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Assessment of quality parameters and microbial characteristics of Cape gooseberry pulp (*Physalis peruviana* L.) subjected to high hydrostatic pressure treatment

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ABSTRACT

The aim of this investigation was to determine the effect of high hydrostatic pressure (HHP) on dietary fiber, total phenolic (TPC), vitamin B and E contents, antioxidant capacity (AC) and microbiological characteristics of gooseberry pulp immediately after processing (300–400–500 MPa/1–3–5 min) and after 30 days of storage at 4 °C. Initially, treatment at 300 MPa/1 min reduced the microbial counts to non-detectable levels (<1.0 log CFU/g). An increased of soluble dietary fiber was observed for all the treatments compared to control samples. Moreover, a notable increase in B_3 and B_6 contents were observed respect to control samples. After refrigerated storage, the insoluble dietary fiber (IDF) to soluble dietary fiber (SDF) ratios were similar to those presented at Day 0. Although the values of TPC were lower than at Day 0, an increasing tendency due to treatments was observed. Regarding antioxidant capacity, maximum values were observed at 500 MPa/5 min. Retentions higher than 84% for (β+γ)-tocopherols and α-tocopherols were reported. Therefore, HHP technology seems to be a good option for microbiological stabilization of gooseberry pulp, while it may also preserve the most quality this pulp including nutritional, antioxidant and physicochemical aspects.

1. Introduction

The application of high hydrostatic pressure (HHP) in food preservation has received particular attention as a viable alternative (economically and technologically) to thermal processes. This technology has been identified as a method for inactivating microorganisms (Patterson, 2005). Thus, this technology could provide consumers with the complete food

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chemical and microbial safety, to minimize or eliminate the need for chemical additives to preserve the sensory and nutritional quality of fresh ingredients (Mújica-Paz et al., 2011). One of the principal advantages of the HHP process is the improvement of both food shelf-life and safety. The loss of viability of microorganisms through HHP is probably the result of a combination of injuries in the cell (Rendueles et al., 2011).

The Cape gooseberry (*Physalis peruviana* L.) belongs to the Solanaceae family and it is an annual plant of short-lived perennials that can grow to one meter (Ramadan, 2011). It is a good source of vitamins A and C, and some of the vitamin B-complex as well as minerals like phosphorus, iron, potassium and zinc (Salazar et al., 2008; Restrepo, 2008). The main benefits associated with gooseberries are their nutritional composition and bioactive components (Hassanien, 2011). It has considerable functional properties due to its antioxidant content and high levels of vitamin E (Ramadan and Mörsel, 2003). Moreover, the health properties of gooseberry phenolic compounds have been extensively studied from the epidemiological point of view and their functionality depends on their content, intake, and bioavailability (Marin et al., 2002). Bioavailability of these compounds can also be affected by microstructure and binding of phenolic compounds within the food matrix which are directly related to fruit processing (Balasundram et al., 2006).

Therefore, the aim of this investigation was to determine the effect of high hydrostatic pressure on microbiological aspects, dietary fiber, tocopherols, total phenolic content, antioxidant capacity and vitamins B of the gooseberry pulp immediately after processing and after 30 days of storage at 4 °C.

2. Materials and methods

2.1. Sample preparation and high hydrostatic pressure treatment

Cape gooseberry was cultivated and purchased in the region of La Serena city, Chile. The samples were selected to provide a homogenous group based on date of harvest, color, size, and freshness according to visual analysis. Before pressurization, the fruits were pressed and homogenized in a blender (Philips, HR1720, The Netherlands, Amsterdam). The gooseberry pulp was packed in polyethylene flexible pouches, and kept under chilling conditions in a refrigerated room (4 ± 2 °C) until processing.

Pressure treatments were performed in an isostatic pressing system (Avure Inc., Kent, WA, USA), with a cylindrical pressure chamber (length 700 mm, diameter 60 mm). Water was used as the pressurizing medium. A pressure level of 300, 400 and 500 MPa with holding times of 1, 3 and 5 min at room temperature were used. Samples were packed in polyethylene bag then heat-sealed and exposed to high hydrostatic pressure treatment. For each experiment trial, a control sample was prepared. The analyses of the proximal composition as well as quality parameters were performed immediately after processing (Day 0) and after 30 days of storage (Day 30). All experiments were done in triplicate. The HHP treated pulp was stored at 4 ± 2 °C. Microbiological analyses were performed immediately after processing and at intervals for up to 90 days storage.

2.2. Microbiological analysis

The samples were analyzed for mesophilic aerobic microorganisms (MAM) and molds and yeasts (MY) counts. Twenty-five mL or grams of each sample were obtained aseptically and homogenized with a 225 mL peptone saline solution 0.1% (Difco, Detroit, USA) in a filter stomacher bag using a Stomacher® (Biocheck, S.A., Barcelona, Spain) at 240 rpm for 60 s. Further decimal dilutions were made with the same diluent, and duplicates of at least three appropriate dilutions were plated on appropriate media. In order to enumerate the mesophilic aerobic microorganisms, 1 mL of each dilution was pour-plated in Plate Count Agar (PCA, Difco, Detroit, USA). After incubation at 30 °C for 72 h, plates with 30–300 colonies were counted. To count the molds and yeasts, 1 mL of the initial (10–1) dilution was spread on three plates (0.3, 0.3 and 0.4 mL) of Dicloran Rose Bengal Chloramphenicol (DRBC, Difco, Detroit, USA) agar, and 0.1 mL of each subsequent was spread on one DRBC plate. Plates were then incubated at 25 °C for 3–5 days, and plates with 30–300 colonies were counted. Results were transformed into logarithms of the number of colony forming units (log CFU/mL). The detection limit was 10 CFU/mL according to WHO (1999).

