# ORIGINAL PAPER

# Effect of Osmotic Dehydration Under High Hydrostatic Pressure on Microstructure, Functional Properties and Bioactive Compounds of Strawberry (*Fragaria Vesca*)

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Received: 27 September 2012 / Accepted: 13 January 2013 / Published online: 30 January 2013 © Springer Science+Business Media New York 2013

Abstract Sliced strawberries were subjected to combined osmotic dehydration (40 °Brix) and high hydrostatic pressure (HHP) at 100, 200, 300, 400 and 500 MPa for 10 min. This research was carried out to study the effects of pressure on firmness, polysaccharides, total dietary fibre and microstructure, functional properties (rehydration ratio and water holding capacity) and bioactive compounds (anthocyanins, flavonoid and total phenolic). HHP affected the texture of the fruits leading to soft fruits due to increasing pressure. Fruit microstructure evidenced influence of pressure presenting the pressurised samples irregular matrices compared to samples treated at 0.1 MPa (control samples). Polysaccharides increased with pressure. Total dietary fibre, anthocyanins, flavonoids and total phenolic content showed a decrease with pressure when compared to control samples.

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CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas), Buenos Aires, Argentina e-mail: kdiscala@fi.mdp.edu.ar Based on results, minor alterations of the mentioned quality parameters were evidenced when working in the range of 300–500 MPa.

**Keywords** Strawberry · High hydrostatic pressure · Osmotic dehydration · Microstructure · Anthocyanins · Firmness

## Introduction

Strawberries (Fragaria vesca cv. camarosa) are nonclimatic fruits, susceptible to mechanical damage, physiological deterioration, water lost and thus, they have a very short post-harvest life (Del-Valle et al. 2005). Strawberries are widely consumed mainly as fresh fruit, juice, nectar, puree and juice concentrate as well as jam (Patras et al. 2009). During processing, their delicate texture is damaged. The cell wall is mainly composed of cellulose microfibrils, embedded in a matrix of pectin, hemicellulose, proteins and phenolic compounds (Fraeye et al. 2010). The colour of strawberry is due to the presence of water-soluble anthocyanin pigments which are responsible for its attractive colour (Da Silva et al. 2008). In addition, antioxidant compounds are very susceptible to degradation when subjected to physical stress in berry products (Patras et al. 2011). The role of antioxidant compounds in reducing the risk of many chronic diseases has been well documented (Patras et al. 2009). Therefore, there is a need for alternative fruit-processing methods for extending the shelf-life of these fruits.

Combined osmotic dehydration and high hydrostatic pressure (OD-HHP) processing is an innovative processing technique (Nuñez-Mancilla et al. 2011). Osmotic dehydration is used widely for the partial removal of water from plant tissues by immersion in a hypertonic (osmotic) solution (Rastogi et al. 2000). High hydrostatic pressure processing is a nonthermal food preservation technique that has emerged as a commercial alternative to traditional thermal food processing (Nuñez-Mancilla et al. 2012). The effect of HHP can vary depending on processing conditions (pressure and holding time) and form (whole, puree and juice). The food matrix can be altered by these variables consequently impacting on the effectiveness of HHP. Moreover, the functional properties of the processed food like the rehydration ability is related to its structure. Thus, some rehydration indices were proposed in order to estimate the tissue damage caused by processing (Zura-Bravo et al. 2011).

The aim of this work was to study the influence of combined osmotic dehydration and high hydrostatic pressure on the rehydration indices, polysaccharides content, firmness, dietary fibre, microstructure and bioactive compounds (flavonoid, anthocyanins and phenolics) in strawberry after 14 days of storage at 5 °C.

#### **Materials and Method**

#### Raw Material

Strawberries (cv. camarosa) were acquired in a local market from the city of La Serena (Chile). The fruits were selected by colour, size and with no signs of mechanical damage and maturity level. Then, the fruits were washed, dried with absorbent tissue paper and manually cut transversely with a stainless steel knife (Tramontina, Chile) into slices of 1 cm thickness. From each fruit, three slices were obtained.

