Using high hydrostatic pressures to retain the antioxidant compounds and to reduce the enzymatic activity of a pitaya–pineapple (*Stenocereus* sp.–*Fragaria ananassa*) beverage

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Abstract Pitaya (*Stenocereus* sp.) is a fruit native to arid and semiarid areas of Mexico. It has high antioxidant activity mainly due to its contents of betalains and phenolics, but its consumption is limited due to very short shelf-life and not very recognized flavor. A beverage of pitaya and pineapple was formulated to improve sensory properties. A high hydrostatic pressure (HHP) study at 400–600 MPa and 25 °C for 2–10 min was applied in the beverage and the effect on the contents of vitamin C, total phenolics and betalains, and the pectin methylesterase (PME) activity of pitaya–pineapple beverages, was evaluated. The effect of the come up time (CUT) was also studied. Vitamin C contents increased from 5% at 600 MPa-CUT to 64% at 400 MPa/CUT. Total phenolic concentrations decreased (20–48%) at all processing conditions tested at 400 MPa/CUT, total betacyanins were retained. At 500 MPa/10 min losses of betaxanthins of up to 6% occurred. The maximum PME activity decrease was 23% at 600 MPa 5 min, but an increase of PME activity 7% was observed at 400 MPa/10 min. HHP seem to be a good option to retain most of the antioxidant compounds in pitaya–pineapple beverage, but more studies are necessary to inactivate PME.

Keywords Pitaya (*Stenocereus* sp.) · High hydrostatic pressure · Betacyanin · Betaxanthins · Vitamin C · Pectin methylesterase

Introduction

Several epidemiological studies suggest that diet plays an important role in preventing chronic diseases. It has been reported that polyphenols, carotenoids, tocopherols and glucosinolates, commonly found in fruits, vegetables and grains, provide a chemopreventive effect to combat oxidative stress in the body, which can improve health (Bauman 2004).

Pitaya (*Stenocereus* sp.) is a fruit of to the Cactaceae family, and it is a crop of paramount importance for the inhabitants of semiarid zones of Mexico (Chuck-Hernández et al. 2016). Pitaya has recently attracted much attention from the scientific community not only because of its jazzy red–purple color and economic value, but also because of its antioxidant activity derived mainly from betalains, vitamin C and phenolic compounds which are known to combat oxidative stress related to some chronic diseases (Chavez-Santoscoy et al. 2009; García-Cruz et al. 2013). Then pitaya has great potential as a functional food or as a source of phytochemicals. A combination with another fruit of the region was searched to create a beverage with a high content of antioxidants and good flavor. Pineapple is a tropical fruit with health benefits because of its content of vitamin C, polyphenols, flavonoids and other phytochemicals (Chakraborty et al. 2015). Pineapple puree has been proven to reduce the incidence of cardiovascular disease and some chronic and degenerative diseases associated with oxidative damage (Dragsted 2003). Mexico is one of the main producers of pineapple in the world. 70%

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of the production is consumed locally as fresh fruit, 25% is used to produce juices and slices, and 5% is exported (Rebolledo et al. 2000).

It is generally known that enzymes are more resistant to pressure than vegetative microorganisms, thus enzymes are often used as the target issue when designing HHP treatments (Ludikhuyze et al. 2001). Both pineapple and pitaya contain enzymes that can cause deterioration problems. Pectin methylesterase (PME) is an enzyme of great concern in the fruit juice industry due to its contribution to the loss of cloudiness. As turbidity is considered a desirable characteristic by consumers in products like pineapple juice, pasteurization is frequently used to decrease PME activity (Castaldo et al. 1997). Nevertheless, one of the main drawbacks of traditional preservation techniques, especially of those based on heat like pasteurization, is the effect of high temperatures on heat-sensitive antioxidant compounds and sensory characteristics of fruits and fruit based beverages. Betalains and vitamin C are susceptible to changes, losing functionality when exposed to high temperatures and other physical factors such as oxygen (Herbach et al. 2007). High hydrostatic pressure (HHP) is an alternative to thermal processes to treat heat sensitive products, due to its non-thermal nature, it allows obtaining safe foods while minimizing undesirable changes in the sensory, physicochemical, and nutritional properties. Fruit juices and beverages are especially adequate to be treated by HHP due to its low pH (Escobedo-Avellaneda et al. 2011). Until now, there are not beverages containing pitaya processed by HHP. Thus, the aim of this work was to study the feasibility of HHP to retain the vitamin C, total phenolic compounds, and betalains of a pitaya–pineapple based beverage. The effect on the inactivation of PME was also evaluated.