2.3. Microbial growth curve modeling

The experimental data obtained were fitted to the reparameterized version of the modified Gompertz equation according to the work of Briones et al. (2010).

$$\log(N_{(t)}) = \log(N_{max})A \cdot \exp\left\{-\exp\left\{\left[\mu_{max} \cdot 2.71822) \cdot \frac{\lambda - SL}{A}\right] + 1\right\}\right\} + A\exp\left\{-\exp\left\{\left[\mu_{max} \cdot 2.71822) \cdot \frac{\lambda - t}{A}\right] + 1\right\}\right\} \quad (1)$$

where $N_{(t)}$ is the viable cell concentration at time t. A is related to the difference decimal logarithm of maximum bacterial growth attained at the stationary phase and decimal logarithm of the initial value of cell concentration, μ_{max} is the maximal specific growth rate, λ is the lag time, N_{max} is the microbial threshold value, SL is the microbiological acceptability limit (i.e., the time at which $N_{(t)}$ is equal to N_{max}), and t is the storage time. The value of N_{max} was set to 5×10^5 CFU/g for mesophilic aerobic microorganisms and of 5×10^3 CFU/g for molds and yeasts. The modified Gompertz equation was fitted to microbiological data using the nonlinear regression toolbox of the GraphPad Prism v. 4.03 (GraphPad Software, Inc., San Diego, CA, USA). The goodness of fit was evaluated using the coefficient of determination (r^2).

2.4. Quality parameters

2.4.1. Determination of tocopherols

The vitamin E content was determined by means of the HPLC-fluorescence method described by Ubaldi et al. (2005). A liquid chromatograph (Shimadzu Instruments, Inc., Shimadzu LC-10 AD) was used for all determinations. Content of α-tocopherol was assessed by means of a fluorescence detector (Shimadzu Instruments, Inc., Shimadzu RF-10 A xL). All measurements were done in triplicate. Vitamin E content was expressed as g/kg total lipids.

2.4.2. Determination of dietary fiber

Samples of cape gooseberry pulp were analyzed for soluble and insoluble dietary fiber fractions according to a gravimetric-enzymatic method (AOAC no 991.43) by using a Total Dietary Fiber Assay Kit (TDF100A; Sigma–Aldrich, Missouri, USA). Briefly, the samples were suspended in MES-TRIS buffer pH 8.2 and digested sequentially with heat stable α-amylase at 95–100 °C, protease at 60 °C, and amyloglucosidase at 60 °C. Enzyme digestates were filtered through tared fritted glass crucibles. Crucibles containing insoluble dietary fiber were rinsed with dilute alcohol followed by acetone, and dried overnight in a 105 °C oven. Filtrates plus washing were mixed with 4 volume of ethanol to precipitate materials that were soluble in the digestate. After allowing standing overnight, precipitates were filtered through tarred fritted glass crucibles. One of each set of duplicate insoluble fiber residues and soluble fiber residues was ashed in a muffle furnace at 525 °C for 5 h. Another set of residues was used to determine protein as Kjeldahl nitrogen (6.25). Total dietary fiber was calculated as the sum of soluble and insoluble dietary fiber, and expressed as g/100 g dry matter (d.m.).

2.4.3. Determination of total phenolic content (TPC)

TPC were determined colorimetrically using the Folin-Ciocalteau reagent (FC) according to previous work with modifications (Chuah et al., 2008). 0.5 mL aliquot of the cape gosseberry pulp extract solution was transferred to a glass tube; 0.5 mL of reactive FC was added after 5 min with 2 mL of Na_2CO_3 solution (200 mg/mL) 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 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™, IL, USA) at 725 nm and compared to a gallic acid equivalent (GAE) calibration curve. Results were expressed as mg GAE/100 g d.m. All reagents were purchased from Merck (Merck KGaA, Darmstadt, Germany). All measurements were done in triplicate.

2.4.4. Antioxidant capacity

Radical scavenging assay (DPPH): the antioxidant capacity determined by DPPH assay followed the procedure described by Turkmen et al. (2005) with some modifications. Different dilutions of the extracts were prepared in triplicate. An aliquot of 3.9 mL of 0.15 mM DPPH radical in methanol was added to a test tube with 0.1 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™, IL, USA). 80% (v/v) methanol was used to calibrate the spectrophotometer. Calibration curves were made for each assay using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). The results were expressed as μmoles of Trolox equivalents (TE)/100 g d.m. All measurements were done in triplicate.

Ferric reducing/antioxidant power assay (FRAP): the antioxidant activity of the extracts was determined by a modified method of FRAP assay according to Benzie and Strain (1999). To prepare the FRAP reagent, a mixture of 0.1 mol/L acetate buffer (pH 3.6), 10 mmol/L 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), and 20 mmol/L ferric chloride (10:1:1, v:v:v) was made. Then, 1.8 mL reagent, 0.18 mL water and 0.06 mL sample were added. The reaction mixture was incubated at 37 °C for 120 min. The increase in absorbance was monitored at 593 nm vs. a blank. FRAP reagent was freshly made up each measuring day and the blank consisted in 1.8 mL reagent 0.18 mL water and 0.06 mL methanol (80%, v/v). The final absorbance of each sample was compared with those obtained from the standard curve made from Trolox equivalents (TE) and the results were expressed as μmol TE/100 g d.m. All measurements were done in triplicate.

2.4.5. Determinations of vitamins B

Water-soluble vitamins were extracted by a combination of acid and enzymatic hydrolysis according to the method of Tayade et al. (2013) with some modifications. Gooseberry pulp (10 g) was suspended in 10 mL of 0.1 N HCl and then treated at 100 °C for 20 min. It was then allowed to cool and the pH was adjusted to 4.0 with 2 N sodium acetate. After that 5 ml of 2% Clara-diastase suspension was added to the sample and it was submitted to enzymatic digestion for 18 h at 37 °C. Then, it was cooled to ambient temperature and centrifuged by 15 min to 5000 rpm, followed by filtered through a 0.45 μm membrane filters and 10 μL injected for HPLC analysis.

Water-soluble vitamins (B_1, B_2, B_3 and B_6) detection and quantification were performed using the Agilent 1200 Series with Zorbax Eclipse XDB-C18, 4.6 × 150 mm, 5 μm reversed-phase column, a mobile phase containing 10 mM phosphate di-sodium buffer (pH 2.6) and 5 mM sodium 1-hexanesulfonate/acetonitrile (9:1, v/v). All measurements were done at 40 °C with a flow-rate of 0.7 ml/min, isocratic elution and detection at 210 and 285 nm. A high-performance liquid chromatography system, equipped with a high pressure pump; automatic injector; a UV–visible diode array detector and controlled by ChemStation software were used for the analysis. Vitamins contents were expressed as mg/100 g d.m. All measurements were done in triplicate.

2.5. Statistical analysis

Analysis of variance (ANOVA; Statgraphics Plus® 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 (MRT), included in the statistical program, was used to prove the existence of homogeneous groups within each of the parameters analyzed.

