Vol. 19, No. 1 (2020) 387-399
Revista Mexicana de Ingeniería Química

REFRIGERATED STORAGE OF HIGH HYDROSTATIC PRESSURE (HHP) TREATED PITAYA (*Stenocereus pruinosus*) JUICE

ALMACENAMIENTO REFRIGERADO DE JUGO DE PITAYA (*Stenocereus pruinosus*) TRATADO CON ALTAS PRESIONES HIDROSTÁTICAS (APH)

B. Quiroz-González[1], V. Rodriguez-Martinez[2], J. Welti-Chanes[2], M. del R. García-Mateos[3]*, J. Corrales-García[1], Ma. C. Ybarra-Moncada[1], G. Leyva-Ruelas[1], J.A. Torres[2]

[1]*Departamento de Ingeniería Agroindustrial. Universidad Autónoma Chapingo. Carretera Federal México-Texcoco km 38.5, Texcoco, Edo. de México 56230, México.*
[2]*Centro de Biotecnología-FEMSA, Escuela de Ingeniería y Ciencias, Tecnológico de Monterrey. Av. Eugenio Garza Sada 2501, Monterrey, NL 64849 México.*
[3]*Departamento de Fitotecnia, Universidad Autónoma Chapingo. Carretera Federal México-Texcoco km 38.5, Texcoco, Edo. de México 56230, México.*

Received: April 11, 2019; Accepted: July 13, 2019

Abstract

This work focuses on HHP and refrigerated storage effects on the quality and acceptability of pitaya juice (pH 5.2, 10°Bx, 9.5% solids) stored under refrigeration. Pitaya is a spheroid fruit from native Mexican cactus, with a juicy and sweet pulp. After 550 and 600MPa treatments for 16 and 12 min, respectively, pitaya juice was stored 60 d at 4±1°C. No aerobic mesophiles were found immediately after HHP treatments, and during storage, population remained below 2 $\log_{10}$ CFU mL^{-1}. In HHP treated juice, yeast and molds were undetectable and remained so during storage. After HHP treatments and during storage, no changes were observed in total soluble solids (9.8 10.0 °Bx), luminosity (4.3 4.4%), and *chroma* (18.9-20.7). Depending on treatment condition, HHP lowered residual PME activity by 59-63%. A further reduction reaching 25% was observed during storage. Acidity, phenolic compounds, betalains concentrations, and antioxidant activity were not affected by HHP but a 43, 10, 14, and 5% decrease, respectively, was observed after 60 d of storage. Finally, sensorial acceptability was not affected by HHP but increased during storage reflecting an increased sweetness perception associated with lower acidity values. This study confirmed HHP as a viable alternative to commercialize pitaya juice with a refrigerated distribution shelf life exceeding 60 d.

Keywords: antioxidant, pitaya juice, sensory quality, refrigerated storage.

Resumen

En este estudio se evalúo el efecto del tratamiento APH en la estabilidad del jugo de pitaya (pH 5.2, 10°Bx, 9.5% sólidos) almacenado bajo refrigeración. La pitaya es una fruta esferoide con pulpa jugosa y dulce de una cactácea nativa de México. Después de tratamientos a 550MPa-16min y 600MPa-12min, el jugo se almacenó 60 d a 4±1°C. No se encontraron mesófilos aerobios después de aplicar APH, y durante el almacenamiento los valores fueron inferiores a 2 $\log_{10}$ UFC mL^{-1}. Tampoco se detectaron levaduras, ni hongos y no hubo cambios en los sólidos solubles totales (9.8 10.0°Bx), luminosidad (4.3 4.4%), e índice de saturación (18.9-20.7) en los jugos tratados y almacenados. Dependiendo del tratamiento APH, la actividad residual de pectínmetilesterasa disminuyó 59-63%, y un 25% adicional durante el almacenamiento. La acidez, compuestos fenólicos, concentración de betalaínas, y actividad antioxidante no fueron afectadas por APH, pero después de 60 d decrecieron en 43, 10, 14 y 5%, respectivamente. El tratamiento APH no afectó la aceptabilidad del jugo, pero incrementó durante el almacenamiento reflejando una mayor percepción de dulzor asociado al decremento de acidez. Este estudio confirma APH como una alternativa viable para la producción comercial y distribución refrigerada de jugo de pitaya con vida útil superior a 60 d.