#### Physicochemical Analysis

The moisture content was determined following the AOAC methodology no. 934.06 (AOAC 1990), using an analytical balance (CHYO, Jex-120, Kyoto, Japan) with an accuracy of  $\pm 0.0001$  g and a vacuum drying oven (Gallenkamp, OVL570, Leicester, UK). The crude protein content was determined using the Kjeldahl method with a conversion factor of 6.25 (AOAC no. 960.52). The lipid content was analysed gravimetrically following Soxhlet extraction (AOAC no. 960.39). The crude fibre was estimated by acid/alkaline hydrolysis of insoluble residues (AOAC no. 962.09). The crude ash content was estimated by incineration in a muffle furnace at 550 °C (AOAC no. 923.03). All methodologies followed the recommendations of the Official Method of Analysis (AOAC 1990). The available carbohydrate was estimated by the difference. The pH was measured using an EXTECH instruments microcomputer pH-vision 246072 (Waltham, Massachusetts, USA); the level of titrimetric acidity was expressed as citric acid. The water activity (a<sub>w</sub>) was measured at 25 °C by means of a water activity meter (Novasina, model TH-500, Pfäffikon, Lachen, Switzerland). All measurements were done in triplicate.

Osmotic Dehydration and High Hydrostatic Pressure Treatments

The concentration of sugar (sucrose, Iansa Co. Chile) used was 40 g/100 g total soluble solids, measured with a refractometer (ABBE, ATAGO model 1T, Tokyo, Japan). The slices obtained were weighed individually and submerged in the hypertonic solution in a 1:4 (w/w) fruit to syrup ratio and packaged in polyethylene in high-density polyethylene pouches (0.14± 0.01 mm, Curbell Plastics Inc. USA) prior to high hydrostatic pressure (HHP) processing. HHP treatment was carried out in a cylindrical loading container at room temperature in a 2-1 pilot high-pressure unit (Avure Technologies Incorporated, Kent, WA, USA). Samples were pressurised at 100, 200, 300, 400 and 500 MPa during 10 min according to previous work (Nuñez-Mancilla et al. 2011). Water was employed as pressure-transmitting medium, working at 17 MPas<sup>-1</sup> ramp rate; decompression time was less than 5 s at room temperature (15 °C), the fruits were removed from the solution after HHP treatment and the excess of syrup solution in the surface was eliminated with tissue paper. Each experiment series was carried out in triplicate. Samples were stored for 14 days in refrigeration (5 °C) until quality analysis.

## **Evaluation of Functional Parameters**

## Rehydration Indices

The lyophilised samples (VirTis, -40 °C) were placed in distilled water at 5 °C for 8 h, using a solid to liquid ratio of 1:50. The samples were then removed, drained for 3 s and weighed. The rehydration ratio (RR) was calculated by means of Eq. 1 and it is expressed as gadsorbed waterg<sup>-1</sup>d.m. The water holding capacity (WHC) was determined by centrifuging the rehydrated samples at  $3,500 \times g$  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. The water holding capacity was calculated from the amount of water removed according to Eq. 2, and is expressed as gretained water 100 g<sup>-1</sup> water. All measurements were done in triplicate.

$$RR = \frac{W_{reh} \times X_{reh} - W_{dried} \times X_{dried}}{W_{dried} \times (1 - X_{dried})}$$
(1)

$$WHC = \frac{W_{reh} \times X_{reh} - W_l}{W_{reh} \times X_{reh}} \times 100$$
(2)

Where  $W_{\text{reh}}$  is the weight of the sample after the rehydration process,  $X_{\text{reh}}$  is the corresponding moisture content on a wet basis,  $W_{\text{dried}}$  is the weight of the sample after the drying process,  $X_{\text{dried}}$  is the corresponding moisture content on a wet matter and  $W_{\text{I}}$  is the weight of the drained liquid after centrifugation.