3. Results and discussion

3.1. Effect of HHP on microbiological behavior

The mean initial populations of total aerobic mesophilic microorganisms (AMM) and molds and yeasts (M&Y) in untreated (control) gooseberry pulp were 2.20 and 1.59 log CFU/g, respectively (Figs. 1 and 2). Immediately after HHP treatment, the AMM count and the M&Y count were significantly reduced ($p < 0.05$). Thus, HHP-treatment at 300 MPa/1 min reduced the microbial counts to non-detectable levels (<1.0 log CFU/g), reaching reductions of 1.59–2.20 log units (Fig. 1). As expected, further increase in the pressure-holding time (300 MPa/3 and 5 min) or pressure (400 MPa/1, 3 and 5 min) resulted in similar values. In fact, the initial populations of natural microbiota in gooseberry pulp were not sufficiently large to evaluate the extent of log reductions

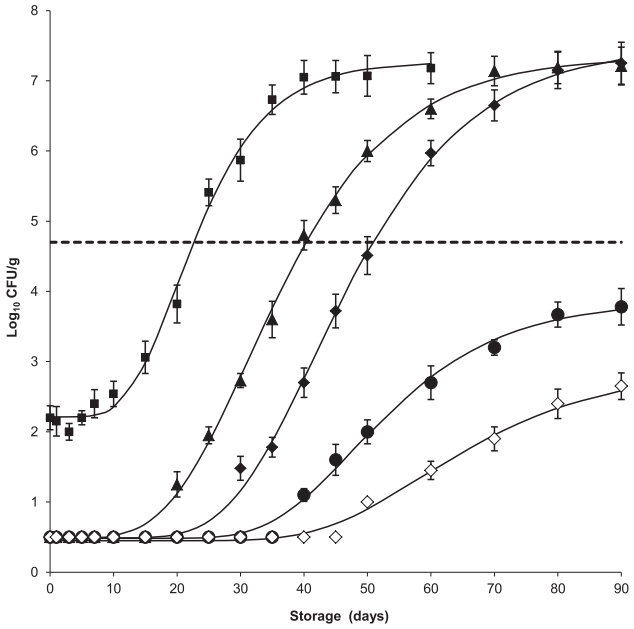

Fig. 1 – Growth curve of mesophilic microorganisms in untreated and HHP-treated Physalis during storage at 4 °C for 90 days. Symbols and lines represent observed and modeled (re-parameterized version of the Gompertz equation) values respectively. Symbols are means of three measurements ± sd. Control (■), 300 MPa/1 min (▲), 300 MPa/3 min (♦), 300 MPa/5 min (●), 400 MPa/1 min (◊). Data for treatments at 400 MPa/3 min and 400 MPa/5 min are not presented because both microbial counts were not detected during the whole storage period. The dotted line shows upper acceptable limit (5×10^4 CFU/g = 4.70 log CFU/g).

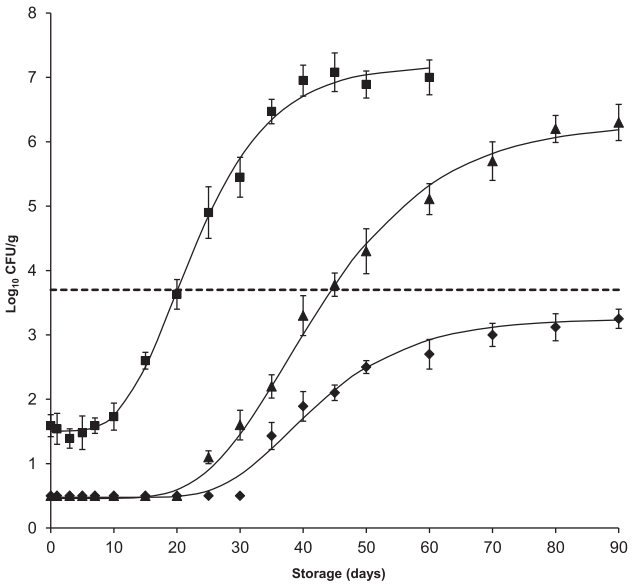

Fig. 2 – Growth curve of molds and yeasts in untreated and HHP-treated Physalis during storage at 4 °C for 90 days. Symbols and lines represent observed and modeled (re-parameterized version of the Gompertz equation) values respectively. Symbols are means of three measurements ± sd. Control (■), 300 MPa/1 min (▲), 300 MPa/3 min (♦). Data for treatments between 300 MPa/5 min and 400 MPa/5 min are not presented because both microbial counts were not detected during the whole storage period. The dotted line shows upper acceptable limit (5×10^3 CFU/g = 3.70 log CFU/g).

that resulted from pressures applied at or above 300 MPa for 3 min.

Other studies have also reported that HHP treatment reduced the natural microbiota of fruit and vegetable products to non-detectable levels immediately after pressurization. In mango pulp, pressure treatment at 483 or 552 MPa/2 s reduced the microbial load to below the detection limit (Guerrero-Beltrán et al., 2006). Similar result was reported by Liu et al. (2014), showing that yeasts, molds and bacterial counts in mango pulp were reduced to <10 CFU/g after HHP treatments at 300 MPa/15 min, 400 MPa/5 min, 500 MPa/2.5 min and 600 MPa/1 min. Reyes et al. (2012) also reported the reduction of mesophilic/psychrophilic bacteria and yeasts counts to non-detectable levels in Aloe vera gel by applying a pressure of 300 MPa/3 min, 400 MPa/1 and 3 min, and 500 MPa/1 and 3 min. The significant impact exerted by HHP treatment on natural microbiota of gooseberry pulp could be attributed to low initial microbial load, as well as to the microbiota composition or types of microorganisms present. In fact, the natural microbiota present in gooseberry pulp was dominated exclusively by Gram-negative bacteria and yeasts (data not shown), which are generally pressure-sensitive (Patterson, 2005).