Palabras clave: antioxidante, jugo de pitaya, calidad sensorial, almacenamiento refrigerado.

* *Corresponding author. E-mail*: rosgar08@hotmail.com
https://doi.org/10.24275/rmiq/Alim588
issn-e: 2395-8472

1 Introduction

Pitaya, a fruit from the pitayo, a columnar cactus belonging to the genus *Stenocereus* is a rounded or ovoid shaped polyspermatic pigmented berry with exocarp (peel) areoles with small needles or thorns (Buxbaum, 1961; Bravo & Sánchez-Mejorada, 1991; Pimienta-Barrios & Nobel, 1994; Arnaud *et al*., 1997; Quiroz-González *et al*., 2018). Its juicy pulp with pleasant sweet, fresh and delicate taste (Yáñez-López *et al*., 2005) has desirable nutritional and antioxidant properties (García-Cruz *et al*., 2016). Currently, the fruit is not widely commercialized due to its perishability (Ochoa-Velasco & Guerrero Beltrán, 2013; García-Cruz, Valle-Guadarrama *et al*., 2016).

Although many nonthermal treatments for fruit juice preservation have been developed such as UV-C, high hydrostatic pressure (HHP), ozone, ultrasound, cold plasma, and membrane processing (Ochoa-Velasco & Guerrero Beltrán, 2012; Ochoa-Velasco & Guerrero Beltrán, 2013; Akgün & Ünlütürk, 2017; Bevilacqua *et al*., 2018; Pérez-Grijalba *et al*., 2018), very few studies report the application of these technologies on pitaya juice. For example, UV-C treatments of pitaya juice lowered the population of aerobic mesophiles by 2.1 $\log_{10}$ UFC mL^{-1}, molds and yeast by 1.1 $\log_{10}$ UFC mL^{-1}, and *Zygosaccharomyces bailii* by 1.8 $\log_{10}$ UFC mL^{-1} (Ochoa-Velasco & Guerrero Beltrán, 2012; Ochoa-Velasco & Guerrero Beltrán, 2013). However, the microbial load (aerobic mesophiles and *Z. bailii*) reached 3.5 $\log_{10}$ UFC mL^{-1} after 5 d of storage at 4 °C, and after 25 d they observed a 25, 11, and 42 % reduction of betalains, phenolic compounds, and antioxidant activity, respectively. Sandate-Flores *et al*. (2017) treated a pineapple-pitaya beverage at 400-600 MPa for 2-10 min. While the treatments retained betacyanins and increased the vitamin C content (5-65 %), a decrease was observed in the concentration of phenolic compounds (20-48 %) and betaxanthins (6 %). Although these results were promising, the microbial stability of the juice mix during storage was not evaluated.

Previous work on pitaya juice without acidification showed that the pressure increase during come-up time (CUT) to 400 MPa (112 s) inactivated *Saccharomyces cerevisiae* to undetectable levels (Quiroz-González *et al*., 2018). Furthermore, 550 MPa/16 min and 600 MPa/12 min treatments achieved 5-log reductions of *Listeria innocua*. Further decreases in *L. innocua* population during storage at 4±1 °C confirmed that HHP-treated pitaya juice would meet current regulations for the commercialization of refrigerated pasteurized juice. However, the sensorial, physicochemical, and nutraceutical characteristics of HPP-treated juice were not evaluated. Therefore, the aim of this work was to assess the effects of these previously tested HPP-treatments, and of subsequent storage under refrigeration, on the native microbiota, pectin methylesterase (PME) activity, physicochemical properties, nutraceuticals content, and sensory properties of pitaya juice without acidification.

