IMPACT OF HIGH HYDROSTATIC PRESSURE ON PHYSICOCHEMICAL CHARACTERISTICS, NUTRITIONAL CONTENT AND FUNCTIONAL PROPERTIES OF CAPE GOOSEBERRY PULP (*PHYSALIS PERUVIANA* L.)

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ABSTRACT

The aim of this research was to assess the effect of high hydrostatic pressure (HHP) treatments (300, 400 and 500 MPa/5 min) on physicochemical, nutritional and functional properties of Cape gooseberry pulp immediately after processing and after 30 days of refrigerated storage. Results showed that HHP as well as storage time clearly influenced sugars, vitamin C, β-carotene, mineral contents and antioxidant capacity. However, the physicochemical characteristics were not influenced by HHP. Pressurization led to a significant increase in fructose and glucose at 300–500 MPa both on day 0 and day 30 ($P < 0.05$). Potassium content increased 8–10% at 300 and 500 MPa, while phosphorus content increased 65% compared with control sample at 400 MPa at day 0 ($P < 0.05$). The antioxidant activity (2,2-diphenyl-1-picrylhydrazyl) increased after 30 days of storage in pressurized samples at 300 and 500 MPa. A maximum antioxidant activity value was observed at 300 MPa by means of the oxygen radical absorbance capacity analysis ($P < 0.05$).

PRACTICAL APPLICATIONS

Influence of high hydrostatic pressure (HHP) on Cape gooseberry pulp quality was evaluated immediately after processing and after 30 days of storage at 4C. Physicochemical, nutritional and functional properties of the gooseberry pulp were determined. The results of this work may contribute to a better assessment of the benefits of high pressure processed gooseberry pulp. HHP processing preserves and enhances quality of bioactive compounds as well as improves their stability during storage, which may lead to the development of new functional food products with this pulp as basic ingredient.

INTRODUCTION

A substantial amount of epidemiological data points to the consumption of plant-based diet as an essential factor for human health (Chen *et al.* 2014). Thus, the increase in awareness of the importance of consuming fruits contributed greatly to the development of better quality processed products. New processing technologies are required to provide consumers with the complete food 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). Emerging technologies have been investigated so as to replace or complement conventional methods being employed in food

processing. In particular, high pressure processing (HPP) is an innovative technology with great potential for optimizing the intake of nutrients and phytochemicals with foods. HPP leads to improvement in the overall quality of foods, especially antioxidant activity, and it can be integrated in food with novel functional properties (Vega-Galvez *et al.* 2011). Retention of sensory attributes and other characteristics of freshness, combined with increased convenience and extended shelf life, will undoubtedly increase the appeal of HPP-preserved foods to consumers (Oey *et al.* 2008; Vega-Galvez *et al.* 2011; Nuñez-Mancilla *et al.* 2014). Thus, due to the importance of fruits in health diets, application of HPP can provide nutritionally improved fruit-based products, leading to safe food with minimal processing effects on food quality (Cao *et al.* 2012; Tadapaneni *et al.* 2014). In some cases, HPP may enhance the bioaccessibility of fruit components; however, the biological importance of the retention of nutrients and bioactive phytochemical compounds imparted by HPP remains to be elucidated. Therefore, it is necessary to study each matrix separately, as the behavior of bioactive compounds and certain quality parameters may differ. Moreover, the effectiveness of HPP treatment depends on the intensity of treatment and the food matrix (Barba *et al.* 2012).

The Cape gooseberry (*Physalis peruviana* L.) belongs to the Solanaceae family and it is a good source of vitamins A and C, as well as minerals such as phosphorus, iron, potassium and zinc (Restrepo 2008). It is an annual short-lived perennial plant, rather hairy, with dome-shaped shrub that can grow up to 1 m (Ramadan 2011). One remarkable aspect of the fruit is its color, being the carotenoids responsible for the orange hues of the gooseberry. However, the level of pigments depends on the stage of fruit ripeness, the extraction process and the storage conditions (Ramadan and Mörsel 2003). Cape gooseberry has considerable functional properties due to its content of antioxidant compounds. The high levels of vitamin E in the oil extracted from the fruit pulp and skin are extremely high compared with the amount present in the seed oil (Ramadan and Mörsel 2003). Therefore, the aim of this investigation was to determine the effect of high hydrostatic pressure (HHP) on physicochemical, nutritional and functional properties of the gooseberry pulp immediately after processing and after 30 days of storage at 4C. The quality indices evaluated were β-carotene, vitamin C, minerals and sugar contents as well as antioxidant capacity.

MATERIAL AND METHODS

Sample Preparation

Cape gooseberry was cultivated and purchased in the region of Coquimbo, Chile. The samples were selected to provide a homogeneous 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 for 3 min (Philips, HR1720, Amsterdam, The Netherlands). The pulp was packed in polyethylene flexible bags 10 cm × 30 cm × 25.4 μm (with O_2, CO_2 and water vapor transmission rates of 3.08×10^{-4}, 2.05×10^{-3} and 2.05×10^{-6} $mmol^3/m^2/s$, respectively, at $P = 101325$ Pa, T = 4C) until further HHP processing.

HHP Treatment and Storage Conditions

HHP treatment was carried out in a 2-L cylindrical loading container of a high pressure equipment (Avure Technologies Incorporated, Kent, WA). Samples were subjected to three different HHP (300, 400 and 500 MPa) for 5 min at room temperature (20 ± 2C). Control samples were analyzed at 0.1 MPa (atmospheric pressure) and room temperature. Water was used as the pressurizing medium. Quality analyses were performed immediately after processing (day 0) and after 30 days of storage (day 30). All experiments were performed in triplicate. Samples were stored at 4 ± 2C until quality analysis.

Physicochemical Analysis

The moisture content was determined by means of Association of Official Analytical Chemists' method (AOAC 1990). The pH was measured using an EXTECH Instruments microcomputer pH vision (model 246072, Waltham, MA). The percentage of titratable acidity was expressed as gram citric acid per 100 g sample. The water activity was measured at 25C 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.

