



Osmotic dehydration under high hydrostatic pressure: Effects on antioxidant activity, total phenolics compounds, vitamin C and colour of strawberry (*Fragaria vesca*)

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ABSTRACT

Simultaneous application of osmotic dehydration under high hydrostatic pressure conditions of strawberries was studied with the purpose of analyzing the effect of the combined process on the antioxidant capacity, phenolic compounds, colour and vitamin C of strawberries during refrigerated storage. The osmotic solution was prepared using commercial sugar at 40 °Brix. Samples were pressurized between 100 and 500 MPa for 10 min. The radical scavenging activity showed higher antioxidant activity at 400 MPa rather than at low pressure (100, 200 and 300 MPa). The total phenolic content increased with pressure presenting a maximum at 400 MPa. Pressurized samples retained vitamin C content. Based on these results, working at 400 MPa for 10 min ensures physicochemical and high levels of nutritional parameters in osmo-dried strawberries.

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1. Introduction

Strawberries (*Fragaria vesca*) which are very popular fruits available during the spring and summer period are highly perishable and susceptible to bruises and fungal attacks (Blanda et al., 2009). Strawberry quality for the market is focused on physical qualities but there is an increasing interest in the health benefits of the fruit as a result of consumer's demands that look for food products that taste, smell and look attractive while providing nutritional benefits (Shin, Ryu, Liu, Nock, & Watkins, 2008). Thus, the handling of fresh products in the supply chain is an important component for maintaining product quality and nutritional value (Russell, LeBlanc, McRae & Ryan, 2009). Therefore, the food industry is very interested in improving marketability of strawberries, not only as fresh fruits but also as processed products. In this sense, osmotic dehydration and high pressure processing are alternatives

to create new products with distinctive final characteristics. Application of high hydrostatic pressure (HHP) is an innovative, emerging technology with potential for optimizing intake of nutrients in human diets (McInerney, Seccafien, Stewart, & Bird, 2007; Vega-Gálvez et al., 2011).

During osmotic dehydration, a cell placed in a hypertonic solution (osmotic pressure higher than that of the cell) will lose water. As the cell wall is permeable the volume between the cell wall and plasmalemma fills with the hypertonic solution (Lewicki & Lenart, 2006). Combination of high hydrostatic pressure with osmotic dehydration leads to cell dehydration while gaining in soluble solids until equilibrium in which net transport phenomena is negligible. Moreover, osmotic dehydration of fruits provokes changes in the product through physical and chemical changes occurring throughout the process (Chiralt et al., 2001). Physiological and biochemical changes in such products occur at a faster rate than in intact fruits resulting in the rapid onset of enzymatic browning and excessive tissue softening. Enzymatic browning and the resultant discolouration of cut fruit products upon exposure to air is a major problem for the food industry impairing not only the colour of fresh-cut fruits but also the flavour and the nutritional

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quality (Perera, Gamage, Wakeling, Gamlath, & Versteeg, 2010). The colour of many fruit and vegetable products such as jams, fruit juices and purée is generally preserved during high hydrostatic pressure at ambient temperature (Landl, Abadias, Sárraga, Viñas, & Picouet, 2010). Changes in colour appearance would be more expected rather than the changes in pigment concentration (Oey, Lille, Van-Loey, & Hendrickx, 2008). Several studies on strawberries and strawberry-based products indicate that colour, flavour, aroma and vitamin C content were only minimally affected by high pressure treatments (Fraeye et al., 2010). Fruit purées are used in a variety of products including jams, conserves and smoothies and contain many health-promoting antioxidants. The role of antioxidant compounds in reducing the risk of many chronic diseases such as cancer, coronary heart disease, and immune system decline has been well documented. Several studies have demonstrated a relationship between consumption of fruits and a lower incidence of degenerative diseases such as heart disease, arthritis and ageing (Blumberg, 2003; Kaur & Kapoor, 2001; Patras, Brunton, Da Pieve & Butle, 2009). Therefore, the aim of this work was to analyze the effects of combined osmotic dehydration and high hydrostatic pressure on physicochemical and quality parameters (colour, antioxidant capacity, total phenolic content and vitamin C) on strawberries stored at 5 °C.