2 Materials and methods

2.1 Sample preparation

Pitaya fruits (*Stenocereus* sp.) at commercial maturity were harvested in Santa Clara Huitziltepec (Puebla, Mexico, Latitude: 18°46 'N, Longitude: 97°53' W). After harvesting, pitaya fruits were stored and transported in an air-conditioned vehicle (12±2 °C) to the FEMSA Biotechnology Center laboratories (Monterrey, NL, Mexico). Prior to juice extraction at room temperature (16±2 °C), the pitaya thorns and pericarp were removed manually. The peeled pitaya fruits were placed in a pulper (Polinox^{TM}, Agrícola Industrial, Ciudad de México, Mexico; with a 0.3 mm sieve pore diameter) to remove seeds. Finally, the juice collected (pH 5.2, 10 °Bx and 9.5% solids) was frozen and stored at -20 °C for later use within 4 months. Pitaya juice was not acidified to avoid changes in the organoleptic properties and to generate a clean label product.

2.2 High hydrostatic pressure (HHP) treatments

Samples of juice (15 mL) were placed in polyethylene bags (Filmpack S.A. de C.V., Puebla, Mexico), vacuum-sealed and treated at 550 and 600 MPa for 16 and 12 min, respectively (Flow Autoclave Systems, Model 2 L, Columbus, OH), using water at room temperature (20 °C) as pressurizing medium. These processing conditions were previously shown to reduce by 5-log the population of *L. innocua*, a suitable surrogate for *L. monocytogenes* due to its physiological, biochemical, metabolic,

and barotolerance similarity (Quiroz-González, Rodríguez-Martínez *et al*., 2018). Adiabatic heating during compression increased the pressurizing medium temperatures reaching values in the 26 to 33 °C range. Treated juices were analyzed after 0 (immediately after treatment), 5, 10, 15, 25, 30, 35, 45 and 60 d of storage at 4±1 °C while untreated controls were analyzed at 0, 5, 10 and 15 d of storage at 4±1 °C. In addition, untreated and treated juice samples collected at each sampling day were frozen, stored at -80 °C, and then thawed for sensory evaluation on day 63 after HHP treatment.

2.3 TSS and acidity evaluation

The total soluble solids (TSS), moisture content and acidity (expressed as g malic acid 100 g^{-1} db) of pitaya juice were measured following Methods 932.12, 920.151, and 942.15 (AOAC, 1995), respectively. All measurements are reported as the average and standard deviation for three replicates.

2.4 Microbial evaluation

HHP-treated and untreated juice was decimally diluted using respectively 5 and 1 mL for the first and subsequent aliquots into 45 and 9 mL 0.1 % peptone-water (BD Bioxon, Becton Dickinson, Estado de México, Mexico). Aerobic mesophilic bacteria dilutions were pour-plated onto Plate Count Agar (PCA, BD Difco^{TM}, Sparks, MD) plates and incubated at 37 °C for 48 h. Yeast and molds (Y&M) populations were obtained on Potato Dextrose Agar (PDA, BD Bioxon, pH adjusted to 3.5 with tartaric acid) plates incubated 5 d at 27 °C (Leyva-Daniel *et al*., 2017). Total coliforms dilutions were pour-plated onto Violet Red Bile Agar (VRBA, Merck, Darmstadt, Hesse) plates and incubated at 37 °C for 24 h (Akgün & Ünlütürk, 2017). Values in juice were expressed as log CFU mL^{-1} and then averaged (n = 6).