Materials and methods

Chemicals

Acetic acid >99.9%, L-ascorbic acid (AA), methanol, pectin from citrus peel, ammonium acetate, gallic acid (GA) and Folin and Ciocalteu's phenol reagent were purchased from Sigma-Aldrich (St. Louis, USA). Sodium carbonate, sodium chloride, and sodium phosphate dibasic were purchased from J.T. Baker, (Ecatepec de Morelos, Mexico). Potassium chloride and sodium hydroxide were purchased from Fermont, (Mexico City, Mexico). Water was provided by a Milli-Q water purification system (Millipore, Germany).

Raw materials and beverage preparation

Pitaya (*Stenocereus* spp) red variant fruits of commercial maturity were collected from a field in Ahuatlán [18°34' latitude (North) and 98°15' longitude (West)], in the state of Puebla, México, in April 2015. Pineapples (*Fragaria ananassa*) in a commercial maturity index were acquired in Monterrey, Nuevo León, Mexico. The fruits were washed, disinfected, and the peels removed. Prickles from pitaya were manually removed. The pulps of both fruits were processed separately in an extractor (Turmix, TU05, USA) excluding the seeds of pitaya. Based on a sensory analysis of color, flavor and general acceptability (data do not show) a beverage composed of 55% pitaya pulp and 45% (w/w) pineapple pulp was prepared, homogenized and kept frozen at −23 °C until processed.

Physicochemical analysis

The pH, total soluble solids (TSS) and moisture content of the beverage were measured. pH was determined with Thermo Fisher Scientific, Orion 3 star, U.S.A. Soluble solids (°Brix) were determined with a refractometer (HANNA, U.S.A.), and moisture content (%) was obtained according to the gravimetric method 934.06 (AOAC 1990).

High hydrostatic pressure

Samples of the beverage were placed in 12 × 5 cm polyethylene bags (Filmpack S.A. de C.V., Guadalupe, Nuevo León, Mexico) and vacuum-sealed for HHP treatments (Model 2 L, Flow Autoclave Systems, Columbus, OH, USA) using water as pressurizing medium at room temperature as initial condition. Samples were treated at 400, 500, and 600 MPa for 2, 5, and 10 min based on previous studies for PME inactivation and maximum pressure level reached by the HHP unit used (Tejada-Ortigoza et al. 2015). Triplicate runs of each treatment conditions were performed by using 55 mL of the beverage. The effect of the come up time (CUT) was also evaluated by placing samples in the HHP vessel and once the desired pressure level was reached the experiments were interrupted. Untreated beverages were used as controls at 0.1013 MPa by triplicate. The initial temperature of water was 20 °C. Due to adiabatic heating during the holding time the process temperatures were 27, 29, and 31 °C at 400, 500 and 600 MPa, respectively. Immediately after processed, the contents of vitamin C, total phenolic compounds, betalains (betacyanins and betaxanthins) and the PME activity were quantified in the beverages.