2. Materials and methods

2.1. Preparation of raw material

Strawberries (*F. vesca* cv. *Camarosa*) were purchased in a local market from La Serena city (Chile) and stored at 5.0 ± 1.0 °C until further processing. The fruits were selected visually according to size and maturity level. Then, the fruits were washed and dried with absorbent tissue paper. After that, they were manually cut with a stainless steel knife into 1 cm thick slices. The osmotic solutions were prepared using commercial sugar (Iansa Co. Chile) and distilled water. The concentration of sugar used was 40 °Brix. From each fruit, 3 slices were obtained. Then, they were weighed individually and submerged in the hypertonic solution in a 1/4 (g/g) fruits to syrup ratio at ambient temperature and packaged in polyethylene bags prior to HHP processing (Nuñez-Mancilla et al., 2011).

2.1.1. High hydrostatic pressure

HHP treatment was carried out in a cylindrical loading container at room temperature in a 2 litter-pilot high pressure unit (Avure Technologies Incorporated, Kent, WA, USA). Samples were subjected to five different HHP conditions, 100, 200, 300, 400 and 500 MPa for a period of 10 min according to previous work (Nuñez-Mancilla et al., 2011). All treatments were performed in an isostatic pressing system within a cylindrical pressure chamber of 700 mm of length and 60 mm of diameter. Water was the pressurizing medium at room temperature (15 °C) during pressure processing. The strawberries slices 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 under refrigerated conditions (5 °C) until quality analysis.

2.1.2. Physico-chemical analysis

The equilibrium moisture content was determined by means of AOAC method No.934.06 (AOAC, 1990). The pH was measured, previous calibration of the instrument, with a buffer solution of pH 4.0 and 7.0, using an EXTECH Instruments microcomputer pH-vision (model 246072, Waltham, MA, USA). The percentage of titratable acidity was expressed as g citric acid/100 g sample. The

water activity (a_w) was measured at 25 °C by means of a water activity instrument (Novasina, model TH-500, Pfäffikon, Switzerland). Soluble solids (°Brix) were measured using a refractometer (ABBE, 1T, Tokyo, Japan). All measurements were carried out in triplicate.

2.2. Quality parameters

2.2.1. Colour

Surface 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, standard illuminant D_{65} and observer 10° (Vega-Gálvez et al., 2011). Five replicate measurements were performed and the results were averaged. In addition, total colour difference (ΔE) was calculated using the following Eq. (1), where L_0 , a_0 and b_0 are the control values for fresh strawberries.

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

2.2.2. Non-enzymatic browning compounds (NEB)

The methodology applied for determination of non-enzymatic browning compounds (NEB) dissolved in the rehydration water was determined as proposed for citrus fruits with some modifications (Meydav, 1977). Clarification of the rehydration water was achieved by centrifugation at $4000 \times g$ for 10 min. The supernatant was diluted 1:1 with 95% ethanol (Sigma Chemical CO., St. Louis, MO, USA), homogenized further, and centrifuged at $4000 \times g$ for 10 min (Greetmed, GT211-80-2, Hejiang, China). The absorbance of the supernatant was read at a wavelength of 420 nm (Spectronic® 20 Genesys™, Illinois, USA) following calibration of the equipment with a blank (1:1 dilution of distilled water and 95 g/L ethanol) using quartz cuvettes. All measures were done in triplicate. NEB was expressed as absorbance for gram of sample.

2.2.3. Total antioxidant activity

Free radical scavenging activity of the samples was determined using the 2,2-diphenyl-2-picryl-hydrazyl (DPPH) method (Turkmen, Sari, & Velioglu, 2005). Different dilutions of the extracts were prepared in triplicate. An aliquot of 2 mL of 0.15 mmol L^{-1} DPPH radical in ethanol was added to a test tube with 1 mL of the sample extract. The reaction mixture was vortex-mixed for 30 s and left to stand at room temperature in the dark for 20 min. The absorbance was measured at 517 nm, using a spectrophotometer (Spectronic® 20 Genesys™, Illinois, USA). To calibrate the spectrophotometer 80 g/L ethanol was used. Control sample was prepared without adding extract. All solvents and reagents were purchased from Sigma (Sigma Chemical CO., St. Louis, MO, USA). Total antioxidant activity was expressed as the percentage inhibition of the DPPH radical and was determined by Eq. (2). All measurements were done in triplicate.

$$\%(\text{TAA}) = \left(1 - \frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right) \times 100 \quad (2)$$

where TAA is the total antioxidant activity and Abs is the Absorbance. IC_{50} , which is the concentration required to obtain a 50% antioxidant capacity, is typically employed to express the antioxidant activity and to compare the antioxidant capacity of various samples. IC_{50} was determined from a graph of antioxidant capacity (%) against extract concentration (mg mL^{-1})