2.5 Residual pectin methylesterase (PME) activity determination

Residual PME (EC 3.1.1.11) activity potentially causing an undesirable loss in juice turbidity (Liu *et al*., 2012; Sandate-Flores, Rostro-Alanis *et al*., 2017) was evaluated as described by Sandate-Flores *et al*. (2017). Briefly, pitaya juice (10 g) was mixed with 10 mL of citrus pectin (1 % w/v, > 6.7 % methoxyl groups, Sigma-Aldrich, St. Louis, MO) and NaCl solution (0.1 M, J.T. Baker, Luis I, Madrid, Spain). After mixing (200 rpm at 37 °C, hot plate, CTR Scientific, Monterrey, Nuevo León, Mexico), pH was adjusted to 7.7 with NaOH (0.1 M, J.T. Baker) and immediately after adding 20 μL (v) of 0.1 M NaOH (c), the reaction time (t) to regain pH 7.7 was measured. PME activity was reported as residual activity percentage.

2.6 Color changes evaluation

Net color change (ΔE), *hue*, brightness and *chroma* were measured following the methodology proposed by McGuire (1992) and using a Hunter Lab colorimeter (MiniScanTM XE Plus No. 45/O-L, series 5348, Reston, VA). All measurements are reported as the average and standard deviation for three replicates.

2.7 Betalains, total phenolic concentration antioxidant activity quantification

Juice aliquots (2 g) were diluted in 8 mL methanol (Sigma-Aldrich, 99.8 %). After 5 min sonication at 5±1 °C (Ultrasonic Cleaner Model 2510R-MTH, Branson, Gaithersburg, MD) and centrifugation at 10062 g (calculated for the specific rotor diameter and RPM used) and 4±2 °C for 5 min (Model SL 16R, Thermo Scientific, Osterode, Germany), the supernatant methanolic extract fraction was used to quantify betalains, phenolic compounds, and antioxidant activity as described next.

2.7.1 Betalains

These were quantified as described by Pérez-Loredo *et al*. (2016). Samples (300 μL) of methanolic extract, previously diluted 1:9 (v/v) with tridistilled water, were analyzed using a 96-well microplate lector (BioTek, Synergy HT, Winooski, VT). As proposed by García-Cruz *et al*. (2016), readings at 535 and 483 nm were used to determine betacyanins ($C_{betacyanin}$) and betaxanthins ($C_{betaxanthins}$) concentrations (Eq. 2), respectively:

$$C_{betacyanins}\ or\ C_{betaxanthins}(mgg^{-1}, db) = \frac{(1000 \cdot A \cdot DF \cdot MW \cdot V)}{(\varepsilon \cdot W \cdot L)} \quad (1)$$

where: A = absorbance reading, DF = dilution factor, MW = molecular weight (betacyanin and betaxanthin, 550 and 308 g moL^{-1}, respectively), V = extract volume (mL), ε = molar extinction coefficient (betacyanin and betaxanthin, 60 000 and 48 000 mL

mol^{-1} cm^{-1}, respectively) as reported by García-Cruz *et al*. (2016), W = juice weight (g), and L = cell path length (cm). All measurements are reported as the average and standard deviation for three replicates.

2.7.2 Total phenolic compounds

These metabolites were quantified as proposed by Sánchez-Rangel *et al*. (2013). Methanolic extract (15 μL), Folin-Ciocalteu reagent (0.25 N, 15 μL, Sigma-Aldrich), and tridistilled water (240 μL) were placed in a microplate. After keeping it under darkness for 3 min and then adding Na_2CO_3 (30 μL, 1N, J.T. Baker), the mixture was kept under darkness for an additional 2 h before absorbance readings at 765 nm. Total phenolic compounds were determined using a gallic acid (GA, 0.016 to 0.099 mg mL^{-1}, Sigma-Aldrich) standard curve. Reported values (mg GA g^{-1} db) are based on three readings for three sample replicates.

2.7.3 Antioxidant activity

Antioxidant activity was evaluated as described by Villarreal-Lozoya *et al*. (2007) with some modifications. Briefly, 234 μL of 160.7 μm DPPH? (2,2-Diphenyl-1-picrylhydrazyl; Sigma-Aldrich) were mixed with 26 μL of methanolic extract. A blank was prepared similarly to the sample but using 234 μL of methanol instead of DPPH. After 15 min, antioxidant activity (AOX) was estimated from sample (ABS_{sample}) and blank (ABS_{blank}) absorbance readings at 515 nm A standard curve using Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, 172.4 to 431 μM; Sigma-Aldrich) was used to estimate AOX values as mM Trolox Equivalent (TE)·g^{-1} db reported as the average for three sample replicates.