Fig. 1 shows the growth curves of AMM of untreated and HHP-treated gooseberry pulp stored at 4 °C for a maximum storage period of 90 days. As it can be seen, the AMM count in untreated samples increased exponentially after a lag phase of around 12 days, and after 22 days of storage reached counts greater than 4.70 log CFU/g (5.0×10^4 CFU/g), which is the upper acceptable limit established by Chilean legislation for fruit pulps (MINSAL, 2010). For treated samples at 300 MPa/1 and 300 MPa/3 min, the upper acceptable limit was exceeded around the day 40th and 51th, respectively. The upper acceptable limit for samples treated at 300 MPa/5 min and 400 MPa/1 min was no exceeded, and was found to be <4.0 log CFU/g at the end of storage period. Moreover, pressurized samples at 400 MPa for 3 and 5 min, AMM count remained below the detection limit (<1.0 log CFU/g) during the whole storage period.

On the other hand, the M&Y counts in untreated samples exceeded the upper acceptable limit of 3.70 log CFU/g (5.0×10^3 CFU/g) established by Chilean legislation around the day 20th, and were found to be >7.0 log CFU/g after 45 days of storage (Fig. 2). For treated samples at 300 MPa/1, the upper acceptable limit was exceeded about the day 44th of storage. The upper acceptable limit for samples treated at 300 MPa/3 min was no exceeded, and was found to be <3.0 log CFU/g at the end of storage period. In contrast, M&Y were not detected after pressure treatment at or above 300 MPa/5 min, and the survivors were kept below the detection limit (<1.0 log CFU/g) during the whole storage period.

Table 1 summarizes the estimated kinetic parameters for maximum specific growth rate (μ_{max}), lag phase (λ), and shelf life (SL) for AMM and M&Y in gooseberry pulp samples stored at 4 °C for 90 days. The re-parameterized version of the Gompertz equation was able to describe the microbial growth, since determination coefficients ($r^2 \geq 0.98$) were found. However, Gompertz parameters for AMM of processed samples at 400 MPa/3 and 5 min, as well as for M&Y on treated samples at or above 300 MPa/5 min were not estimated since microbial growth was not detected throughout storage time. As expected, estimated microbial growth kinetic parameters, including shelf life were affected by HHP treatment.

Table 1 – The effect of HHP treatment on kinetic parameters of mesophilic microorganisms and molds and yeasts in gooseberry pulp stored at 4 °C.

Treatments	Kinetic parameter values					
	Mesophilic microorganisms			Molds and yeasts		
	λ	μ_{max}	SL	λ	μ_{max}	SL
Control	12.12 ± 2.25^{a}	0.24 ± 0.03^{a}	22.59 ± 1.21^{a}	11.24 ± 1.89^{a}	0.25 ± 0.03^{a}	20.04 ± 0.85^{a}
300 MPa/1 min	18.27 ± 1.27^{b}	0.19 ± 0.01^{b}	40.28 ± 1.92^{b}	24.31 ± 2.45^{b}	0.16 ± 0.02^{b}	44.45 ± 1.30^{b}
300 MPa/3 min	27.97 ± 1.44^{c}	0.19 ± 0.02^{b}	50.79 ± 1.46^{c}	27.08 ± 2.23^{b}	0.12 ± 0.03^{b}	$>90^{c}$
300 MPa/5 min	34.61 ± 2.13^{d}	0.1 ± 0.01^{c}	$>90^{d}$	N.D^{c}	N.D^{c}	$>90^{c}$
400 MPa/1 min	42.42 ± 2.71^{e}	0.06 ± 0.01^{d}	$>90^{d}$	N.D^{c}	N.D^{c}	$>90^{c}$
400 MPa/3 min	N.D^{f}	N.D^{e}	$>90^{d}$	N.D^{c}	N.D^{c}	$>90^{c}$
400 MPa/5 min	N.D^{f}	N.D^{e}	$>90^{d}$	N.D^{c}	N.D^{c}	$>90^{c}$
500 MPa/1 min	N.D^{f}	N.D^{e}	$>90^{d}$	N.D^{c}	N.D^{c}	$>90^{c}$
500 MPa/3 min	N.D^{f}	N.D^{e}	$>90^{d}$	N.D^{c}	N.D^{c}	$>90^{c}$
500 MPa/5 min	N.D^{f}	N.D^{e}	$>90^{d}$	N.D^{c}	N.D^{c}	$>90^{c}$

SL, shelf-life (days); λ, lag phase (days); μ_{max}, maximum specific growth rate (1/days).
[a–f] Values in the same column with different superscripts differ significantly ($p < 0.05$; LSD).
N.D, not determined (parameters were not estimated due to microbial counts were < 1.0 log cfu/g throughout storage.

Table 2 – Effect of pressure on tocopherols of Cape gooseberry pulp for Day 0 and after storage at 4 °C (Day 30).

Treatments	g/kg of total lipids		
	α-Tocopherol	(β+γ)-Tocopherols	δ-Tocopherol
Day 0			
Control	$10.70^{a} \pm 0.28$	$0.77^{a,b,c} \pm 0.03$	nd
300 MPa/1 min	$10.73^{a} \pm 0.21$	$0.79^{b} \pm 0.01$	nd
300 MPa/3 min	$10.57^{a} \pm 0.21$	$0.77^{a,b,c} \pm 0.01$	nd
300 MPa/5 min	$10.57^{a} \pm 0.06$	$0.79^{b,c} \pm 0.01$	nd
400 MPa/1 min	$10.71^{a} \pm 0.01$	$0.74^{a} \pm 0.03$	nd
400 MPa/3 min	$10.15^{b} \pm 0.07$	$0.75^{a} \pm 0.01$	nd
400 MPa/5 min	$9.16^{c} \pm 0.02$	$0.75^{a} \pm 0.01$	nd
500 MPa/1 min	$9.34^{c} \pm 0.12$	$0.66^{d} \pm 0.01$	nd
500 MPa/3 min	$9.20^{c} \pm 0.17$	$0.69^{d} \pm 0.01$	nd
500 MPa/5 min	$9.86^{b} \pm 0.06$	$0.76^{a,c} \pm 0.01$	nd
Day 30			
Control	$10.57^{a} \pm 0.32$	$0.69^{a,d} \pm 0.03$	nd
300 MPa/1 min	$10.05^{b} \pm 0.07$	$0.73^{b,c} \pm 0.01$	nd
300 MPa/3 min	$9.96^{b,c} \pm 0.06$	$0.71^{b,c,d} \pm 0.01$	nd
300 MPa/5 min	$10.40^{a} \pm 0.10$	$0.70^{a,b,d} \pm 0.01$	nd
400 MPa/1 min	$9.71^{c} \pm 0.18$	$0.69^{a,d} \pm 0.01$	nd
400 MPa/3 min	$8.94^{d} \pm 0.03$	$0.61^{e} \pm 0.01$	nd
400 MPa/5 min	$9.21^{d} \pm 0.13$	$0.74^{c} \pm 0.02$	nd
500 MPa/1 min	$9.16^{d} \pm 0.06$	$0.68^{a} \pm 0.02$	nd
500 MPa/3 min	$9.12^{d} \pm 0.09$	$0.69^{a,d} \pm 0.03$	nd
500 MPa/5 min	$8.90^{d} \pm 0.09$	$0.68^{a} \pm 0.02$	nd

nd, shows non-detected value or no peak found. Values are mean ± sd ($n = 3$).
[a–d] Different letters in the same column indicate significant differences ($p < 0.05$).