Minerals

Minerals (P, Cu, Fe, Mn, Zn, Ca, Mg, Na and K) were determined by using an atomic absorption spectrophotometer (Shimadzu Instruments, Inc., SpectrAA-220, Kyoto, Japan) after digestion in a mixture of H_2SO_4, HNO_3 and $HClO_4$. The P content was estimated using a phospho-vanadium–molybdenum complex, measuring the absorbance at 466 nm (spectrophotometer UV-120-02; Shimadzu Instruments, Inc.) as described in a previous work (Vega-Galvez *et al.* 2011). All measurements were carried out in triplicate.

Vitamin C

The vitamin C extraction was performed using 5% metaphosphoric acid (MPA) as described in a previous work

(Campos *et al*. 2009) with some modifications. Fifteen milliliters of the extracting solutions was added to a 2.5-g sample that was then mixed in a shaker for 20 min. The sample was ground and filtered through a double layer cheese cloth and the filtrates were centrifuged for 15 min at 3,075 × g at 10C. The supernatant was injected for chromatographic analysis.

Chromatographic conditions. The method used a Zorbax Eclipse XDB-C18 (Agilent, Santa Clara, CA), 4.6 × 150 mm, 5 μm reversed-phase column, a mobile phase containing 5 mM cetyltrimethylammonium bromide as the ion-pairing agent and 50 mM potassium dihydrogen phosphate as buffer, at pH 3.9 (the solution was filtered through a 0.22-μm filter for use in the chromatograph). All measurements were performed at 20C and a flow rate of 0.8 mL/min, isocratic elution and detection at 245 nm. Peak areas were considered for quantitative analysis. The calibration curve was prepared for concentration between 15 and 120 μg of ascorbic acid per milliliter in 5% MPA. Vitamin C was estimated after reduction of dehydroascorbic acid with dithiothreitol. Results were expressed as mg vitamin C/100 g sample. All measurements were carried out in triplicate.

β-Carotene

Conventional extraction was performed in triplicate using the method described in a previous work (Briones-Labarca *et al*. 2013) with some modifications. Cape gooseberry pulp (5 g) was extracted in 50 mL of a mixture of hexane : acetone : ethanol (50:25:25, v/v/v) using an orbital shaker. The extract was vacuum-filtered through a Büchner funnel. The residue was re-extracted until it became colorless. The filtrates were combined in a separatory funnel and washed with 50 mL distilled water. The water phase was discarded and Na_2SO_4 (2 g) was added as a desiccant. The hexane phase was transferred into a 250 mL round-bottom flask. The solvent was evaporated using a rotary evaporator under reduced pressure at 40C. The Cape gooseberry extract was dissolved in hexane and diluted to a final volume of 50 mL. Chromatographic conditions were set according to the work published by Laur and Tian (2011). Analyses of β-carotene were performed using an Agilent 1200 series high-performance liquid chromatography (HPLC) system (Agilent, Singapore City, Singapore), including a quaternary pump (model G1311A), an autosampler (model G1329B), a column oven (model G1316A) and a photodiode array detector (model G1315D). The column used was a Kromasil 100-5C18 (Eka Chemical AB, Bohus, Sweden), 250 × 4.6 mm, connected to a Kromasil guard column. The column temperature was controlled at 30C during the HPLC runs. Data were processed using the Agilent ChemStation software. The flow rate was set at 1 mL/min and the mobile phases were (A) acetonitrile : H_2O : triethylamine (900:99:1, v/v/v), adjusted with a 1 M phosphoric acid solution at pH 8.5, and ethyl acetate (B). The gradient elution program was: 0–5 min, 100–75% A; 5–10 min, 75–30% A; 10–14 min, 30–0% A; 14–15 min, 0–100% A; 15–20 min, 100% A. The absorbance was read at 450 nm, β-carotene was identified based on the retention time and peak areas compared with authentic standards. The calibration curve was prepared between 50 and 500 μg of β-carotene/mL. Results were expressed as mg β-carotene/100 g sample. All measurements were carried out in triplicate.

Sugars (Glucose, Fructose and Sucrose)

Five grams of Cape gooseberry pulp was dissolved in 10 mL of methanol (80%) and agitated on a shaker at 200 ×g 3075 for 30 min according a previous method with some modifications (Djendoubi *et al*. 2012). The extracted sample was centrifuged for 3 min and supernatant was filtered through a 0.45-μm membrane filter and 10 μL was injected in the HPLC.

Analysis of sugars was determined using a HPLC unit (Perkin Elmer Flexar LC model, Shelton, WA) with a refractive index detector, including a Flexar binary LC Pump, a Flexar LC auto sampler and Flexar column oven. Separation of sugars was carried out using an Supersosil LC-NH_2, 5 μm (25cm × 4.6 mm) with column temperature maintained at 25C, the mobile phase was acetonitrile : water (75:25; pH 5.1) and the flow rate was set at 1 mL/min for isocratic elution. Sugars in fruit extracts were identified by their characteristic retention times compared with those of standards. Sugar contents were expressed in g/100 g sample. All measurements were carried out in triplicate.

Antioxidant Capacity

Oxygen Radical Absorbance Capacity Assay. Extraction of the antioxidant compounds to determine antioxidant capacity by oxygen radical absorbance capacity (ORAC) method was performed as described by Ou *et al*. (2002) with some modifications. Two grams of pulp was weighed using an analytical balance (model CHYO Jex-120, Tokyo, Japan) with a precision of 0.0001 g and dissolved in 20 mL of acetone/water (50:50, v/v). The mixture was stirred for 1 h at 200 ×g 3075 on an orbital shaker (OS-20, BOECO, Hamburg, Germany). The extract was centrifuged (Eppendorf 5804, Hamburg, Germany) at 4,000 ×g 3075 for 15 min and the supernatant was filtered through a Whatman No. 1 filter paper. This process was repeated and the supernatants were brought together to dryness in a rotary evaporator (IKA RV 10, Staufen, Germany) at 40C. The extract was reconstituted with phosphate buffer pH 7.4 and brought to a final volume of 25 mL that was then stored at −20C until further analysis.