2.8 Effect of HHP on overall sensory acceptability of juices

The juice overall sensory acceptability was assessed by a panel of 64 males and 80 females (18-33 yrs) using a 9-point hedonic scale where 9 = like extremely, 5 = neither like nor dislike, and 1 = dislike extremely. Each panelist evaluated four samples in balanced randomized order, including a control (untreated juiced) and three HHP-treated juice samples. The HHP-treated samples were from either of the two HHP treatment conditions (550 MPa/16 min; 600MPa/12 min) and each of the storage times at 4±1 °C. Untreated juiced (0 d) and treated samples were kept at 80 °C after the corresponding refrigerated storage time (0, 15 and 60 d), and then thawed on day 63 for sensory evaluation.

2.9 Statistical analysis

Experimental values were analyzed by a mixed model for repeated measures (Eq. 2), with post-hoc Tukey test (Statistical Analysis System V9, for Windows, SAS Institute Inc., Cary, NC).

$$y_{ijt} = \mu + \alpha_i + d_{j(i)} + \gamma_t + (\alpha\gamma)_{it} + e_{ijt} \qquad (2)$$

Where y_{ijt} = response at time t on the j^{th} experimental unit assigned to the i^{th} treatment and assuming normal independent distribution approximation with mean 0 and variance σ_y^2, μ = overall mean effect, α_i = i^{th} fixed treatment effect, $d_{j(i)}$ = random effect of j^{th} experimental unit within the i^{th} treatment and assuming normal independent distribution approximation with mean 0 and variance σ_d^2, Y_t = fixed t^{th} time effect when measurement is taken, $(\alpha\gamma)_{it}$ = fixed interaction effect between treatment and time, e_{ijt} = random error associated with the jth experimental unit assigned to the i^{th} treatment at time t, e_{ijt} assuming normal independent distribution approximation with mean 0 and variance σ_e^2. Finally, $d_{j(i)}$ and e_{ijt} were assumed independent (Wang & Goonewardene, 2004). The covariance structures used were compound symmetry, variance components, self-regressive, Toeplitz, and unstructured while the method used was multiple linear regression. The estimated variance-covariance matrix was used to model the natural variation of the data in order to improve the prediction of treatment effects and the comparison between treatments over time. The χ^2 (Chi-square) Friedman's test was used to determine significant sensorial differences (Eq. 3):

$$\chi^2 = \frac{12}{[p(t)(t+1)]} \sum_{i=1}^{t} x.j - [3(p)(t+1)] \qquad (3)$$

where p = number of panelists; t = number of treatments, $x.j$ = sum of the j treatment arrangement; the degrees of freedom for χ^2 are $t-1$. The minimum significant order difference (MSOD) was determined to compare two rank sums ($p < 0.05$) according to Eq. 4:

$$MSOD = z_{\alpha/2}\sqrt{(pt(t+1)/6} \qquad (4)$$

where $z_{\alpha/2}$ is the critical value of normal distribution with $\alpha/2$ (Hernández-Montes, 2007; Valdivia-Nájar *et al*., 2018).