2.2.4. Total phenolic content

Total phenolic content (TPC) was determined colorimetrically using the Folin–Ciocalteu reagent (FC) according to Chuah et al. (2008) with modifications. 0.5 mL 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 Na₂CO₃ solution (200 mg mL⁻¹) 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 ultrapure water was added and the formed precipitate was removed by centrifugation during 5 min at 4000× *g*. Finally, the absorbance was measured in a spectrophotometer (Spectronic® 20 Genesys™, Illinois, USA) at 725 nm and compared to a Gallic acid (GA) calibration curve. Results were expressed as mg GA 100 g⁻¹ matter. All reagents were purchased from Merck (Merck KGaA, Darmstadt, Germany). All measurements were done in triplicate.

2.2.5. Vitamin C content

The determination of vitamin C was performed by certification of NBS (N-Bromosuccinimide) according to Barakat, El-Wahab, and El-Sadr (1995) with modifications. The oxidizing agent (NBS) was standardized by taking an aliquot of 10 mL of a standard solution of ascorbic acid (0.2 mg mL⁻¹), which is placed in a 250 mL Erlenmeyer flask containing 2 mL of a solution of KI (4 g/L), 0.8 mL of a solution of acetic acid (10 g/L), drops of a solution of starch (1 g/L) as indicator and 12 mL of distilled water, then titrated with a solution of NBS (0.2 g/L). The end point was reached when a permanent blue colour was observed. For the determination of ascorbic acid in fresh and treated samples, 0.2 g oxalic acid was added, crushed, homogenized and filtered. The samples solution was placed in an Erlenmeyer flask containing 5 mL of KI solution, 2 mL of acetic acid solution, drops of starch solution and 30 mL of distilled water, then titrated with the NBS solution. Each determination was performed in triplicate. Vitamin C content, expressed as mg Vitamin C 100 g⁻¹, was calculated as follows:

$$\text{Vit C} = \left(\frac{2 \times \text{AA} \times B}{T \times M} \right) \times 100 \quad (3)$$

where *T* (mL) is the volume of N-Bromosuccinimide (NBS) of the standard solution of Vitamin C; AA is the concentration of the standard solution of ascorbic acid (mg mL⁻¹); *B* (mL) is the volume of NBS corresponding to the sample and *M* (g) is the sample mass.

3. Statistical analysis

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 mean values 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 of the parameters.

4. Results and discussion

4.1. Physico-chemical characterization

Table 1 shows the mean values and standard deviations of the moisture content, water activity, soluble solids, % acidity and pH for both fresh and pressurized samples. A significant decrease in the moisture content relative to the untreated sample was observed for the treated samples at different pressures ($P < 0.05$). An increase in the soluble solids content was observed for the treated samples at different pressures ($P < 0.05$). In addition, some of the osmotic syrup may not actively migrate into the cells but may simply penetrate into the intercellular spaces of tissue (Falade & Igbeka, 2007).

The water activity (a_w) of the untreated sample showed a significant decrease when compared to samples at different pressures in the whole range of pressure ($P < 0.05$). Using the Multiple Range Test (MRT) included in the statistical program, two homogenous groups were observed (0.1 and 100, 200, 300, 400 and 500 MPa). Cruz-Romero, Kelly, and Kerry (2007) observed changes in oyster's water activity treated with high pressures; however, the parameter did not show statistical differences. Moreno et al. (2011) working with ohmic heating and vacuum impregnation of osmotic dehydrated strawberry reported that a_w decreases in treated samples compared to control samples. In the same table, values of pH showed significant differences ($P < 0.05$) between the control and the pressurized samples in the range of 3.27 ± 0.05 – 3.48 ± 0.02 . Three homogenous groups were observed (0.1–100–300; 200–300–500 and 200–400–500 MPa). Regarding to acidity, significant differences were observed between treated and control samples ($P < 0.05$) in the range of 0.91 ± 0.01 – $1.40 \pm 0.07\%$. Four homogenous groups were observed (0.1; 100 MPa; 200–400 MPa; 300–500 MPa). Landl et al. (2010) reported similar results working with high hydrostatic pressure on the quality of acidified granny smith apple puree product.