Analysis of phytochemicals

Vitamin C

The vitamin C was extracted according to Santos et al. (2012) with some modifications. Briefly, 0.5 g of sample was extracted with 4 mL of 10 mM ammonium acetate/methanol 50:50 (v/v). After 1 min of agitation, samples were placed in an ultrasound bath (Ultrasonic Cleaner, Auto science, China) at a frequency of 40 kHz for 15 min. Bath temperature was always controlled with ice to guarantee 20 °C. The samples were centrifuged at 4415g 7 min and the supernatant was removed and placed in a volumetric flask. The solid residue of these extractions was re-extracted as explained above. The two extracts were placed in the same volumetric flask and were filled to 10 mL. 2 mL were filtered through a 0.20 µm nylon filter, and placed in HPLC vials with nitrogen headspace. 25 µL sample were injected into an HPLC-FLD system (Agilent 1200 HPLC, Santa Clara, USA) using a Zorbax Eclipse XDB C18 column (5 µm, 150 × 4.6 mm i.d.) (Agilent, Santa Clara, USA). 10 mM ammonium acetate solution (pH 4.5) (phase A) and methanol with 0.1% acetic acid (phase B) were used as eluent solvents. Elution was carried out in isocratic mode with 80% phase A for 7 min at a flow rate of 1 mL/min. Chromatograms were monitored at Exc = 280 and Em = 440 nm for ascorbic acid. Quantification of vitamin C was performed using a calibration curve of six points using an external standard of L-ascorbic acid (0.01 to 10 mg/L) (Santos et al. 2012). Results were expressed as mg AA/g beverage on a wet basis (wb).

Total phenolic compounds

Total phenolic compounds were measured according to the method described by (Escobedo-Avellaneda et al. 2014). Pitaya–pineapple beverage samples were diluted 1:100 in distilled water. Fifty µL of diluted sample were mixed with 650 µL water and 50 µL of the Folin–Ciocalteu reagent. After 5 min, 250 µL sodium carbonate 0.5 M were added. The samples were then incubated for 2 h at 37 °C, and then 200 µL of each sample were transferred to a Costar clear 96-flat bottom well microplate. The absorbance was measured at 765 nm in a microplate reader (VarioskanFlash, Thermo Scientific, Finland). A six-points calibration curve in a 5.7–285.3 µmol/g range of GA was used to obtain the phenolic concentration as µmol/g beverage on a wet basis (wb).

Betalains quantification

Betalains in the pulp were quantified according to Sandate-Flores et al. (2016). The concentration of the betalains was

calculated by the extinction coefficient for betanin ($\epsilon = 1120$) and vulgaxanthin I ($\epsilon = 750$), these pigments have maximum absorption at 540 and 480 nm, respectively (Sandate-Flores et al. 2016). To compare the betalains result with literature, the concentration of betalains were showed on a dry basis in milligram per gram (mg/g).

Pectin methyl esterase (PME)

For the evaluation of PME activity in treated and untreated beverages, 20 g beverage (m) was weighed and mixed with 20 mL of a solution (pectin 1% (w/v) and 0.1 M sodium chloride). The mixture was stirred at 1500 rpm on hot plate (30 °C) (CTR scientific, Mexico City, Mexico). The pH of the mixture was adjusted to 7 using different concentrations of NaOH, and then the mixture was readjusted to pH 7.7. Then 20 µL (v) of 0.5 N NaOH (c = concentration of NaOH) were added. The time (t) needed to return the pH to 7.7 was measured and the PME residual activity was calculated as follows (Eq. 1) (Bermúdez-Aguirre et al. 2011). The residual PME activity (%) was calculated with the Eq. 2, where PME_{HHP} is the PME activity in each treatment and PME_0 is the PME activity in untreated beverages.

$$PME \text{ activity} = \frac{c \cdot v}{m \cdot t} \quad (1)$$

$$Residual \text{ PME activity} = \frac{PME_{HHP}}{PME_0} \times 100 \quad (2)$$

Experimental design and statistical analyses

A 2x4x3 bifactorial analysis was carried on where the two factors were time (CUT, 2, 5 and 10 min) and pressure (control, 400, 500 and 600 MPa). The bifactorial analysis was performed to establish if the parameters of time and pressure in the HHP have an effect on the content of phytochemical compounds and PME activity. Results were reported as the mean ± standard deviation of three independent replicate samples. The analyses of variance (ANOVA) were performed using Minitab® 16.1.0 with $\alpha = 0.05$. The analysis multivariate analysis of variance (MANOVA) was performed by applying IBM SSPA Statistics 19 with $\alpha = 0.05$.