## Total Polysaccharides Content

Polysaccharides content was estimated by a colorimetric analvsis. Five grams of pressurised strawberries were extracted with 80 mL of water in bath at 100 °C for 2 h, with constant agitation and the samples were vacuum filtered. The filtrate was diluted to 100 mL in a beaker according to the methodology suggested by Hu et al. (2003). Two milliliters of the solution and 10 mL of absolute ethanol were added in plastic tubes; samples were centrifuged at 2,500×g for 30 min, and the supernatant was removed; the precipitate was dissolved in a final volume of 50 mL water. One milliliter of the filtered solution, 1 mL of phenol at 5 g100<sup>-1</sup>mL and 5 mL of concentrated sulphuric acid were added to the top of the tubes. It was allowed to settle for 30 min. Sample absorbance was determined at 490 nm (Spectronic<sup>®</sup> 20 Genesys<sup>TM</sup>, IL, USA). Total polysaccharide content was estimated by comparison with a standard curve generated from D-+-glucose analysis. All measurements were done in triplicate. It was expressed as mg polysaccharides/100 g sample.

## Determination of Dietary Fibre

The total dietary fibre was determined by the gravimetricenzymatic method 32-07(n° 991.43) suggested by the Official Method of Analysis (AOAC 1990) using a Total Dietary Fibre Assay Kit (TDF100A; Sigma-Aldrich). Duplicate 1.0 g of treated samples were lyophilised and suspended in MES/TRIS buffer with 40 mL a pH of 8.2 (24 °C). Then, samples were digested with 50  $\mu$ L of heat-stable  $\alpha$ amylase for 35 min between 95 and 100 °C and added 5 mg of protease for 30 min at 60 °C. A 0.561 M HCl solution was added to the suspension to reduce the pH to 4.2-4.8 prior to digesting the sample with 200 µL amyloglucosidase by 30 min at 60 °C. The enzyme digest was filtered through tarred fritted glass crucibles. Crucibles were rinsed with dilute ethanol (95 %) followed by acetone and dried overnight in a 105 °C oven. For insoluble dietary fibre (IDF), enzyme digestates were filtered, without precipitation, and the crucibles containing IDF were rinsed with alcohol and acetone. One set of duplicate residues was ashed in a muffle furnace at 525  $^\circ$ C for 5 h. The other set of residues was analyzed for protein as Kjeldahl nitrogen ×6.25. Soluble dietary fibre (SDF) was calculated by subtracting the IDF proportion from the TDF.

## Determination of Maximum Puncture Force

Firmness, which is the maximum force applied to puncture the strawberries fruit tissue, was measured as an indicator of texture. Firmness of samples was measured using a Texture Analyses (Texture Technologies Corp, TA, XT2, Scardale, NY, USA). The puncture diameter was 1 mm, the distance was 3 mm and test speed was 1.5 mms<sup>-1</sup>. The maximum force

was measured by making one puncture in each slice of strawberries fruit sample, using ten slabs per treatments (Di Scala et al. 2011). The mean values of maximum force for each treatment were then calculated and the results were expressed as *N*.

#### Cryo-SEM Observations

Sample microstructure was observed by Cryo-SEM in a JEOL JSM-5410 microscope (Jeol, Tokyo, Japan). Lyophilised strawberry samples were rehydrated for 10 h at room temperature. Square rehydrated samples of more or less  $4 \times 1.5 \times$ 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 ×350 magnification to observe changes in cell structure.