4.2. Colour and non-enzymatic browning compounds (NEB)

Colour changes of strawberry associated with chromatic coordinates (L^* , a^* and b^*) as affected by high hydrostatic pressure processing are presented in Fig. 1. The values of the chromatic coordinates for the control samples (L^* , a^* and b^*) were 34.60 ± 0.24 , 34.24 ± 0.20 and 18.64 ± 0.01 , respectively. The redness (a^*) and the yellowness (b^*) should be considered as the physical parameters to describe the visual colour degradation in the samples. From the point of view of chromatic coordinates, there are significant differences between the fresh and the treated samples ($P < 0.05$). HHP-processed samples have significantly lower a^* values as compared to untreated sample ($P < 0.05$). The values b^* decrease with high hydrostatic pressure treatment ($P < 0.05$) compared to untreated sample. All treatments decreased minimally the browning index or lightness (L^*) indicating that fresh strawberry presented a darker colour compared to samples treated with

Table 1
Physicochemical characterization of fresh and pressurized strawberries during osmotic dehydration.

Treatment pressure (MPa)	Moisture (g 100 g ⁻¹)	Soluble solids (°Brix)	pH	Acidity (g citric 100 g ⁻¹)	a_w
0.1	90.66 ± 0.02 ^a	7.3 ± 0.6 ^a	3.53 ± 0.01 ^a	1.40 ± 0.07 ^a	0.955 ± 0.002 ^a
100	86.24 ± 0.01 ^{ab}	10.0 ± 0.0 ^b	3.48 ± 0.02 ^a	0.91 ± 0.01 ^b	0.945 ± 0.002 ^b
200	84.11 ± 0.06 ^{ab}	10.8 ± 0.0 ^c	3.30 ± 0.03 ^{bc}	1.00 ± 0.01 ^c	0.945 ± 0.002 ^b
300	83.84 ± 0.05 ^{ab}	11.5 ± 0.0 ^d	3.42 ± 0.04 ^{ab}	1.10 ± 0.02 ^d	0.944 ± 0.001 ^b
400	82.23 ± 0.05 ^b	12.2 ± 0.3 ^e	3.27 ± 0.05 ^c	1.00 ± 0.03 ^c	0.944 ± 0.001 ^b
500	81.81 ± 0.09 ^b	12.3 ± 0.3 ^e	3.34 ± 0.06 ^{bc}	1.09 ± 0.05 ^d	0.947 ± 0.003 ^b

Values are mean ± standard deviation of triplicates. Values followed by the same letter in the same column are not significantly different ($P < 0.05$).

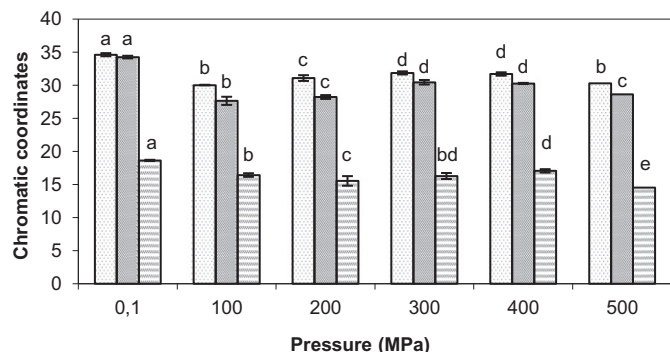


Fig. 1. Effect of osmotic dehydration under high hydrostatic pressure conditions on chromatic coordinates (□ L*, ▤ a*, ▨ b*) of strawberries samples. Bars represent mean \pm standard deviation of triplicates. Identical letters above the bars indicate no significant differences ($P < 0.05$).

osmotic dehydration and high hydrostatic pressure. Some investigation on the stability of colour of fruits demonstrated that insignificant changes occur during HHP but a decrease in colour is reported during storage (Perera et al., 2010).

Fig. 2 shows the total colour changes (ΔE) of processed strawberries as function of process pressure ($P < 0.05$). The ΔE values of strawberry samples decreased with the treatments at 100 and 200 MPa. However, working between 300 and 500 MPa did not show a significant difference in the colour of pressurized samples related to control samples (fresh fruits). The colour of pressurized products depends on the inhibition of browning due to chemical reaction involving enzymes and the stability of the pigments as affected by HHP treatment (Perera et al., 2010). Non-enzymatic browning may result from the condensation of a carbonyl group with amino acids, reactions of sugars and ascorbic acid in the absence of free amino acids (Valdramidis, Cullen, Tiwari & Donnell, 2010). In fruits, the browning is caused mainly by enzymatic oxidation of endogenous phenols into quinines, which then polymerize into brown products (Ding, Chachin, Ueda, & Wang, 2002). Fig. 2 also presents the colour changes of slices of strawberries related to non-enzymatic browning reaction. It can be observed a clear increase in the NEB values as a result of increasing process pressure ($P < 0.05$). The pressures 300, 400 and 500 MPa present the highest browning compared with 0.1, 200 and 300 MPa. Previous works reported different results regarding effect of HHP on colour. Lopez-Malo, Paloue, Barbosa-Canovas, Welte-Chanes, and Swanson (1998) reported that HHP helped to preserve colour in

