbosa-Cánovas 2005).

Total dietary fibre content

Table 1 shows the total dietary fibre content of the aloe vera gel samples. TDF content of all stored and treated samples were significantly different to that of the fresh (F0) aloe vera gel sample ($p<0.05$). TDF content in sample (F35) stored for 35 days increased 18 % with respect to the untreated fresh aloe vera gel (F0). In general, TDF content remained at a constant value of 0.56 ± 0.01 % w. b. for samples treated at 400 and 500 MPa, with a slight decrease compared to the fresh gel stored for 35 days (F35). In contrast, the sample treated at 300 MPa had a TDF content of 0.60 ± 0.01 % w. b. that is not significantly different to that of the fresh gel stored for 35 days (F35). A similar result was reported by Ramula and Rao (2003) working with different fruits (watermelon, orange, pineapple, etc). Grossi et al. (2011) worked on the synergistic cooperation between high pressure treatment and carrot dietary fibre. Two formulations of pork sausages containing different percentages of carrot dietary fibre were pressurized at 500 and 600 MPa and showed an increase of dietary fibre with pressure. Wennberg and Nyman (2004) working with white cabbage showed only a slight affect of HHP on the total dietary fibre content. Mateo-Aparicio et al. (2010) worked on the combined effect of high hydrostatic pressure and controlled temperature on total dietary fibre content. They showed that TDF content in dry and hydrated okra samples would increase with increasing hydrostatic pressure and temperature.

Determination of gel firmness

Table 1 shows the observed changes in firmness of aloe vera gel treated at different pressures after 35 days storage compared to the fresh gel (F0) and the stored gel (F35). Results showed that there are least significant differences ($p<0.05$) among samples F0, F35 and HHP-treated gel. These firmness values varied from 3.44 ± 1.13 to 5.69 ± 1.52 N / mm. It was observed that gel firmness of all HHP-treated samples was significantly greater than that of the fresh and the untreated stored samples (F0 and F35). These differences might be due to the development of a compressed structure in the parenchymal tissue mainly formed by structural polysaccharides (pectin, cellulose, and hemicellulose) located within the cell wall, which provide stiffness to the latter (Miranda et al. 2009; Vega-Gálvez et al. 2011b). Miranda et al. (2009) reported presence of calcium in aloe vera, in the form of calcium pectate that might have formed three-dimensional networks with the pectins, which provide firmness to the parenchymal tissue. Miao et al. (2011) observed that an increase in pressure increased texture of water bamboo shoots during storage period compared to control sample. Basak and Ramashamy (1998) showed a loss in texture as a result of HHP (100–400 MPa) treatment for some

vegetables up to 67 %, which is ascribed to the expeditious action of pressure. They have found a recovery of firmness to the initial level during long pressure holding time (60 min), also ascribed to the firming action of pectin methylesterase (PME). Tangwongchai et al. (2000) studied the effect of high pressure in tomatoes (200–600 MPa for 20 min) which presented an increased and decreased cell disruption for treatments at 500 and 600 MPa. Stute et al. (1996) and Kasai et al. (1997) also hypothesized that the improved texture properties of vegetables after pressurization could be due to the enzymatic demethylation of pectins, followed by the formation of calcium pectate.

Microstructural changes

Microstructure images of the aloe vera gel were observed by Cryo-SEM. Fig. 1a shows the cell structure of fresh gel and Fig. 1b–d shows morphological and microstructural changes observed in HHP-treated samples. The effect of high pressure on the tissue structure of aloe vera gel is shown in Fig. 1b–d, where it is observed that treated samples show a cell structure with a minimal irregular shape and a minor reduction on intracellular integrity compared to control sample. Fig. 1a (control sample) shows an aloe vera gel structure with an intact parenchymal cell, of well-rounded shape with characteristic diameter, whose dimensions are in the range of 300–400 μm . Similarly, close contact among the cell walls of adjacent cells can be observed (Miranda et al. 2009; Vega-Gálvez et al. 2011b). The cell wall is represented as a

heterogeneous and dynamic polymeric unit comprising of a three dimensional interlinked fibrous structure of cellulose. The cellulose of the cell wall gives stiffness and strength to the structure, whereas pectin and hemicelluloses of the middle lamella give plasticity and dictate the degree to which the cells can be pulled apart during deformation (Lewicki and Porzecka-Palak 2005; Miranda et al. 2009).

Due to the degradation of the tissue caused by pressure, cells were deformed and a higher number of intercellular spaces were filled with soluble material from the inside of the cells. When pressure was applied, intracellular liquid spread throughout the tissue. The tonoplast or plasmalemma could now be observed as a consequence of the compression during the HHP treatment (Vásquez-Gutiérrez et al. 2011). Knowledge of the microstructure in which mass transfer takes place may assist in finding the mechanisms and their relative contributions to the transport phenomena. In this sense, work still needs to be done on how the structure of processed food affects the actual availability of bioactive components as well as its properties and functionality (Aguilera 2005; Vega-Gálvez et al. 2011b).

Conclusions

Effects of high hydrostatic pressure treatment on physico-chemical and structural properties of aloe vera gel after 35 days of storage were investigated in the range of 300–500 MPa for 3 min. Proximate composition showed that the

Fig. 1 Cryo-SEM micrographs of Aloe vera gel fresh and treated at different pressures (x 350): (a) fresh (b) 300 MPa (c) 400 MPa and (d) 500 MPa

main characteristic of aloe vera gel is its high water content. The parameters crude fibre and carbohydrate contents of treated samples showed significant differences ($p < 0.05$) with respect to the control sample. However, no significant differences were found in the case of moisture, protein, ash and fat contents with respect to the control sample as pressure increased. Vitamin C content did not show any significant difference after 35 days of storage with a final mean value of 131.17 ± 12.71 mg vitamin C / 100 g d.m.. Polysaccharides content decreased with an increase in pressure ($p < 0.05$). The hydration ratio (HR) decreased with pressure showing a lower HR of 14.62 ± 0.96 (g absorbed water / g dry matter) at 400 MPa. The maximum water holding capacity was 74.17 ± 4.38 (g retained water / 100 g water) at 500 MPa. The dietary fibre content increased with an increase in pressure at 300 MPa, decreasing slightly its content at 400 and 500 MPa. An increase of tissue firmness was observed at high pressures. HHP-treated aloe vera gel presented minor changes in the cellular structure. According to these results, aloe vera gel treated over 400 MPa would maintain its physico-chemical and structural properties; therefore, this research provides useful information on HHP processing of aloe vera gel.

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