#### **Evaluation of Bioactive Compounds**

#### Anthocyanins

The anthocyanins content of strawberry sample was determined according to the work of Odriozola-Serrano et al. (2009) with a slight modification. Five grams of strawberries were homogenised (Ultra turrax T18 basic) for 5 min. The homogenate was transferred to a 50-mL volumetric flask and made up with each buffer. The absorbance of the mixtures at pH 1 and 4.5 was then measured with spectrophotometer (Spectronic<sup>®</sup> 20 Genesys<sup>TM</sup>, IL, USA) at 510 and 700 nm. The anthocyanins content was calculated according to Eq. 3 and expressed as g pelargonidin-3-glucoside by grams.

$$TA = \frac{\left[ (A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5} \right] \times MW \times DF \times 1,000}{\varepsilon \times L}$$
(3)

Where MW is the molecular weight of pelargonidin-3glucoside (433.0 g/mol), DF is the dilution factor, *L* is the path length in centimeters and  $\varepsilon$  is the molar extinction coefficient for pelargonidin-3-glucoside (22,400 Lmol<sup>-1</sup> cm<sup>-1</sup>). Results were expressed as pelargonidin-3-glucoside content compared to the untreated sample.

#### Extraction of Flavonoids and Total Phenolic

The extraction of flavonids and total phenolic was carried out using 80 % acetone method described by Liu et al. (2002) with a slight modification. Five grams of processed fruits was homogenised with 50 mL of 80 % acetone using an Ultra Turrax (Ultra turrax T18 basic) for 5 min. Then, it was shaken during 1 h in the dark. The homogenate was filtered through #1 Whatman paper. The filtrate was recovered and the acetone was evaporated by a rotary evaporator at 40 °C. The samples were then brought to the desired volume (100 mL) with deionised water and refrigerated at 5 °C prior to analysis. The extracts were used for the measurement of flavonoids and phenolics.

#### Flavonoids

The total flavonoid content was determined by calorimetric assay with some modifications (Shin et al. 2007). A 0.5-mL aliquot appropriately diluted sample was added to a 5-mL tube containing 2 mL of dionised water. Then 0.15 mL of 5 % NaNO<sub>2</sub> was added to this mixture, which was allowed to stand for 5 min at room temperature, and 0.15 mL of 10 % ALCL<sub>3</sub>·6H<sub>2</sub>O was added. The mixture was allowed to stand for 6 min at room temperature, and 1 mL of 1 molL<sup>-1</sup> NaOH was added and the total was made up to 5 mL with deionised water. The absorbance of the solution versus a blank at 415 nm was measured immediately. The results are expressed as quercetin equivalents using a standard curve prepared from authentic quercetin (Sigma Q4951).

## Total Phenolic Content

Total phenolic content (TPC) was determined colorimetrically using the Folin-Ciocalteau reagent (FC) according to Chuah et al. (2008) with modifications. Half milliliter aliquot of the strawberry extract solution is transferred to a glass tube; 0.5 mL of reactive FC is added after 5 min and 2 mL of Na<sub>2</sub>CO<sub>3</sub> solution (200 mg/mL) were added and shaken. The sample was then mixed on a vortex mixer and the reaction proceeded for 15 min at ambient temperature. Then, 10 mL of ultra pure water were added and the formed precipitate was removed by centrifugation during 5 min at  $4,000 \times g$ . Finally, the absorbance was measured in a spectrophotometer (Spectronic® 20 Genesys<sup>TM</sup>, Illinois, USA) at 725 nm and compared to a gallic acid (GA) calibration curve. Results were expressed as mgGA100<sup>-1</sup>g matter. All measurements were done in triplicate.

#### Statistical Analysis

Analysis of variance (ANOVA; Statgraphics Plus<sup>®</sup> 5.1 software, Statistical Graphics Corp., Herndon, USA) was used to indicate significant differences among samples. Significance testing was performed using Fisher's least significant difference test and differences were taken as statistically significant (p<0.05). The multiple ranges test, included in the statistical program, were used to prove the existence of homogeneous groups within each of the parameters analysed.