avocado purée, by preventing browning and facilitating retention of green colour (Krebbes, Matser, Koets, & Van den Berg, 2002). Green beans, HHP-treated at 500 MPa/1 min at ambient temperature, have shown to maintain a more intense bright green colour on the vegetable's surface. Ahmed, Ramaswamy, and Hiremath (2005) observed that there was no visual colour change in mango pulps after high pressure treatments at 100–400 MPa/20 °C/15–30 min. They observed that colour parameters of mango pulps remained constant after high pressure treatment indicating pigment stability.

4.3. Total antioxidant activity (TAA) and total phenolic content (TPC)

The radical scavenging activity was investigated based on high hydrostatic pressure as observed in Fig. 3. It can be seen that this process variable has an important effect on antioxidant activity of the strawberries sample. All treatments showed a decrease in the TAA (231.24 ± 0.03 – $181.73 \pm 0.06 \mu\text{g mL}^{-1}$ sample) when related to the initial value ($P < 0.05$). Samples pressurized at 400 MPa have increased the antioxidant capacities when compared to control samples ($P < 0.05$). Other authors have also reported that high hydrostatic pressure processing either increases or maintains the antioxidant activity of liquid foods. For example, total scavenging activity (DPHH) in aqueous and organic fractions of tomato purée was unaffected by a HHP treatment of 400 MPa at 25 °C for 15 min (Sánchez-Moreno, Plaza, De Ancos, & Cano, 2006). Patras et al. (2009) indicated that HHP-treated samples of strawberry purées at 400 MPa had significantly lower antioxidant capacities when compared to unprocessed samples.

A different trend was observed for the total phenolic content, also shown in Fig. 3, presenting significant differences with control ($P < 0.05$). However, in the case of 100 and 200 MPa, there were not significant differences compared to control samples. The content of total polyphenols was in the range of 252.21 – $337.30 \text{ mg GA } 100 \text{ g}^{-1}$ sample for both the control and the treated samples. This increase in total phenolic content may be related to an increased extractability of some of the antioxidant components following high pressure processing (Patras et al., 2009). Previous works have reported an increase in antioxidant capacity when applying high pressure, ultrasonic and pulsed electric field processes in grape by-products (Corrales, Toepfl, Butz, Knorr, & Tauscher, 2008) and fresh-cut water melon (Oms-Oliu, Odriozola-Serrano, Soliva-Fortuny & Martín-Belloso, 2009).

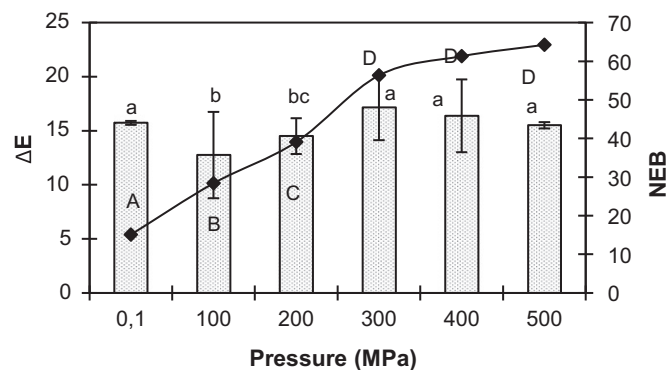


Fig. 2. Effect of osmotic dehydration under high hydrostatic pressure conditions on colour differences (□ ΔE), and non-enzymatic browning (—●— NEB) of strawberries samples. Bars represent mean \pm standard deviation of triplicates. Identical letters above the bars indicate no significant differences ($P < 0.05$).