Table 1. Changes in pitaya acidity juice during storage at 4±1 °C

Storage time (days)	Acidity (Expressed as g malic acid/100 g)		
	Untreated	550 MPa 16 min	600 MPa 12 min
0	0.14 ± 0.01^{a}	0.14 ± 0.01^{aA}	0.15 ± 0.01^{aA}
5	0.11 ± 0.0^{b}	0.11 ± 0.01^{bB}	0.10 ± 0.01^{bB}
10	0.10 ± 0.01^{c}	0.10 ± 0.01^{cC}	0.10 ± 0.01^{cC}
15	0.09 ± 0.00^{d}	0.08 ± 0.00^{dD}	0.08 ± 0.00^{dD}
25	-	0.08 ± 0.00^{D}	0.08 ± 0.00^{D}
30	-	0.07 ± 0.00^{D}	0.08 ± 0.00^{D}
35	-	0.08 ± 0.00^{D}	0.08 ± 0.00^{D}
45	-	0.07 ± 0.00^{D}	0.07 ± 0.00^{D}
60	-	0.08 ± 0.00^{D}	0.08 ± 0.00^{D}

Values sharing same letter indicate no significant differences (Tukey mean comparison, $p < 0.05$). Lowercase and uppercase letters indicate the evaluation of all or only treated juices stored 15 and 60 d, respectively. After 15 d, untreated samples showed significant spoilage and were discarded.

3 Results and discussion

3.1 *Effect of HHP on TSS and acidity*

There was no HHP processing effect ($p < 0.05$) on juice TSS and acidity. While storage time had no effect on TSS values (10.0-9.9 °Bx, data not shown), acidity reduced ($p < 0.05$) from an initial 0.14-0.15 value to 0.07-0.08 % malic acid at 60 d (Table 1). A decline in the concentration of organic acids (malic, ascorbic and citric) during storage has been reported for apple, orange, grape, and strawberry juice (Cao *et al*., 2012; Juarez-Enriquez *et al*., 2015; Garcia *et al*., 2018; Mezey & Mezeyová, 2018). These reductions could be caused by oxidative reactions due to presence of residual oxygen, oxygen permeation across the polyethylene bag (Cao, Bi *et al*., 2012; Juarez-Enriquez, Salmeron-Ochoa *et al*., 2015), residual enzymatic activity (González-Agüero *et al*., 2016), or presence of microorganisms using malic acid as an energy source (García *et al*., 2018).

3.2 *Effect of HHP on native microbiota*

Previous work (Quiroz-González, Rodríguez-Martínez *et al*., 2018) showed that the HPP treatments selected for this study achieve a 5-log reduction in the *L. innocua* population. To comply with US legislation ("Pathogen Reduction," *Code of Federal Regulations*, Title 21 §120-Subpart B 2018), *Listeria monocytogenes* was considered to be the "pertinent microorganism" in the pitaya juice investigated in this study. Non-pathogenic *L. innocua* was used as surrogate due to its physiological, biochemical, metabolic, and barotolerance resemblance to this pathogen (Tay *et al*., 2003; Sokołowska *et al*., 2014). Comparisons with *Escherichia coli* and *Salmonella* strains have shown that *L. innocua* is more barotolerant than these pathogens (e.g., Alpas *et al*., 2000; Guerrero-Beltrán *et al*., 2011). The initial aerobic mesophilic bacteria population in pitaya juice was 2.87 $\log_{10}$ CFU mL^{-1}. HHP processing reduced it to below detection levels and remained so for 10 and 15 d for 550 MPa/16 min and 600 MPa/12 min treated juice, respectively (Figure 1). The microbial inactivation for longer time at the higher pressurization level may reflect an increased protein denaturation, rupture, or lipid phase change of cell membranes (Cheftel, 1995; Lado & Yousef, 2002; Huang *et al*., 2014). After 15 d, the aerobic mesophilic population increase for untreated juice exceeded 8 log CFU mL^{-1}, and thus data collection for untreated juice samples was discontinued. In the case of treated samples, the mesophilic population remained at 2 log CFU mL^{-1} or lower. As previously stated, the same treatments lowered *L. innocua* population by more than 5 $\log_{10}$ CFU mL^{-1} and reduced *Saccharomyces cerevisiae* counts to below detectable levels (Quiroz-González, Rodríguez-Martínez *et al*., 2018). In the case of mold and yeast population, untreated juice showed around 5 log CFU mL^{-1} at 15 d of storage (Table 2) while for HHP-treated juice their population reached below detection level after all treatments and remained so