Extension of λ and reduction of μ_{max} in HHP-processed gooseberry pulp were observed compared with untreated samples. For example, λ of AMM was extended around 18 days in pressurized samples at 300 MPa/1 min, which was significantly higher ($p < 0.05$) than that estimated in control samples (12 days); whereas, μ_{max} decreased from 0.24 to 0.19 1/days. The λ and μ_{max} for M&Y showed a similar behavior to AMM. An extension of λ and decrease in μ_{max} of microbial populations as a consequence of HHP treatment has also been reported by other researchers (Briones et al., 2010; Reyes et al., 2012; Slongo et al., 2009). This effect may be attributed to more severe cell damage and the increase of injured bacteria, which delayed the onset of microbial growth of sublethally, damaged populations during refrigerated storage (Briones et al., 2010; Cruz-Romero et al., 2008; Reyes et al., 2012).

HHP-treatments also significantly ($p < 0.05$) increased the microbiological shelf-life (SL) of gooseberry pulp stored at 4 °C (Table 1). Microbiological shelf life, based on AMM count (4.7 log CFU/g) and M&Y count (3.7 log CFU/g), estimated in the present study for unpressurized gooseberry pulp stored at 4 °C was around 23 and 20 days, respectively; whereas for pressure treated samples at 300 MPa/1 and 3 min ranged between around 40 and 51 days. In contrast, HHP treatment at 300 MPa/5 min and 400 MPa/1 min were able to keep the AMM count under the upper limit of acceptability and the M&Y count below the detection limit (<1.0 log CFU/g) during the entire storage time, thus showing a shelf-life of at least 90 days. Moreover, HHP treatment at 400 MPa/3 and 5 min kept levels of both microbial counts below the detection limit throughout the cold storage period

Table 3 – Effect of HHP on insoluble dietary fiber (IDF), soluble dietary fiber (SDF) and total dietary fiber (TDF) of Cape gooseberry pulp immediately after HHP (Day 0) and after storage at 4 °C (Day 30).

	g/100 g d.m.		
Treatments	IDF	SDF	TDF
Day 0			
Control	$35.86^{a} \pm 0.53$	$2.59^{a,d} \pm 0.10$	$38.45^{a} \pm 0.62$
300 MPa/1 min	$36.03^{a} \pm 0.45$	$2.66^{a,d} \pm 0.49$	$38.69^{a} \pm 0.93$
300 MPa/3 min	$32.12^{b} \pm 1.22$	$1.85^{b,c} \pm 0.29$	$33.97^{b} \pm 0.93$
300 MPa/5 min	$36.84^{a} \pm 0.63$	$1.59^{b} \pm 0.04$	$38.43^{a} \pm 0.67$
400 MPa/1 min	$36.84^{a} \pm 2.62$	$2.19^{a,c} \pm 0.03$	$39.03^{a} \pm 2.65$
400 MPa/3 min	$44.09^{c} \pm 0.62$	$3.03^{d} \pm 0.16$	$47.13^{c} \pm 0.78$
400 MPa/5 min	$44.39^{c} \pm 0.32$	$3.79^{e} \pm 0.42$	$48.18^{c} \pm 0.10$
500 MPa/1 min	$44.07^{c} \pm 1.04$	$5.02^{f} \pm 0.11$	$49.09^{c} \pm 1.15$
500 MPa/3 min	$44.19^{c} \pm 0.68$	$4.73^{f} \pm 0.04$	$48.92^{c} \pm 0.72$
500 MPa/5 min	$51.98^{d} \pm 0.83$	$7.37^{g} \pm 0.02$	$59.35^{d} \pm 0.84$
Day 30			
Control	$33.57^{a,b,c} \pm 0.21$	$2.14^{a} \pm 0.02$	$35.71^{a,b,c} \pm 0.19$
300 MPa/1 min	$34.62^{b} \pm 0.63$	$2.19^{a} \pm 0.26$	$36.81^{b,e} \pm 0.37$
300 MPa/3 min	$33.71^{a,b,c} \pm 0.17$	$2.27^{a} \pm 0.21$	$35.98^{b,c,e} \pm 0.03$
300 MPa/5 min	$32.07^{d} \pm 0.42$	$3.59^{b} \pm 0.19$	$35.66^{a,c,d} \pm 0.23$
400 MPa/1 min	$33.42^{a,c} \pm 0.84$	$1.98^{a} \pm 0.01$	$35.40^{a,c,d} \pm 0.85$
400 MPa/3 min	$32.69^{a,d} \pm 0.02$	$1.86^{a} \pm 0.16$	$34.55^{d} \pm 0.14$
400 MPa/5 min	$30.92^{e} \pm 0.55$	$4.10^{c} \pm 0.02$	$35.02^{a,c,d} \pm 0.52$
500 MPa/1 min	$32.03^{d} \pm 0.82$	$4.93^{d} \pm 0.32$	$36.96^{e} \pm 1.14$
500 MPa/3 min	$30.18^{e} \pm 0.05$	$4.58^{b} \pm 0.16$	$34.76^{a,d} \pm 0.10$
500 MPa/5 min	$34.29^{b,c} \pm 0.08$	$3.99^{d} \pm 0.23$	$38.27^{f} \pm 0.15$

Values are mean ± sd ($n = 3$).
[a–g] Different letters in the same column indicate significant differences ($p < 0.05$).

and, as a result, the shelf life was extended for more than 90 days.