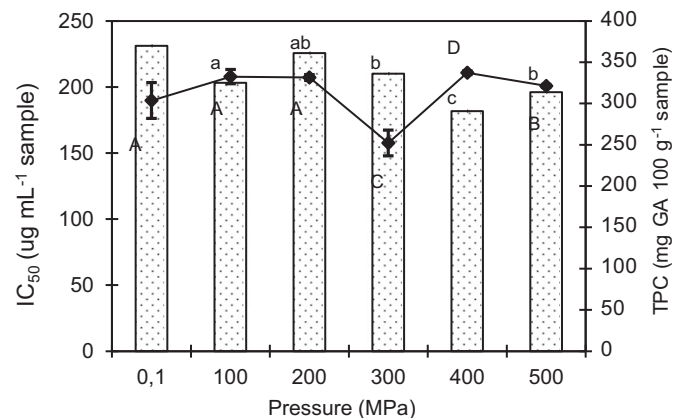


Fig. 3. Effect of osmotic dehydration under high hydrostatic pressure conditions on DPPH free radical scavenging activity (□ IC₅₀) and (—●— TPC) total phenolic contents of strawberries. Bars represent mean \pm standard deviation of triplicates. Identical letters above the bars indicate no significant differences ($P < 0.05$).

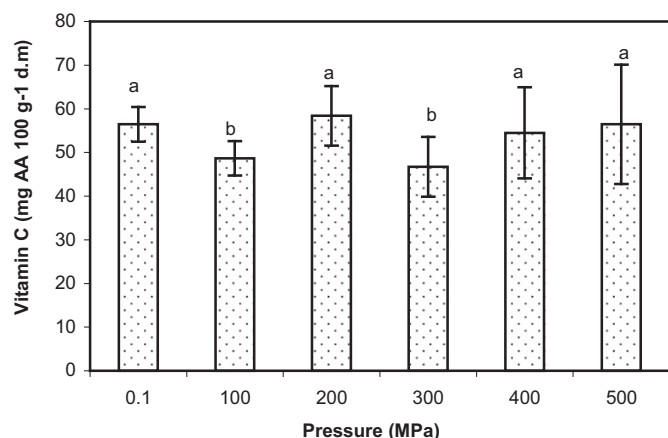


Fig. 4. Effect of osmotic dehydration under high hydrostatic pressure on the vitamin C content of strawberries. Bars represent mean \pm standard deviation of triplicates. Identical letters above the bars indicate no significant differences ($P < 0.05$).

4.4. Vitamin C content (Vit C)

Vitamin C content in control and processed strawberry ranged from 47.09 to 56.37 mg Vit C 100 g⁻¹ sample. These values are in the range of those previously reported by Vocá et al. (2008). Vitamin C, which is thermo-labile, is easily destroyed during processing and storage (Wolbang, Fitos, & Treeby, 2008). Fig. 4 shows the values of vitamin C as function of process pressure. Samples treated at 200, 400 and 500 MPa for 10 min preserved the initial vitamin C content (98% retention). However, levels of vitamin C in samples treated at 100 and 300 MPa for 10 min were significantly lower than in fresh samples ($P < 0.05$). A possible explanation for these differences could be pressure-induced enzyme activation during processing like oxidative reactions by enzymes such as cytochrome oxidase, ascorbic acid oxidase and peroxidase found in fruits, aerobic and non-enzymatic anaerobic reactions (Patras et al., 2009). Similar result reported for Patras et al. (2009) working with strawberries purées indicated that pressurization preserved 94% in the samples. Barba, Esteve, and Frigola (2011) indicated that pressurization preserved 92% in the samples of blueberry juice.

5. Conclusion

Simultaneous application of osmotic dehydration and high hydrostatic pressure on strawberry was investigated in the range from 100 to 500 MPa. When comparing the fresh sample with the pressurized strawberries ($P < 0.05$), results indicated that this combine method is particularly interesting due to the content of bioactive components (antioxidant capacity, phenolic content and vitamin C) in treated samples. Changes in chromatic coordinates due to increasing pressure led to a noticeable modification of surface fruit colour as indicated through the ΔE parameters. Non-enzymatic browning was more pronounced for pressure higher than 200 MPa. The radical scavenging activity showed higher antioxidant activity at 400 MPa rather than at low pressure (100, 200, 300) and 500 MPa. The total phenolic content increased with an increase in the pressure. The vitamin C content did not show changes with increasing pressure. Therefore, the results of this work indicate that the report quality profiles of strawberry osmotically dehydrated under high hydrostatic pressure between 300 and 500 MPa showed minimal differences when compared to untreated samples. Thus, it is recommended working at 400 MPa/10 min to obtain processed strawberries with high levels of both nutritional and antioxidant characteristics.

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