## **Results and Discussion**

## Raw Material Characterisation

Table 1 shows the composition results obtained for fresh strawberry. As expected, fruits presented high water content (91.43 g/100 g) (Ozcan & Haciseferogullari 2007; El-Beltagy et al. 2007). The ranges of pH and acidity were comparable to those reported by previous investigations (Kafkas et al. 2007, El-Beltagy et al. 2007; Zheng et al. 2007). Furthermore, the strawberries showed  $a_w>0.90$  being very susceptible to microbial deterioration (Moraga et al. 2004). Regarding protein, fat and ash contents, the values were comparable with those reported in the work of Garcia, Pereira, De Luca Sarantopoulos and Hubinger (2010). Differences in fruit composition varies depending on the cultivar and other agronomic factors.

#### **Rehydration Indices**

Figure 1 presents the rehydration ratio (RR) as well as the water holding capacity (WHC) for each working pressure. WHC increased as pressure was increased. The maximum WHC was observed at 500 MPa ( $51.83\pm0.99$  g retained water  $100^{-1}$ g water), while the minimum was noted at 200 MPa ( $26.22\pm3.37$  g retained water  $100^{-1}$ g water). Based on an ANOVA, significant differences were observed among control and treated samples (p<0.05). Regarding RR, an increase in working pressure resulted in an increase from  $1.91\pm0.02$  to  $4.10\pm0.05$  (gabsorbed waterg<sup>-1</sup>d.m.). The maximum RR values were observed working at 300–400–500 MPa, in contrast to 100–200 MPa (p<0.05). Rehydration success is based on the preservation of the structures along both dehydration and rehydration treatments. Although most dehydrated cells presented a protoplast apparently able to reincorporate water,

Table 1 Fresh strawberry characterisation

Parameters	(g/100 g)
Moisture	91.43±0.01
Protein	$0.45 {\pm} 0.02$
Fat	$0.49 {\pm} 0.02$
Crude fibre	$0.54 {\pm} 0.16$
Ash	$0.37 {\pm} 0.01$
Available carbohydrates <sup>a</sup>	6.67
Energy (Kcal/g sample)	32.89
a <sub>w</sub>	$0.96 {\pm} 0.01$
pH	$3.52 {\pm} 0.01$
Acidity (% citric acid)	$1.45 {\pm} 0.01$
Soluble solids (°Brix)	$8.7 {\pm} 0.07$
Vitamin C (mg/100 g)	$56.85 {\pm} 1.18$

<sup>a</sup> By difference

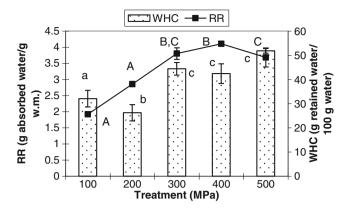


Fig. 1 Effect of osmotic dehydration under high hydrostatic pressure conditions on rehydration ratio (*RR*) and water holding capacity (*WHC*) of strawberries samples. *Bars* represent mean  $\pm$  standard deviation of triplicates. *Identical letters above the bars* indicate no significant differences (p<0.05)

some of them lose their rehydration ability or even lyse during rehydration, as a consequence of the loss of connections between the protoplast and the cell wall during both dehydration and rehydration processes (Seguí et al. 2013). In addition, application of increasing working pressure tend to increase the cell permeability resulting in an increase of the rehydration ability of the fruit. Thus, there is a combined effect of cell permeabilisation due to osmotic stress (as the dehydration proceeds) and high-pressure-induced permeabilisation (Rastogi 2010, 2000).