The ORAC assay was performed following the procedure described previously by Zhang *et al.* (2010). A fluorescein stock solution (100 mmol/L) in phosphate buffer (75 mM, pH 7.4) was prepared and kept at 4C in the dark. A fresh working fluorescein solution (100 nM) was prepared daily by diluting the stock solution in a phosphate buffer. Next, 200 μL (100 nM) of the working fluorescein solution was added to each 40 μL of pulp sample or Trolox standard prepared in phosphate buffer in a black 96-well plate and incubated at 37C for 20 min. The assay was initiated by adding the peroxyl radical generator prepared in phosphate buffer. Specifically, 35 μL of 2,20-azobis-2-amidinopropane (0.36 mol/L) was added and the fluorescence was measured (ex = 485 nm and em = 535 nm) every minute using a Victor X3 Multilabel Plate Reader (Perkin–Elmer, Turku, Finland) maintained at 37C until the reading had declined to less than 5% of the initial reading. Standards and samples were run in triplicate. Results for ORAC were determined using a regression equation relating Trolox concentrations and the net area under the kinetic fluorescein decay curve. The ORAC value of the gooseberry extract was expressed in micromoles of Trolox equivalents (TE)/100g sample. All measurements were carried out in triplicate.

2,2-Diphenyl-1-Picrylhydrazyl Assay. Extraction of the antioxidant compounds to determine antioxidant capacity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method was carried out by a solvent extraction method. This extraction was determined according to previous methods (Vasco *et al.* 2008; Rojas-Barquera 2009) with some modifications. Six grams of the pulp was extracted twice at room temperature under continuous stirring on an orbital shaker (OS-20 BOECO, Hamburg, Germany) for 1 h, initially with 20 mL of a methanol : water (50:50 v/v) mixture and then with 20 mL of acetone : water (70:30 v/v) with an intermittent centrifugation (4,000 ×g 3075, 15 min, 5C). On the whole, three acetone : water (70:30 v/v) extractions were performed. Four supernatants were collected and concentrated to dryness in a rotary evaporator (RV 10; IKA) at 40C. The extract was reconstituted with 80% methanol and analyzed for antioxidant activity.

The antioxidant capacity determined by DPPH assay followed the procedure described in the previous work (Turkmen *et al.* 2005) with some modifications. Different dilutions of the extracts were prepared in triplicate. An aliquot of 2 mL of 0.15 mM DPPH radical in methanol 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, IL, Rochester, NY). Eighty percent (v/v) of 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 micromoles TE/100 g sample. All measurements were carried out in triplicate.

Statistical Analysis

Two-way analysis of variance (Statgraphics Plus 5.1 software, Statistical Graphics Corp., Herndon, VA) 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 range test included in the statistical program was used to prove the existence of homogeneous groups within each of the parameters analyzed.

RESULTS AND DISCUSSION

Effect on Physicochemical Characterization

In Table 1, the moisture content, soluble solids, pH, percentage of acidity and water activity of untreated (control samples, 0.1 MPa) and HHP-treated samples at day 0 and 30 are shown. A significant increase ($P < 0.05$) in the moisture content of the treated samples, pressurized at 400 and 500 MPa, was observed on day 0. This increase in moisture content may be due to the increased water absorption capacity of proteins, as it is known that HHP can increase the hydration of proteins (Briones-Labarca *et al.* 2011a, 2013). The initial pH of the untreated sample was 3.93 ± 0.06 on day 0 (Table 1). Sample treated at 300 MPa showed no significant difference compared with untreated sample on day 0 ($P > 0.05$). However, the pH value increased significantly for samples treated at 400 MPa ($P < 0.05$), which may be due to conformational changes associated with the unfolding and denaturation of proteins and exposure of basic amino acids to the medium (Kaur *et al.* 2013). The soluble solids content was significantly lower in the samples treated at 300, 400 and 500 MPa compared with the untreated sample (0.1 MPa; $P < 0.05$). This decrease may be due to degradation during pressure treatment of sucrose, identified as the main sugar in Cape gooseberry pulp. Neither acidity nor water activity in the treated pulps presented significant difference ($P > 0.05$) when compared with the untreated sample.

In Table 1, the mean values and standard deviations of moisture content, pH, percentage of acidity, soluble solids and water activity after 30 days of refrigerated storage are also presented. Changes in moisture content, pH, percentage of acidity and soluble solids became apparent in the untreated sample (0.1 MPa). Solid soluble content of the control sample showed a decrease after 30 days of storage;

TABLE 1. EFFECT OF HIGH HYDROSTATIC PRESSURE (HHP) ON PHYSICOCHEMICAL CHARACTERISTICS: MOISTURE, pH, PERCENTAGE OF ACIDITY, SOLUBLE SOLIDS AND WATER ACTIVITY IN UNTREATED AND TREATED CAPE GOOSEBERRY PULP