Results and discussion

Characteristics of unprocessed beverages

Table 1 shows the pH, TSS, content of antioxidant compounds and PME activity of the untreated pitaya–pineapple beverage. The pH of the beverage was 4.3, so it can be considered as a high acid product. The concentration of

Table 1 Physicochemical characteristics, phytochemical composition, and pectin methylesterase activity of the unprocessed pitaya–pineapple beverage

Parameter	
pH	4.3
TSS (°Brix)	10.6
Vitamin C	2.9 ± 0.3^a
Total phenolics content	7.6 ± 1.1^b
Betacyanins	1.5 ± 0.01^c
Betaxanthins	4.0 ± 0.02^d
PME	0.002985 ± 0.00004^e

^a mg AA/g juice wet basis^b $\mu\text{mol/g}$ wet weight juice^c mg betanin equivalents/g dry basis^d mg betaxanthin equivalents/g dry basis^e s^{-1}

vitamin C was 2.9 ± 0.3 mg AA/g wb. Previous studies have reported vitamin C contents of pitaya from 0.084 to 0.170 mg AA/g wb (*S. stellatus*) and up to 0.217 mg AA/g wb for *S. griseus* (Chuck-Hernández et al. 2016). The content of vitamin C in the beverage is higher than the reported one in the literature for pitaya due to the combination with pineapple [0.48 mg AA/g, (USDA 2015)]. In additions, factors such as maturity, production zone, climate, and other factors could cause the differences in vitamin C contents. Compared with other cactus fruits, the untreated pitaya–pineapple beverage had a lower vitamin C content. Jimenez Aguilar et al. (2015a, b) reported 6.73 ± 0.39 mg AA/g wb for prickly pear from the Rojo cenizo variety and 3.46 ± 0.43 mg AA/g wb for the Rojo San Martín variety (Jiménez-Aguilar et al. 2015b).

Total phenolic compounds in the untreated pitaya–pineapple beverage was 7.6 ± 1.1 $\mu\text{mol/g}$ wb. This concentration is similar to fruits considered as important sources of phenolic like plum (7.8 ± 0.7 $\mu\text{mol/g}$ wb) or blueberry (8.9 ± 1.1 $\mu\text{mol/g}$ wb) (Vinson et al. 2001), but lower than the content reported for prickly pear (38.6 ± 0.6 $\mu\text{mol/g}$ wb for the Rojo Cenizo variety) (Jimenez-Aguilar et al. 2015a, b).

Betacyanins concentration in the beverages was 1.5 ± 0.01 mg/g db while betaxanthins content was 4.0 ± 0.02 mg/g db. Betacyanins contents found in this work differ slightly to values reported in literature for red pitaya (2.9 ± 0.38 mg/g db) due to the addition of pineapple juice (García-Cruz et al. (2013). Betacyanin and betaxanthin concentrations of 7.8 ± 0.7 and 4.3 ± 0.4 mg/g db, respectively, were reported for beet (*Beta vulgaris*) (García-Cruz et al. 2013). This value depends on the ratio applied. The content of betaxanthins in the pitaya–pineapple beverage is similar to the reported value for red beet.

Effect of HHP on functional compounds and PME activity

Vitamin C

The percentage of retention of vitamin C after HHP treatments is shown in Fig. 1a. The vitamin C content ranged from 3.0 ± 0.2 (CUT/600 MPa) to 4.7 ± 0.5 mg AA/juice (CUT/400 MPa), what represents increments from 5 to 64% with respect to the initial content. According to the statistical analysis performed both pressure and time significantly affected vitamin C concentration ($P < 0.05$) (Fig. 1b). In the case of CUT treatments, the higher the pressure the lower the vitamin C contents obtained, while for 2–10 min the pressure seems not have a significant effect. The time greatly influenced the vitamin C content at 400 MPa/CUT, while at the other processing conditions it seems to have an insignificant effect showing similar vitamin C concentrations. The higher vitamin C content observed at some processing conditions could be due to the capacity of HHP to increase the permeability due to rupture of plant cell membranes. Specifically, the pressure could affect the cell organelles and compartments containing high concentrations of ascorbate (cytosol 30–40% and vacuole 20–30%) resulting in the release of vitamin C (Horemans et al. 2000; Patras et al. 2009). Other cacti fruits beverages had been treated with HHP. In *Opuntia ficus-indica* beverages prepared with 10% peel and 90% pulp, the vitamin C retention in beverages pressurized at 400 MPa (CUT-16 min) was close to 100%. At 550 MPa vitamin C content decreased to 21%, and to 39% with the increment of time (Jiménez-Aguilar et al. 2015a). In *Opuntia macro-rhiza* juice the HHP application did not affect the content of vitamin C.