## Polysaccharides

Figure 2 shows the polysaccharides content of fresh as well as pressurised samples. It was noted that polysaccharides content in fresh samples was  $330.53\pm74.10$  (mgpolysaccharides $100^{-1}$ gd.m.). An increase of process pressure resulted in an increase of polysaccharides compared to the initial value. The maximum polysaccharides content in

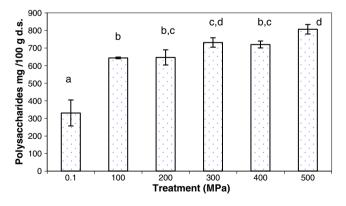


Fig. 2 Effect of osmotic dehydration under high hydrostatic pressure conditions on polysaccharides content of strawberries samples. *Bars* represent mean  $\pm$  standard deviation of triplicates. *Identical letters above the bars* indicate no significant differences (p<0.05)

processed samples was shown at 500 MPa (p < 0.05). Previous investigations have reported similar results related to the increase of polysaccharides content in treated samples. In the work of Yang et al. (2009), increased in polysaccharides content of longan fruit is associated to the inactivation of pectinmethylesterase and polygalacturonase caused by HHP treatment. These enzymes are responsible for the redistribution of polysaccharides, which are pectinmethylesterase and polygalacturonase, respectively. In addition, increased in polysaccharides content could be related to transition phase, like changes of the molar volume in response to pressure induced by HHP which depends on the level and time of pressurisation (Kasapis & Sablani 2008).

## Dietary Fibre

Table 2 presents the mean and standard deviation of dietary fibre (insoluble (IDF), soluble (SDF) and total (TDF) for all the samples. The insoluble dietary fibre content in pressurised samples decreased 1–30 %. However, significant differences were not found between control and treated samples (p<0.05). When comparing SFD from processed and control samples (p<0.05), it was observed a decreased of 15– 40 %, being the minimum loss at 400 MPa (15.5 %). On the other hand, TDF did not show significant differences with control samples (p<0.05), being the minimum loss of TDF at 400 MPa (5 %). Similar results were reported by other authors working with watermelon, orange, pineapple (Ramulu & Rao 2003); cabbage (Wennberg & Nyman 2004) and okara samples (Mateo-Aparicio et al. 2010).

#### **Firmness**

Firmness is one of the most desirable attributes in fresh as well as processed fruits. The behaviour of this physical property affected by processing is illustrated in Fig. 3. The firmness of control sample was  $1.18\pm0.38$  N. Significant

 Table 2
 Effect of osmotic dehydration under high hydrostatic pressure conditions on dietary fibre of strawberries samples

	-	-	
Pressure (MPa)	IDF (g/100 g)	SDF (g/100 g)	TDF (g/100 g)
0.1	2.07±0.05a	1.03±0.40a	3.10±0.36a
100	$1.55 {\pm} 0.84a$	0.60±0.01a	2.16±0.83a
200	1.40±1.09a	$0.60 {\pm} 0.03 a$	2.00±1.06a
300	1.68±0.21a	0.70±0.24a	2.38±0.46a
400	$2.05 \pm 0.01a$	0.87±0.63a	$2.92{\pm}0.62a$
500	1.75±1.18a	$0.80 {\pm} 0.43a$	$2.25{\pm}0.75^a$

Values are means  $\pm$  standard deviation. Identical letters in the same column indicate no significant differences (p<0.05)

IDF insoluble dietary fibre, SDF soluble dietary fibre, TDF total dietary fibre

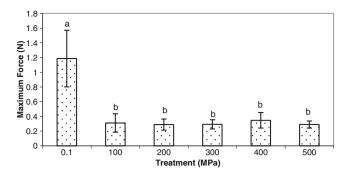


Fig. 3 Effect of osmotic dehydration under high hydrostatic pressure conditions on firmness of strawberries samples. *Bars* represent mean  $\pm$  standard deviation of triplicates. *Identical letters above the bars* indicate no significant differences (p < 0.05)