	Treatments							
	0.1 MPa		300 MPa		400 MPa		500 MPa	
Parameters	Day 0	Day 30	Day 0	Day 30	Day 0	Day 30	Day 0	Day 30
Moisture (g/100 g)	78.61 ± 0.24^{a}	82.68 ± 1.73^{A}	76.92 ± 3.14^{a}	80.02 ± 0.88^{B}	83.76 ± 1.74^{b}	79.02 ± 1.53^{B}	88.23 ± 3.34^{b}	80.91 ± 0.43^{AB}
pH*	3.93 ± 0.06^{a}	3.98 ± 0.00^{A}	3.90 ± 0.10^{a}	3.78 ± 0.01^{B}	4.10 ± 0.00^{b}	3.69 ± 0.03^{C}	3.73 ± 0.06^{c}	3.98 ± 0.01^{A}
Acidity†	1.61 ± 0.56^{a}	1.56 ± 0.02^{A}	1.93 ± 0.03^{a}	1.64 ± 0.01^{B}	1.69 ± 0.02^{a}	1.66 ± 0.03^{BC}	1.69 ± 0.02^{a}	1.69 ± 0.02^{C}
Soluble solids (°Brix)	14.93 ± 0.12^{a}	14.5 ± 0.01^{A}	13.97 ± 0.06^{b}	14.07 ± 0.12^{B}	14.27 ± 0.12^{c}	14.33 ± 0.12^{C}	14.13 ± 0.12^{cb}	14.47 ± 0.12^{A}
Water activity*	0.93 ± 0.002^{a}	0.94 ± 0.003^{A}	0.93 ± 0.008^{a}	0.94 ± 0.002^{AB}	0.93 ± 0.014^{a}	0.94 ± 0.002^{A}	0.93 ± 0.001^{a}	0.94 ± 0.002^{B}

For each storage time, mean values followed by different lowercase superscript letters (a–c) indicate significant ($P < 0.05$) differences as a result of HHP in the day 0. Mean values followed by different uppercase superscript letters (A–C) indicate significant ($P < 0.05$) differences as a result of HHP in the day 30. Values are mean ± standard deviation carried out in triplicate.

* Dimensionless.

† Percentage of citric acid.

acidity, moisture content and pH showed a decrease ($P < 0.05$). In particular, the percentage of acidity did not present significant differences during the storage in pressurized samples treated at 300, 400 and 500 MPa. Comparable results were reported by other authors working with carrot and tomato juice pressurized samples at 250 MPa/35C/15 min (Dede *et al.* 2007). Water activity with a mean value of 0.942 did not show any significant difference ($P < 0.05$) among the pressurized samples.

Effect on Sugars

Sugars are strongly related to the sweetness of Cape gooseberry pulp, which is one of the most important quality attributes that significantly affects consumer acceptance. The identified sugars in the Cape gooseberry pulp were fructose, glucose and sucrose. Changes in sucrose, fructose and glucose immediately after HHP treatments are presented in Table 2. The control sample presented the highest level of sucrose (2.40 ± 0.02 g/100 g sample) followed by glucose and fructose. Similar results have been reported for fresh gooseberry (Novoa *et al.* 2006). However, in other reports it was shown that fresh Cape gooseberry contains 15% soluble solids (mainly sugars) with a high level of fructose, which makes it valuable to diabetics (Puente *et al.* 2011).

TABLE 2. SUGAR CONTENT OF CAPE GOOSEBERRY PULP IMMEDIATELY AFTER PRESSURIZATION (DAY 0)

	High hydrostatic pressure treatments			
Sugars*	0.1 MPa	300 MPa	400 MPa	500 MPa
Fructose	0.99 ± 0.01^{a}	1.54 ± 0.01^{b}	1.55 ± 0.04^{b}	1.55 ± 0.01^{b}
Glucose	1.02 ± 0.01^{a}	1.61 ± 0.01^{b}	1.59 ± 0.01^{b}	1.62 ± 0.00^{b}
Sucrose	2.40 ± 0.02^{a}	1.34 ± 0.01^{b}	1.31 ± 0.03^{b}	1.38 ± 0.00^{b}

Note: Values are mean ± standard deviation ($n = 3$). Values followed by the same superscript letter in the same row are not significantly different ($P < 0.05$).

* Content of sugars expressed in g/100 g sample.

On day 0, a significant increase in fructose and glucose content was observed at three pressure levels (300, 400 and 500 MPa) compared with untreated sample (0.1 MPa; Table 2). However, in the pressurized samples, significant differences ($P < 0.05$) were not noticeable. Therefore, fructose and glucose were the most abundant sugars in the treated samples. As for the sucrose content, an opposite effect occurred. Sucrose content in the samples treated at 300, 400 and 500 MPa decreased significantly compared with untreated samples (0.1 MPa), although at the three pressure levels, the differences in sucrose content was not significant ($P < 0.05$). The decrease in sucrose content of the treated samples may be attributed to enzymatic hydrolysis (e.g. by invertase) that took place when applying pressure to the pulp. Invertase is an enzyme that converts sucrose into equimolar amounts of fructose and glucose. It can be seen in Table 2 that the levels of fructose and glucose increased immediately after HHP treatment, while the sucrose level fell as a result of a typical invertase action (Butz *et al.* 2003). The same effect of pressure in relation to glucose and fructose contents was reported in assays with mango nectars at 600 MPa/1 min (Butz *et al.* 2003).

Table 3 shows the effect of HHP treatment on sugar content after 30 days of storage at 4C. In the untreated sample, a severe decrease (20%) in sucrose content after storage can be observed, whereas in the pressurized samples there was a significantly lower drop compared with the control samples ($P < 0.05$). These results could indicate that the inversion of sucrose was affected by enzymatic

TABLE 3. FRUCTOSE, GLUCOSE AND SUCROSE FROM CAPE GOOSEBERRY PULP AFTER 30 DAYS OF STORAGE

	High hydrostatic pressure treatments day 30			
Sugars*	0.1 MPa	300 MPa	400 MPa	500 MPa
Fructose	0.80 ± 0.01^{a}	1.62 ± 0.02^{b}	1.65 ± 0.02^{b}	1.64 ± 0.00^{b}
Glucose	1.27 ± 0.01^{a}	1.69 ± 0.02^{a}	1.73 ± 0.03^{c}	1.72 ± 0.01^{bc}
Sucrose	0.47 ± 0.01^{a}	1.13 ± 0.02^{b}	1.10 ± 0.01^{c}	1.19 ± 0.01^{d}

Note: Values are mean ± standard deviation ($n = 3$). Values followed by the same superscript letter in the same row are not significantly different ($P < 0.05$).