Other researchers reported the benefits of high pressure treatment on extending the microbiological shelf life of fruit products, including peach purée (Guerrero-Beltrán et al., 2005), mango purée (Guerrero-Beltrán et al., 2006), acidified apple purée (Landl et al., 2010), nectarine purée (García-Parra et al., 2001), kiwi purée (Fernández-Sestelo et al., 2013), pomegranate arils (Ríos-Romero et al., 2012), cashew apple juice (Lavinas et al., 2008) and pomegranate juice (Varela-Santos et al., 2012). The efficacy of HHP processing to inactivate endogenous microorganisms present in gooseberry pulp and extend its microbiological shelf life during the storage at 4 °C could be attributed probably to its low pH and/or presence of antimicrobial compounds such as whitanolides (Ramadan, 2011), which can act synergistically with high pressure leading to increased microbial inactivation. It is known that most of the microorganisms are more susceptible to pressure at low pH values and the survival of sublethally damaged cells is less during subsequent storage in acidic environment (Bayindirli et al., 2006; Garcia-Graells et al., 1998). Likewise, the synergistic effect of high pressure and antimicrobial compounds that occurs naturally in food has been reported by Garcia-Graells et al. (2003) and Patterson et al. (2012). In addition, the effect of refrigerated storage should also be considered, given that HHP-treatment delayed the onset of the microbial growth phase and so contributed to the shelf-life extension (Briones et al., 2010; Cruz-Romero et al., 2008; Reyes et al., 2012).

3.2. *Effect on tocopherols*

Table 2 presents the tocopherols composition of untreated and pressurized pulp at Day 0 and after 30 days of refrigerated storage. The tocopherols identified were the α-tocoferol and β+γ-tocopherols. The α-tocopherol was the major tocopherol for all samples and its value for the control sample was 10.70 ± 0.28 g/kg. The compound (β+γ-tocopherol) was observed in all samples; however δ-tocoferol was not detected in any of the samples. Regarding α-tocopherol, treatments above 400 MPa/3 min presented significant differences with control samples ($p < 0.05$) at Day 0. However, (β+γ)-tocopherols content showed no significance differences between treatments except for 500 MPa/1 and 3 min ($p < 0.05$).

After 30 days of storage, evidence of a decrease in both components was detected at different process conditions, however, high retentions were observed, for example: 84% (500 MPa/5 min) and 87% (400 MPa/3 min) for α-tocopherol and (β+γ)-tocopherols, respectively.

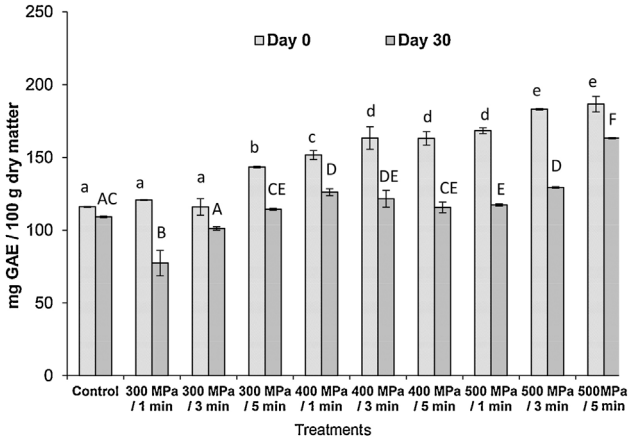

Fig. 3 – Effect of HHP on total phenolic contents of gooseberries pulp. Bars represent mean ± sd of triplicates. Identical letters above the bars indicate no significant differences ($p < 0.05$).

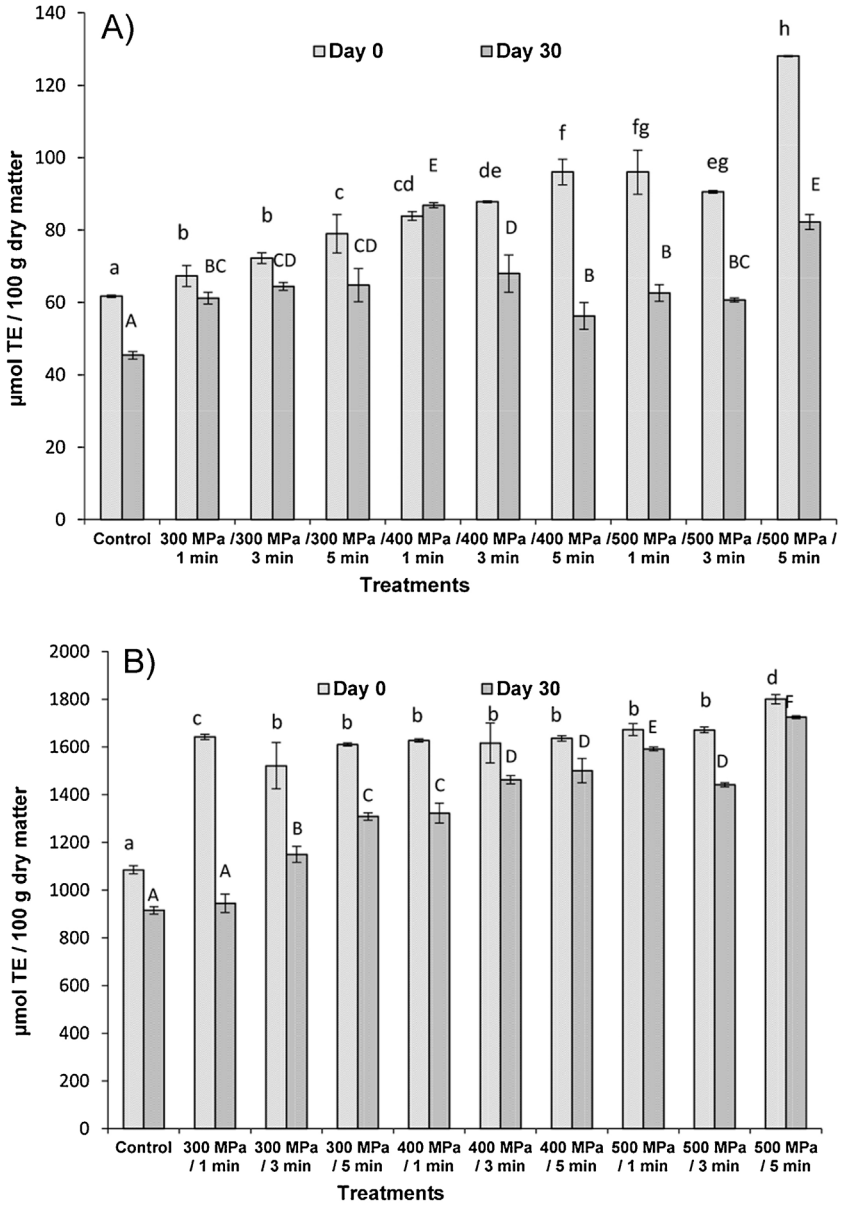

Fig. 4 – Effect of HHP on antioxidant capacity of gooseberries pulp by means of (A) DPPH and (B) FRAP analysis. Bars represent mean ± sd of triplicates. Identical letters above the bars indicate no significant differences ($p < 0.05$).