difference was observed between control and processed samples. However, among pressurised samples, there was no evidence of statistical difference (p < 0.05). Firmness of processed samples decreased  $74.4\pm2.1$  % compared to control samples. This result could be related to volume reduction of osmosed samples that have been explained in terms of the changes of the volumes of liquid phase (water plus soluble solids) and gas phase (in the intercellular spaces) of the tissue (Chiralt & Talens 2005). In addition, high pressure causes folding and corrugation of the cell wall, resulting in changes in the microstructure, which in turn affect the textural properties due to a loss of cellular turgor, firmness and cell wall integrity. Many authors have suggested that changes in cell permeability are a major cause of textural degradation in several fruits (Oey et al. 2008; Rastogi 2010). Previous reports observed that HHP did not have any adverse effect on product texture for green peas treated from 400-900 MPa/5-10 min, potatoes treated at 400 MPa/15 min and pineapple treated from 50-700 MPa/10 min (Quaglia et al. 1996; Al-Khuseibi et al. 2005; Kingsly et al. 2007). On the other hand, some authors reported that vegetables and fruits soften due to HHP in the range of 100-600 MPa (Basak & Ramashamy 1998; Tangwongchai et al. 2000). Thus, the effect of HHP on produce texture is not only determined by the intensity of treatment but also by its structure and physiology.

## Microstructure Changes

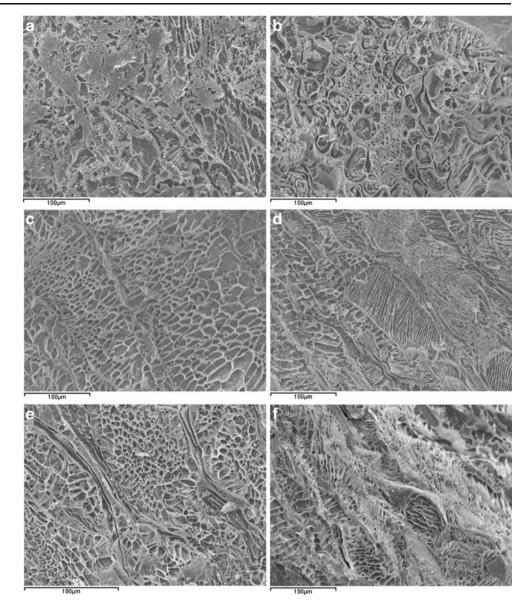
Cryo-SEM observations allow us to observe the structural solids, such as cell wall and membranes, but also the soluble solids of cells in a dentritic structure formed during the ice microcrystal sublimation in the glassy cryoconcentrated cell liquid phase. Bright structural elements in the micrographs correspond to the polymeric structures and water-solute glass, and dark spots to the ice microcrystal sites (Bomben & King 1982). Figure 4 shows the effect of osmotic dehydration under HHP conditions on microstructure of strawberries parenchyma tissue. It is possible to observe that when HHP increase, damage in strawberry cell is more important. During high-pressure processing, turgor loss and cellular changes can take place along with changes in cell conformation, elongation, separation or debonding and/or cell wall disruption (Rastogi 2010). In Fig. 4c until f, the same aspect in the intra- and intercellular volumes can be observed. This could be due to a partial solubilisation of pectic substances of middle lamella in the liquid phase. Moreno et al. (2012) reported similar results. Similar results in microstructure were reported in previous investigations working with strawberries between 150 and 300 MPa and carrots at 100 MPa (Van Buggenhout et al. 2007; Trejo-Araya et al. 2007).

# Anthocyanin Content, Total Flavonoid and Total Phenolic Content

In the present study, levels of the anthocyanins in sliced strawberry range from 33 to 86 mg $100^{-1}$ g pelargonidin-3-glucoside (Table 3). These values are in the range of those reported by other authors (Zheng et al. 2007; Cheel et al. 2007). Although pressure decreased the anthocyanins compared to control samples, for pressurised samples significant differences were not observed (p > 0.05). Degradation of anthocyanin pigments in strawberry samples may have been catalyzed by the presence of oxidase enzymes during or processing, as has been demonstrated in several fruit systems. However, anthocyanins degradation in processed berry products has been reported to arise as a result of indirect oxidation by phenolic quinines generated by polyphenol oxidase and peroxidase (Patras et al. 2009).