It was generally reported that oxygen and the food matrix influence the AA stability during HPP. For example, vitamin C content of the orange and OLC (orange–lemon–carrot) juices processed at 500 and 800 MPa was not, or only insignificantly, reduced compared to unprocessed juice. Nor did the contents of L-ascorbic acid and dehydroascorbic acid change to any statistically significant extent (García et al. 2001). Results confirm those reported about the stability of L-ascorbic acid specifically in orange juice when pressurized at mild temperatures. However, according to (Esteve and Frigola 2008) who studied the effect of HP treatment (400 MPa/42 °C/5 min) and thermal pasteurization (90 °C, 20 s) on orange juice, the vitamin C loss (in all cases) was higher for high-pressurized juice. Similarly, Pozo-Insfran et al. (2007) found that HP treatment of muscadine GJ at 400 and 550 MPa for 15 min produced decreases in vitamin C concentration of 84 and 18%, respectively, immediately after processing. They

Fig. 1 a Vitamin C retention of pitaya-pineapple beverages treated at different HHP conditions. 400 MPa (light gray bar), 500 MPa (dark gray bar) and 600 MPa (black bar). **b** Statistical analysis of vitamin C

attributed the greater degradation of vitamin C in matrices of this kind to enzyme activity, which is produced at pressures below 550 MPa. Some processing conditions may affect negatively the vitamin C retention: the juice was not deaerated before processing, and the presence of oxygen, high shear, and temperature have certainly contributed to oxidize the vitamin. In addition, the equipment used for homogenization (extractor Turnix TU05, USA) had metal blades that could present a small erosion after processing. According to (Ball 2005), the presence of oxygen and trace metals favors the oxidation of the vitamin by creating metal–oxygen–ascorbate with consequent

formation of dehydroascorbic acid that is irreversibly oxidized.

Phenolic compounds

Total phenolic content in HHP-treated beverages ranged from 3.9 ± 0.8 to 6.6 ± 0.8 $\mu\text{mol/g}$ (wb) which corresponds to retentions from 52 (10 min/400 MPa) to 87% (10 min/600 MPa) (Fig. 2a). Pressure and time were statistically significant ($P < 0.05$). For treatments done during 2–10 min, the higher the pressure level the higher the phenolic retention, while for samples processed during the

Fig. 2 a Total phenolics retention of pitaya-pineapple beverages treated at different HHP conditions. 400 MPa (light gray square), 500 MPa (dark gray square) and 600 MPa (black square). **b** Statistical analysis of total phenolics

CUT, a clear effect of pressure level was not observed (Fig. 2b). The lowest loss of total phenolic compounds occurred at 400 MPa/CUT (19%), coinciding with the conditions that produced the highest vitamin C concentrations. Decrement of total phenolic compounds during the treatment could be attributed to enzymatic degradation caused mainly by peroxidases (POD) and polyphenoloxidases (PPO) present in pineapple (Chutintrasri and Noomhorm 2006; Chakraborty et al. 2014). POD oxidize the phenol utilizing H_2O_2 , and PPO catalyze the oxidation of phenolics compounds to quinones producing brown pigments (Eisenmenger and Reyes-De-Corcuera 2009). For prickly pear beverages, significant increments in phenolic contents from 16 to 20% at 550 MPa at 2 and 4 min,

respectively, were observed by Jiménez-Aguilar et al. (2015a, b).