* Sugars content expressed in g/100 g sample.

hydrolysis in this study, which was in accordance with incomplete inactivation of acid invertase. Changes in sucrose could be related to different enzyme activities, including sucrose phosphate synthase, acid invertase, neutral invertase and sucrose synthase (Liu *et al.* 2014). The other sugars (fructose and glucose) increased significantly during storage in the treated samples ($P < 0.05$). The fructose contents at the three levels of pressure studied were not significantly different. Comparable results for sucrose in strawberry jam treated at 400–500 MPa/10–30 min and stored refrigerated at 4C have been reported (Kimura *et al.* 1994). Other authors working with mango nectar reported that content of sucrose and total sugar decreased significantly, while fructose and glucose increased significantly. Moreover, the changes were more pronounced in treated samples, especially for samples stored at 25C (Liu *et al.* 2014).

Effect on Vitamin C

Figure 1 shows the effect of HHP process on vitamin C content immediately after pressurization and after 30 days of storage at 4C. The average vitamin C content of pressurized samples was 19.43 ± 0.253 mg/100 g pulp as compared with 18.65 ± 0.93 mg/100 g pulp in untreated sample (0.1 MPa). The initial vitamin C content of untreated sample was within the range of values reported in previous investigations (Barreto *et al.* 2009; Briones-Labarca *et al.* 2013). Although in all treatments the vitamin C content showed a slight tendency to increase immediately after processing, application of pressure did not lead to significant differences compared with the untreated sample ($P < 0.05$). Different results have been reported from other authors working with Cape gooseberry, where an increase in vitamin C content between 9 and 53% was found after application of 500 MPa/30–90 s (Briones-Labarca *et al.* 2013). The observed increase of vitamin C content in treated samples may be related to better extractability of this component as a result of pressurization and higher stability of ascorbic acid to pressure compared with thermal treatments or with the presence of oxidation inhibitors that inhibit the oxidation of ascorbic acid (Castro *et al.* 2008; Kaushik *et al.* 2014). In another study with red peppers, an increase of about 10–20% in ascorbic acid content compared with control sample was observed (Castro *et al.* 2008). In mango pulp, vitamin C content was reported to increase to a maximum of 129% in a single pressure treatment of 600 MPa (Kaushik *et al.* 2014). These authors attributed the increase in vitamin C content to the extraction effect of high pressure, which causes cells to rupture under compression releasing the cytosol content into the extracellular space. In others reports, it was observed that 90% of the initial vitamin C contents of strawberry and blackberry purees were retained after HPP treatment (400, 500, 600 MPa/15 min/10–30C; Patras *et al.* 2009). However, there are also reports on the vitamin C content in orange juice and in orange-lemon-carrot juice (500 or 800 MPa for 5 min) that showed HHP treatment had no significant effect (Fernández-García *et al.* 2001).

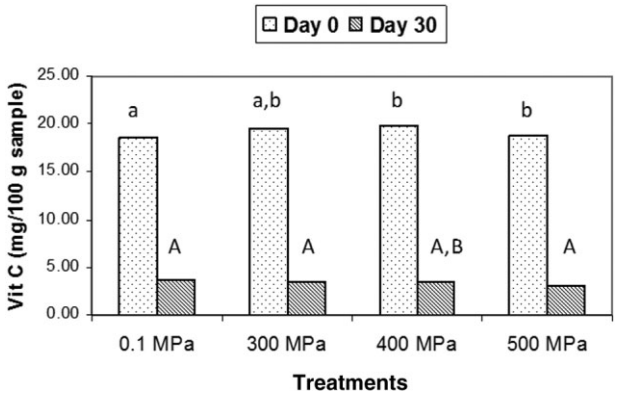

FIG. 1. EFFECT OF HIGH HYDROSTATIC PRESSURE ON VITAMIN C CONTENT OF CAPE GOOSEBERRY PULP AT DAY 0 AND AFTER 30 DAYS OF STORAGE

Values are mean ± standard deviation carried out in triplicate. Identical letters above the bars indicate no significant difference ($P < 0.05$).

The vitamin C content of Cape gooseberry after 30 days of storage presented a decrease in all treatments (Fig. 1), with no significant differences ($P < 0.05$) between untreated samples (0.1 MPa) and pressurized samples. Vega-Galvez *et al.* (2011) reported results with similar tendency for aloe vera gel treated at 300–500 MPa/3 min and stored for 35 days. It is known that ascorbic acid degrades, following two consecutive or parallel pathways, aerobically and anaerobically, which can be explained in a first step by the oxidation of ascorbic acid to dehydroascorbic acid, which is then further irreversibly converted into 2,3-diketogulonic acid (Valdramidis *et al.* 2009; Vega-Galvez *et al.* 2011). Polydera *et al.* (2003) studied vitamin C deterioration kinetics in pasteurized and HPP reconstituted orange juice. They reported a more rapid decrease of ascorbic acid concentration at the beginning of storage that could be attributed to the

TABLE 4. EFFECT OF HIGH HYDROSTATIC PRESSURE (HHP) ON B-CAROTENE CONTENT ON CAPE GOOSEBERRY PULP, ON DAY 0 AND AFTER 30 DAYS STORAGE

	β-carotene (mg 100/g sample)	
Treatment HHP	Day 0	Day 30
0.1 MPa	221.27 ± 12.05[a]	241.94 ± 9.19[A]
300 MPa	194.92 ± 5.15[b]	197.64 ± 8.38[B]
400 MPa	186.28 ± 9.41[b]	135.27 ± 5.68[C]
500 MPa	232.48 ± 12.81[a]	122.15 ± 10.07[C]

Note: Values are mean standard ± deviation ($n = 3$). For each storage time, mean values followed by different lowercase superscript letters (a–c) indicate significant differences ($P < 0.05$) as a result of pressure in the day 0. Mean values followed by different uppercase superscript letters (A–C) indicate significant differences ($P < 0.05$) as a result of pressure in the day 30.

immediate reaction of an amount of ascorbic acid with the dissolved oxygen. Wolbang *et al*. (2008) observed that the vitamin C content was significantly affected at HPP at 600 MPa/10 min during storage. It is known that the effect of HPP on vitamin C has been investigated with different conclusions, a reduction in vitamin C content was observed in this investigation. Vitamin C, which is labile, is easily destroyed during processing and storage period and this may contribute to the varying results reported in the scientific literature. Landl *et al*. (2010), working with apple puree at pressures of 400 and 600 MPa/5 min/20C, observed losses of vitamin C during storage (3 weeks of refrigerated storage). They reported that oxygen and the food matrix influenced the vitamin C stability during HPP and subsequent storage.