There is scarce information about the effect of HHP on the stability of tocopherols in different food matrix, especially in fruit pulp. However, controversial reports were informed. For example: Barba et al. (2012) reported an increase of total tocopherol content in orange juice with milk treated at 100–400 MPa/15–30 °C/9 min, mainly due to an increase in α-tocoferol content, in particular at 200–300 MPa. On the other hand, a significative decrease was observed on milk tocopherol content when applying 100 MPa, while an increase was detected when processing at 200–400 MPa. Moltó-Puigmartí et al. (2011) did not found significative changes in the total tocopherol content when applying 400–600 MPa/22–27 °C/5 min in human milk.

3.3. *Effect on dietary fiber*

Table 3 presents the soluble dietary fiber (SDF), insoluble dietary fiber (IDF) and total dietary fiber (TDF) of untreated and pressurized samples. An increase in SDF was observed at Day 0 for all the treatments compared to control samples ($p < 0.05$), except for treatments at 300 MPa/3 MPa/5 min and 400 MPa/1 min. IDF followed the same pattern of TDF ($p < 0.05$). The insoluble dietary fiber to soluble dietary fiber ratios were in the range of 32–52. In terms of health benefits, both kind of fiber complement each other. It is well documented that dietary fiber is involved in disease prevention and enhanced health of consumers (Foschia et al., 2013). Specifically, SDF and IDF exhibit unique components, structures, and physiological effects. SDFs have been linked to the reduction of cholesterol in blood and the decrease in the intestinal absorption of glucose, whereas IDFs are associated with water absorption and intestinal regulation (Im et al., 2014).

After refrigerated storage, the insoluble dietary fiber to soluble dietary fiber ratios were in the range of 30–34, similar to those presented al Day 0, indicating that although some variations were observed in IDF and SDF during storage ($p < 0.05$), the relation between them were still high. Comparable results were reported by previous investigations (Wennberg and Nyman, 2004). Moreover, the high IDF content indicates considerable amounts of celluloses and

hemicelluloses present in the fruit pulp. A high proportion of IDF content could have beneficial health effects related to increases in satiety and in the volume and weight of fecal mass, thus promoting improved functioning of the digestive system (Peerajit et al., 2012).

3.4. Effect on total phenolics content

The total phenolic content was investigated based on high hydrostatic pressure treatments as observed in Fig. 3. At Day 0, the initial TPC was 116.04 ± 0.32 mg GAE/100 g d.m. while pressurized samples changed in the ranged 120.63 ± 0.23–186.65 ± 5.28 mg GAE/100 g d.m. Comparable values were reported by Repo de Carrasco and Encina Zelada (2008). When comparing control samples with treated samples, an increased was observed with exception of samples treated at 300 MPa/1 and 3 min ($p < 0.05$). Comparable results were informed in previous investigations working with apricot nectars apricot nectars, carrot and tomato puree, and cashew apple (Huang et al., 2013; Patras et al., 2009; Queiroz et al., 2010; Ferrari et al., 2010; Nuñez-Mancilla et al., 2013). Effect of HHP on TPC could be attributed to an increase in extractability of some of the antioxidant components following high pressure processing (Patras et al., 2009).

When analyzing the values of TPC after 30 days of storage, the content of control juice decreased compared to the correspondent value at Day 0. Similar results were reported by Klimczak et al. (2007) working with pressurized orange juice after 4 months of storage and by Piljac-Žegarac et al. (2009) after 13 days of storage at 4 °C working with fruit juices. Regarding treated samples, there were significant differences between control and pressurized samples ($p < 0.05$), at each pressure/time condition. An increasing tendency was observed at Day 0 and Day 30. Comparable results were reported by Zhou et al. (2014) and Vega-Galvez et al. (2012) working with pumpkin and Aloe vera, respectively.

3.5. Effect on antioxidant capacity

Antioxidant activity depends on the sample characteristics and the extraction method applied (Barba et al., 2010). Fig. 4 shows the antioxidant capacity of fresh and pressurized pulp samples determined by means of two performed analyses (DPPH and FRAP). Fresh samples presented values of 61.71 and 1084.9 μmol TE/g d.m. for DPPH and FRAP, respectively. When analyzing the immediate effect of HHP (Day 0) a clear increasing tendency was observed for all treatments with respect to control sample when applying DPPH analysis ($p < 0.05$). Moreover, both DPPH and FRAP analysis showed a maximum of antioxidant capacity at 500 MPa/5 min. This increase could be associated to changes in food matrix due to more intensive treatments (pressure and time), loss of cellular wall resulting in liberation of biocompounds with antioxidant activity (Briones-Labarca et al., 2011; McInerney et al., 2007).

Regarding samples after storage, control samples decreased their antioxidant capacity respect to Day 0. Comparable results were reported by Piljac-Žegarac et al. (2009) by observing an increased of the antioxidant activity of fruit juices during the first two days but a notable decrease after 29 days of storage. Pressurized samples did not present a clear trend when applying DPPH, showing 400 MPa/1 min and 500 MPa/5 min the highest values of antioxidant capacity. However, when analyzing the samples tested by means of FRAP analysis, a maximum value of antioxidant capacity was observed at 500 MPa/5 min (1800.4 μmol TE/g d.m.). Published results are dependant of process conditions, antioxidant test applied and food matrix. For example, Wang et al. (2012) reported loss of antioxidant capacity in potato juice at 400, 500 and 600 MPa during 10, 5 and 2.5 min working with DPPH and FRAP. Barba et al. (2013) informed similar results of antioxidant capacity of treated samples respect to control samples of blueberries juice working at 200, 400 and 600 MPa/5, 9 and 15 min. However, they observed a decreased of antioxidant capacity at 400 MPa and 600 MPa/15 min; and Plaza et al. (2006) reported a decrease of antioxidant capacity during storage processing orange juice at 400 MPa/1 min/40 °C.