Total flavonoids and total phenolic content of fresh fruits were  $101.73\pm8.07$  mg quercetin  $100^{-1}$  g and  $258.57\pm6.39$  mg GA  $100^{-1}$  g, respectively. Flavonoids as one of the most diverse and widespread group of natural compounds are probably the most important natural phenolics (Prasad et al. 2009). Table 3 shows the total flavonoid content derived from quercetin, a decreased with increasing working pressure was evidenced (p < 0.05). The decreased in flavonoids content in treated samples was in the range of 20–50 %, being the maximum lost at 500 MPa. This behaviour could be due to higher pressure levels or longer processing times causing a decrease of the flavonoids content. Quercetin derivatives are the most abundant flavonois in strawberry fruits. In this respect, flavonoi levels are of the same

Fig. 4 Effect of osmotic dehydration under high hydrostatic pressure conditions on microstructure of strawberries samples. Cryo-Sem micrographs (×350): **a** 0.1 MPa, **b** 100 MPa, **c** 200 MPa, **d** 300 MPa, **e** 400 MPa, **f** 500 MPa



**Table 3** Effect of osmotic dehydration under high hydrostatic pressure conditions on bioactive compounds of strawberries samples

Treatment (MPa)	Anthocyanin (mg/100 g d.s) <sup>a</sup>	Flavonoid (mgquercetin/100 g)	Total phenols (mgGA/100 g)
0.1	85.45±2.46a	101.73±8.07a	258.57±6.39a
100	$49.09 {\pm} 0.55 b$	79.15±2.73ab	$249.53 {\pm} 5.86 ab$
200	$50.77 {\pm} 2.72b$	66.63±1.82bc	169.67±2.83c
300	$32.99 {\pm} 1.79 b$	77.98±4.98abc	$239.00{\pm}6.71b$
400	$50.52 {\pm} 1.79b$	64.72±0.76bc	194.08±3.14c
500	$49.73 {\pm} 1.18b$	55.28±2.78c	185.97±1.99c

Values are means  $\pm$  standard deviation. Identical letters in the same column indicate no significant differences (p<0.05)

d.s dry sample, GA gallic acid

<sup>a</sup> Values expressed as pelargonidin-3-glucoside

range as the ones present in other products like grapes, tomatoes or green tea (Pallauf et al. 2008). The total flavonoid concentration of sample strawberries can be maintained or changed by storage conditions (Shin et al. 2007). From Table 3, it can be observed that TPC decreased as working pressure was increased (p < 0.05). Among pressurised samples, the highest TPC, between pressurised samples was observed at 100 MPa. This decrease could be due to the partially soluble polymers formed during storage that react with the FC reagent (Pérez-Vicente et al. 2004). Some authors reported decreased in TPC in fruits working at 450 MPa/20 °C/1-5 min after 30 days storage (Keenan et al. 2010). Others, reported an increase amount of phenolics due to HHP during processing of olive fruits (Tokusoglu et al. 2010).

#### Conclusion

Effects of combined osmotic dehydration and high hydrostatic pressure in the range from 100 to 500 MPa/10 min on quality parameters of sliced strawberries storage for 14 days at 5 °C were investigated in this work. The ability of samples rehydration (water holding capacity and rehydration index) as well as the polysaccharides content increased with pressure (p < 0.05). TDF did not show significant differences with control samples (p < 0.05), being the minimum loss of TDF at 400 MPa. HHP also affected firmness presenting a notable decreased with control samples. However, among pressurised samples, significant differences in firmness were not observed (p < 0.05). The microstructure of strawberries was also influence by HHP resulting in modification of the cellular components at higher pressures. Regarding bioactive compounds, anthocyanins and flavonoids decreased in the pressurised samples showing no significant differences among treatments. However, although total phenolic content decreased in pressurised samples compared to control samples, among treated fruits, those processed at 100 and 300 MPa presented the high TPC values.

Acknowledgements The authors gratefully acknowledge the Research Department of Universidad de La Serena, Chile for providing financial support to this investigation.

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