Effect on β-Carotene

Table 4 shows the β-carotene contents of control (0.1 MPa) and treated samples of Cape gooseberry pulp at different pressure levels, immediately after pressurizing (day 0) and after 30 days of storage at 4C. β-carotene content of the control sample was 221.27 mg β-carotene/100 g sample. Ramadan (2011) reported comparable results (326 mg/100 g sample), while Briones-Labarca *et al*. (2013) and Puente *et al*. (2011) informed high values, 1074.67 mg β-carotene/100 g fresh sample and 1460 mg β-carotene/100 g in pulp, respectively. On day 0, samples treated at 300 and 400 MPa showed significant differences with control samples (0.1 MPa; $P < 0.05$). Although 500 MPa was the treatment showing the highest value of β-carotene, this value was not significantly different with the corresponding control sample (0.1 MPa; $P < 0.05$). The loss of β-carotene content (at 300–400 MPa) was probably due to the fact that carotenoids are highly unsaturated compounds, thus degradation is due mainly to oxidation. Moreover, some authors concluded that HHP could be a useful tool to produce an improvement on the extraction of potentially health-related compounds and, in consequence, to modify their bioaccessibility (Plaza *et al*. 2012). HHP treatment can affect the membranes in fruit cells; in addition, carotenoids are tightly bound to protein and membrane lipids. Moreover, pressure above 300 MPa can cause irreversible protein denaturation (De Ancos *et al*. 2002). De Ancos *et al*. (2002), working with mango nectars at 600 MPa/1 min, reported that the content of total carotenoids in treated samples exhibited no significant changes after HHP treatment. Extrinsic factors, such as presence or absence of light, temperature of storage, packaging, as well as characteristics of the food matrices, such as their chemical composition, the oxygen dissolved in the samples, size of the particles and the physical state of the carotenoid in the food, can affect carotenoids stability.

In Table 4, the changes in β-carotene content after 30 days of storage at 4C are shown. β-carotene content of pressurized samples presented significant differences with the corresponding values of control samples (0.1 MPa; $P < 0.05$). Treatments of 400 and 500 MPa/5 min resulted in a decrease of 44 and 49% in β-carotene content compared with the untreated sample after 30 days of storage, respectively. De Ancos *et al*. (2002) reported comparable results in orange juice treated at 50 MPa/30C/5 min. These authors mentioned that after 30 days of the storage, significant losses of 17 and 42% of total carotenoid content were noticed in the pressurized orange juice as well as the untreated juice, respectively. These losses in the content of β-carotene could be probably due to processing and storage leading to instability of the polyene chain of carotenoids. As a consequence, these compounds may undergo geometric isomerization (promoted by heat, light and acids) and oxidation (stimulated by light, heat, metals, enzymes and peroxides, and inhibited by antioxidants) which are the main causes of carotenoid degradation (Plaza *et al*. 2011). Different results were reported in previous investigations. Plaza *et al*. (2011) reported that orange juice (400 MPa/1 min/36C) was not affected by pressure treatment and the carotenoids remained quite stable during refrigerated storage. Hsu *et al*. (2008) reported that the HHP increased the total carotenoid content up to 62 and 60%, when working with tomato juices pressurized at 300–500 MPa/25C/10 min and stored at 4C for 28 days.

Effect on Antioxidant Capacity

Figure 2 shows the influence of HHP on the antioxidant capacity of control and treated pulp samples determined by means of DPPH and ORAC analysis. On day 0, an antioxidant capacity of 19.34 ± 1.26 μmol TE/100 g sample and