Table 4 – Changes in vitamins B of Cape gooseberry pulp immediately after HHP (Day 0) and after storage at 4 °C (Day 30).

Treatments	B_3 (niacin)	B_6 (pyridoxine)
Day 0		
Control	$26.59^{a} \pm 0.94$	$24.80^{ab} \pm 0.21$
300 MPa/1 min	$36.38^{b} \pm 1.06$	$25.88^{b} \pm 0.45$
300 MPa/3 min	$35.10^{bc} \pm 1.37$	$25.21^{b} \pm 0.59$
300 MPa/5 min	$35.22^{bc} \pm 1.88$	$23.82^{a} \pm 0.64$
400 MPa/1 min	$36.11^{b} \pm 1.46$	$24.87^{ab} \pm 0.69$
400 MPa/3 min	$33.56^{cd} \pm 0.48$	$27.79^{c} \pm 0.36$
400 MPa/5 min	$33.28^{cd} \pm 1.42$	$28.47^{c} \pm 0.63$
500 MPa/1 min	$33.21^{cd} \pm 1.25$	$28.72^{c} \pm 0.30$
500 MPa/3 min	$31.54^{d} \pm 1.24$	$32.13^{d} \pm 0.79$
500 MPa/5 min	$45.48^{e} \pm 0.21$	$32.68^{d} \pm 0.62$
Day 30		
Control	$23.46^{a} \pm 0.84$	$27.84^{ad} \pm 0.51$
300 MPa/1 min	$18.46^{b} \pm 0.46$	$31.14^{b} \pm 0.81$
300 MPa/3 min	$20.09^{c} \pm 0.35$	$32.72^{c} \pm 0.49$
300 MPa/5 min	$20.55^{c} \pm 0.01$	$33.23^{c} \pm 0.56$
400 MPa/1 min	$19.16^{bc} \pm 0.21$	$30.69^{b} \pm 1.38$
400 MPa/3 min	$19.98^{c} \pm 0.96$	$28.96^{d} \pm 0.44$
400 MPa/5 min	$20.46^{c} \pm 1.08$	$25.75^{e} \pm 0.47$
500 MPa/1 min	$25.22^{de} \pm 0.30$	$27.06^{a} \pm 1.19$
500 MPa/3 min	$26.43^{d} \pm 0.40$	$27.22^{a} \pm 0.74$
500 MPa/5 min	$24.79^{e} \pm 0.25$	$27.03^{a} \pm 0.24$

Values are mean ± sd ($n = 3$).
[a–e] Different letters in the same column indicate significant differences ($p < 0.05$).

3.6. Effect on vitamins B

Table 4 presents the vitamin B contents (B_3 and B_6) for untreated samples and pressurized samples of gooseberry pulps. Vitamins B_1 and B_2 were not detected during analysis. Similar results were reported by Oey et al. (2008). Regarding vitamins B_3 and B_6, their initial contents were 26.59 ± 0.94 mg/100 g d.m. and 24.80 ± 0.21 mg/100 g d.m., respectively. Vitamin B_6 is also called pyridoxine and B_3 called niacin help the body convert food (carbohydrates) into fuel (glucose), which is used to produce energy. These B vitamins, often referred to as B complex vitamins, also help the body metabolize fats and protein. B complex vitamins are needed for healthy skin, hair, eyes, and liver. They also help the nervous system function properly (Chiang et al., 2005). After immediate application of HHP, a notable increase in B_3 and B_6 was observed respect to control samples ($p < 0.05$). The treatment of 500 MPa/5 min showed the maximum value of these vitamins (45.48 and 32.68 mg/100 g d.m. for B_3 and B_6, respectively). Regarding B_6, the treatment

at 400 MPa/5 min showed the lower retention of this vitamin. Sancho et al. (1999) reported that at 600 MPa/30 min/20 °C significative differences were not found in B_1 and B_6 with respect to control samples of strawberry juices. No changes in vitamin B_1, B_2, B_3 and B_6 contents were observed after pressurizing orange juice (Donsì et al., 1996). The fact that these water soluble vitamins were treated by a non-thermal treatment minimized the deleterious effect of certain thermal treatments on these chemical compounds (Oms-Oliu et al., 2012).

Regarding storage time, after 30 days of storage at 4 °C treatments between 300 MPa/1 min and 400 MPa/5 min showed a decreased in B_3, however, a maximum was presented at 500 MPa/5 min ($p < 0.05$). Vitamin B_6 presented retention higher than 97% compared to control samples at Day 30. There is scarce information about the effect of HHP on vitamins B after storage time. Sancho et al. (1999) also reported that vitamin B_6 retention was high after 30 days of storage working with strawberry "coulis" at 200–600 MPa/30 min.

4. Conclusions

The impact of high pressure processing on microbiological and quality parameters of Cape gooseberry pulp was evaluated immediately after processing and after 30 days of storage at 4 °C. Microbiological data was successfully fitted to the re-parameterized version of the modified Gompertz equation. Based on results, HHP-treatments applied over 300 MPa/5 min were sufficient to keep microbial populations investigated below the detection limit during the whole storage period. Therefore, these treatments extended the microbiological shelf-life of pulp for more than 90 days at 4 °C. The α-tocopherol was the major tocopherol for all samples. Treatments above 400 MPa/3 min presented significant differences with control samples at Day 0. An increased in TDF was observed at Day 0 for all the treatments compared to control samples. TPC showed a different trend, presenting an increased over 300 MPa/5 min. The highest antioxidant capacity including DPPH and FRAP assays between pressurized samples was observed at 500 MPa/5 min and a notable increase in vitamins B_3 and B_6 contents were observed respect to control samples at Day 0. After refrigerated storage, the insoluble dietary fibers to soluble dietary fiber ratios were in the range of 34–38, similar to those presented al Day 0. These results may contribute to a better assessment of the benefits of high pressure processing to preserve and enhance the quality of bioactive compounds as well as to improve the pulp stability during storage in the scenario of functional food products.

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