Betacyanins

The betacyanins concentration in HHP-treated beverages ranged between 1.4 ± 0.03 (10 min/500 MPa) and 1.5 ± 0.04 mg/g (dw) (CUT/400 MPa) which corresponds to retentions from 92 to 100% without significant statistical difference as proven by the lack of effect of pressure and time ($P > 0.05$) (Fig. 3a, b). Similar to this work, Jimenez-Aguilar et al. (2015a, b) reported that betacyanin concentration in prickly pear (*Opuntia ficus-indica*) did not change significantly after 400 MPa/CUT-

Fig. 3 **a** Betacyanins retention of pitaya-pineapple beverages treated at different HHP conditions. 400 MPa (light gray square), 500 MPa (dark gray square) and 600 MPa (black square). **b** Statistical analysis of betacyanins. **c** Betaxanthin retention of pitaya-pineapple beverages

treated at different HHP conditions. 400 MPa (light gray square), 500 MPa (dark gray square) and 600 MPa (black square). **d** Statistical analysis of betaxanthin

8 min, but at 550 MPa/2 min, they decreased by 8% (Jiménez-Aguilar et al. 2015a). Even though the pH of the pitaya–pineapple beverage is lower (4.3) than the optimal pH range (5.5–5.8) reported for betanin (a type of betacyanin) stability (Herbach et al. 2006), the betacyanins were not affected under HHP because the behavior of systems at atmospheric pressure could change under high pressure.

Betaxanthins

The betaxanthins concentration in HHP treated beverages ranged from 3.8 ± 0.09 to 4.0 ± 0.17 mg/g (dw) which corresponds to retentions from 94 (10 min/500 MPa) to 101% (5 min/500 MPa) (Fig. 3c, d). The time significantly affected the retentions observed ($P < 0.05$). For example, at 500 MPa and treatment times from CUT–5 min retentions of about 100% were observed while at 10 min a retention of 94% was obtained. Similar to this work Jiménez-Aguilar et al. (2015a) reported a loss of 8% in

prickly pear beverages after 2 min of pressurization at 550 MPa.

Pectin methylesterase inactivation

The PME residual activity of the pitaya–pineapple beverage measured immediately after HHP treatments ranged from 77 (600 MPa/2–10 min) to 107% (400 MPa/10 min) (Fig. 4) showing a high resistance to be inactivated by pressure. The increment on PME activity observed at some processing conditions could be related with their release from cell compartments. The high baroresistance of this enzyme was previously observed in products like tomato juice (residual activity of 72% at 200 MPa/10 min/25 °C/pH 3.8–4.2 adjusted to 4.5) (Kuo-Chiang 2008) and mango nectar (55% at 345 MPa/4 min/<17 °C, pH was adjusted to 7.0) (Bermúdez-Aguirre et al. 2011). For beverages treated during 2–10 min, it is noted that the higher the pressure level the lower the residual PME activity. There is not a clear effect of the CUT on the

Fig. 4 PME retention of pitaya-pineapple beverages treated at different HHP conditions. 400 MPa (light gray square), 500 MPa (dark gray square) and 600 MPa (black square)

enzymatic activity. Similar behavior was observed on PME of Greek navel orange juice (during processing at 30 °C/400, 500, 600 and 700 MPa) (Polydera et al. 2004). A combination of high pressure and moderate temperature is recommended to increase the sensitivity of PME to HHP processes (Ludikhuyze et al. 2001; Tejada-Ortigoza et al. 2015). By combining pressure and heat, Chakraborty et al. (2014) obtained a maximum inactivation level of 42% for pineapple puree treated at 500 MPa/60 °C/pH 3.0. The combination of HHP and moderate temperature at not more than 50 °C due to the sensitivity of betalains to temperature, can be used to increase the PME inactivation levels.

Conclusion

The treatment at 400 MPa/CUT had a great impact on the content of vitamin C causing an increase of 64% compared with untreated beverages. Beverages lost 13%–48% of total phenolic compounds at 400–600 MPa/CUT-6 min, probably due to the action of peroxidases and polyphenoloxidases from pineapple. Betacyanin and betaxanthin were barely affected by the treatments showing retentions near to 100%. The maximum PME inactivation level was near to 23% at 600 MPa/2–10 min. A combination of HHP and temperature was suggested for the inactivation of PME, as long as the temperature was below 50 °C, to maintain betalains stability. At 600 MPa/10 min retentions of vitamin C of 133%, total phenolic of 87%, betacyanins and betaxanthins of 96% were obtained, and PME activity decreased by 21%, so HHP (600 MPa/10 min) seem to be a good option to preserve antioxidants in pitaya-pineapple beverage, but more studies or treatment methods must be tested to obtain beverages with higher PME inactivation levels.

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