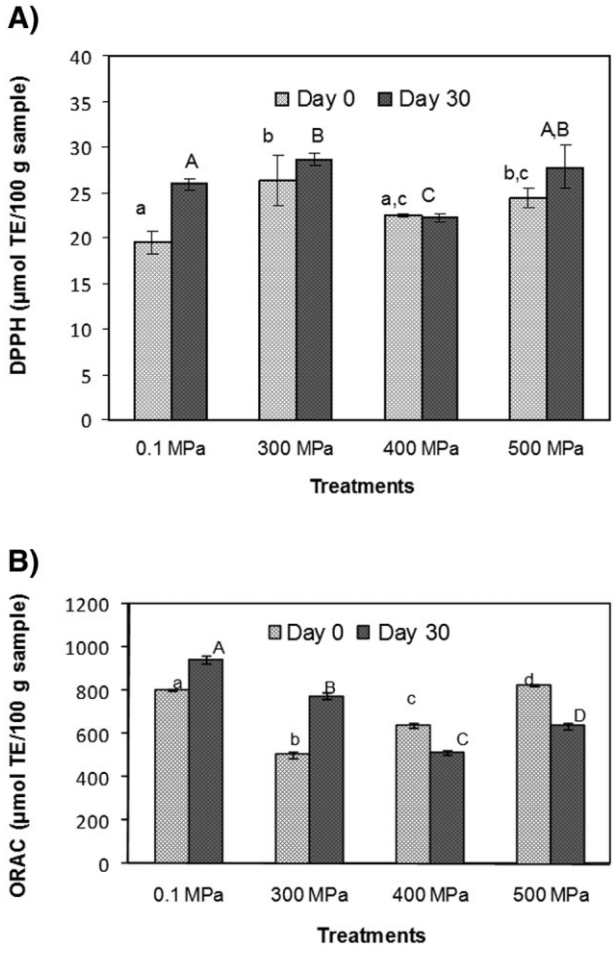

FIG. 2. EFFECT OF HIGH HYDROSTATIC PRESSURE ON ANTIOXIDANT ACTIVITY BY 2,2-DIPHENYL-1-PICRYLHYDRAZYL (DPPH) (A) AND OXYGEN RADICAL ABSORBANCE CAPACITY (ORAC) (B) ON CAPE GOOSEBERRY PULP AT DAY 0 AND AFTER 30 DAYS OF STORAGE
Values are mean ± standard deviation carried out in triplicate. Identical letters above the bars indicate no significant difference ($P < 0.05$).

804.12 ± 1.73 µmol TE/100 g sample for DPPH and ORAC, respectively, were determined in the untreated sample. These results are comparable with those reported in previous investigations on Cape gooseberry (Gironés-Vilaplana *et al.* 2014). As can be observed from Fig. 2A, the application of HHP process caused a significant increase in antioxidant capacity of Cape gooseberry pulp as determined by DPPH assay for the three applied pressure levels. The pressure treatment at 300 MPa led to the highest value of antioxidant capacity, with an increase of 35% compared with the control sample ($P < 0.05$). On the other hand, analysis of the ORAC assays (Fig. 2B) showed a different result. At 300 and 400 MPa, a decrease in the value of antioxidant capacity was observed, while at 500 MPa the antioxidant activity increased compared with the control samples (0.1 MPa; $P < 0.05$). Del Pozo-Insfran *et al.* (2007) also reported higher losses in antioxidant activity using the ORAC assay working with grape juice at 400 and 500 MPa for 15 min. This decrease was attributed to degradation of anthocyanins. The observed differences among the assays can be attributed to the fact that these two methods differ in terms of their assay principle and experimental conditions as mentioned previously. Jung *et al.* (2013), working with carrot and spinach, also found that after application of 500 MPa/20 min, an increase in antioxidant activity occurred with respect to the control sample.

HHP treatments are thought to influence the extraction yield of bioactive compounds with antioxidant activity. Presumably, HHP led to an increased permeability of the pressurized cells and a subsequent improvement of the extraction effect (Briones-Labarca *et al.* 2013). Similar results have been reported in previous investigations (Briones-Labarca *et al.* 2013; Kaushik *et al.* 2014). Therefore, the influence of pressure on antioxidant activity is not the same for all food products. Literature data vary considerably when reporting on the effects of both time/temperature and food matrix dependency (Nuñez-Mancilla *et al.* 2014). Liu *et al.* (2014), working with mango nectars, reported that HHP treatment caused no significant changes in the antioxidant capacity of mango nectars using the ferric reducing ability of plasma (FRAP) assay, but a significant decrease with respect to the fresh products was observed in the DPPH assay.

In Fig. 2, the changes in antioxidant capacity of the pulp after 30 days of storage at 4C, as determined by DPPH (Fig. 2A) and ORAC (Fig. 2B) assays, are shown. The DPPH antioxidant activity increased after 30 days of storage in samples pressurized at 300 and 500 MPa ($P < 0.05$). Increases in antioxidant capacity values during storage can be attributed to the migration of antioxidant compounds from the cell to the pulp matrix, enhancing the extraction of both antioxidants compounds. It is known that the application of HHP induces morphological changes (compression of the vacuoles, cellular enlargement, aggregation of cytoplasmatic proteins, etc.) that lead to rupture of the membrane and the subsequent liberation of intracellular constituents (Jacobo-Velázquez and Hernández-Brenes 2012). Comparable results were reported in previous investigations working with carrots, green beans, broccoli and aloe vera gel (McInerney *et al.* 2007; Vega-Galvez *et al.* 2011). Chen *et al.* (2014), working with cloudy pomegranate juice at 400 MP for 5 min, reported that DPPH antioxidant activity decreased with the extension of storage time (90 days at 4C). The loss of DPPH antioxidant activity was associated with the loss of total anthocyanins and total phenols during storage at 4C.

TABLE 5. TOTAL MINERAL CONTENT OF CAPE GOOSEBERRY PULP ON DAY 0

Treatments day 0				
Mineral*	0.1 MPa	300 MPa	400 MPa	500 MPa
Magnesium	120.14 ± 3.86[a]	57.02 ± 0.71[b]	85.82 ± 2.42[c]	65.11 ± 0.68[d]
Calcium	37.69 ± 1.88[a]	27.29 ± 1.37[b]	32.61 ± 2.55[c]	37.30 ± 2.29[a]
Potassium	501.90 ± 16.12[a]	553.47 ± 9.75[b]	478.19 ± 4.44[c]	546.21 ± 11.16[b]
Sodium	52.70 ± 1.69[a]	26.81 ± 1.38[b]	25.84 ± 0.57[b]	46.45 ± 1.44[a]
Phosphorus	54.91 ± 0.18[a]	61.49 ± 1.65[b]	90.67 ± 1.34[c]	76.27 ± 1.10[d]
Iron	3.83 ± 0.13[a]	1.35 ± 0.07[b]	3.93 ± 0.08[a]	5.77 ± 0.10[c]
Zinc	1.51 ± 0.04[a]	0.47 ± 0.03[b]	1.02 ± 0.06[c]	0.51 ± 0.04[a]
Copper	0.66 ± 0.06[a]	0.28 ± 0.04[b]	0.40 ± 0.01[b]	0.48 ± 0.01[a]
Manganese	0.70 ± 0.02[a]	0.28 ± 0.04[b]	0.39 ± 0.03[a]	0.47 ± 0.03[c]

Note: Values are mean ± standard deviation carried out in triplicate. Values followed by the same superscript letter in the same row are not significantly different ($P < 0.05$).

* Mineral content expressed in mg/100 g sample.

Effect on Minerals

Minerals are essential requirements for the normal functioning of the body and are classified according to their relative amounts or requirements as macronutrient or micronutrient (Puente *et al.* 2011). Table 5 shows the mineral content of control and pressurized samples of Cape gooseberry pulp on day 0. The predominant minerals in the untreated sample (0.1 MPa) were potassium and magnesium. These results were slightly different from those previously reported for Cape gooseberry pulp (Leterme *et al.* 2006; Rodrigues *et al.* 2009). These differences could be due probably to different cultivars of Cape gooseberry, completely different growth conditions or different geographic regions. The macronutrients reported in this work that increased in level as an effect of the HHP in comparison with the untreated sample were potassium and phosphorus. Potassium increased by 8–10% in extraction yields at 300 and 500 MPa/5 min, while phosphorus increased at 400 MPa/5 min by 65% compared with control sample on day 0. The macronutrients, such as magnesium and sodium, and the micronutrients, such as zinc, copper and manganese, presented a significant decrease for all HHP treatments ($P < 0.05$). Repo De Carrasco and Encina (2008) indicated that the presence of zinc would contribute to the antioxidant activity of fruit. These authors also reported iron content close to 1.2 mg/100 g sample in the Cape gooseberry fruit, while in this study, especially at 500 MPa/5 min, a value of 5.77 ± 0.10 mg/100 g was determined. Iron (Fe), a physiologically essential trace element, functions in the hemoglobin in red blood cells to transport oxygen from the lungs to the body's tissues, including muscles and brain. As for calcium, the recommended dietary allowance is 1,300 to 1,000 mg/day according to age and sex group, while recommendations for zinc range between 8 and 11 mg/day (Briones-Labarca *et al.* 2011a). Thus, 100 g serving of Cape gooseberry pulp treated with HHP (500 MPa/5 min) would contribute 37.30 mg of calcium and 0.51 mg of zinc, respectively.

The influence of HHP process on the mineral content of Cape gooseberry after 30 days of storage is shown in Table 6. Pressurized samples presented high contents of calcium (300 MPa), potassium (400 MPa/5 min), zinc (400 MPa/5 min), copper (300 MPa/5 min) and sodium

TABLE 6. TOTAL MINERAL CONTENT OF CAPE GOOSEBERRY PULP AFTER 30 DAYS OF STORAGE

Treatments day 30				
Mineral*	0.1 MPa	300 MPa	400 MPa	500 MPa
Magnesium	79.67 ± 1.15[a]	47.65 ± 0.78[b]	55.89 ± 0.77[c]	51.72 ± 0.63[d]
Calcium	27.26 ± 0.73[a]	39.27 ± 0.81[b]	32.25 ± 0.47[c]	33.72 ± 0.54[d]
Potassium	434.80 ± 5.61[a]	497.44 ± 7.02[b]	532.21 ± 3.02[c]	479.15 ± 7.69[d]
Sodium	31.77 ± 1.54[a]	12.39 ± 0.30[b]	13.00 ± 0.23[b]	42.95 ± 1.92[c]
Phosphorus	98.24 ± 0.71[a]	68.63 ± 0.69[b]	73.34 ± 2.28[c]	72.71 ± 1.18[c]
Iron	10.72 ± 0.22[a]	2.73 ± 0.06[b]	1.84 ± 0.04[c]	1.93 ± 0.05[c]
Zinc	0.95 ± 0.01[a]	0.64 ± 0.01[b]	1.01 ± 0.04[c]	0.92 ± 0.02[a]
Copper	0.46 ± 0.01[a]	0.63 ± 0.01[b]	0.60 ± 0.02[c]	0.45 ± 0.03[a]
Manganese	0.35 ± 0.01[a]	0.21 ± 0.01[b]	0.35 ± 0.01[a]	0.17 ± 0.00[c]

Note: Values are mean ± standard deviation carried out in triplicate. Values followed by the same superscript letter in the same row are not significantly different ($P < 0.05$).

* Mineral content expressed in mg/100 g sample.

(500 MPa/5 min) compared with the control samples after 30 days of storage ($P < 0.05$). Zinc and calcium are essential nutrients that is often lacking in human diets, either due to insufficient intake or to poor absorption of food. In developing countries, deficiencies of these minerals lead to much suffering and death (Briones-Labarca *et al*. 2011a). As mentioned, HHP modified the food matrix leading to disruption of plant cell walls and resulting in the release of compounds into the extracellular environment (Briones-Labarca *et al*. 2011b). This release of minerals due to HHP processing could be an advantage as fruits or derived fruits products are not the main sources of minerals. Vega-Galvez *et al*. (2011) reported comparable results in pressurized aloe vera gel. In their work, these authors observed a significant increase in potassium, calcium and sodium compared with the control sample at 400 MPa for 35 days of storage. Nevertheless, this behavior was not observed in magnesium, phosphorus, manganese and iron ($P < 0.05$) that showed a decrease in content compared with the control samples on day 30.

CONCLUSIONS

Influence of HHP (300, 400 and 500 MPa/5 min) on Cape gooseberry pulp quality was evaluated immediately after processing and after 30 days of storage at 4C. The application of HHP allowed better extraction of the relevant components such as vitamin C, sugar contents as well as minerals such as phosphorous, calcium and potassium. According to the DPPH and ORAC assays, HHP treatments improved antioxidant capacity of Cape gooseberry pulp. In particular, at 300 MPa, the maximum antioxidant activity determined by means of ORAC method was observed. Therefore, the results of this work may contribute to appraise the benefits of high pressure processed Cape gooseberry pulp with preserved and enhanced quality of bioactive compounds, which may lead to the development of new functional food products with this pulp as basic ingredient.

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