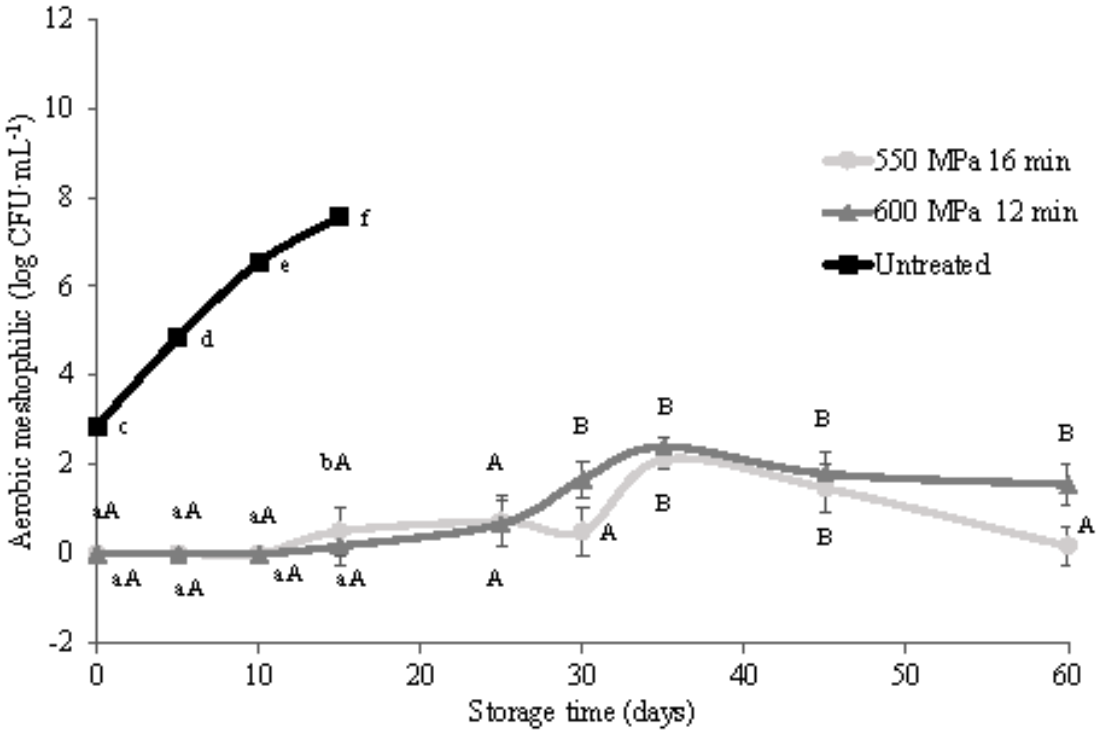

Fig. 1. Aerobic mesophilic counts in pitaya juice stored at 4±1 °C. Values sharing same letter indicate no significant differences (Tukey mean comparison, $p < 0.05$). Lowercase and uppercase letters indicate the evaluation of all or only treated juices stored 15 and 60 d, respectively. Vertical bars: standard deviation

Table 2. Molds and yeast populations (log CFU mL^{-1}) in untreated pitaya juice during storage at 4±1 °C

Storage time (days)	Log (CFU mL^{-1}), mean ± standard deviation (n=6)
0	2.83 ± 0.03 a
5	2.89 ± 0.05 b
10	4.55 ± 0.04 c
15	4.59 ± 0.08 c

No significant difference between values sharing same letter (Tukey mean comparison, $p < 0.05$).

during storage (data not shown). These observations confirmed that the 550 MPa/16 min and 600 MPa/12 min treatments are safe processing options allowing the commercial distribution of refrigerated non-acidified pitaya juice.

3.3 Effect of HHP on residual pectin methylesterase (PME) activity

The reduction in residual PME activity in untreated juice reached 58 % after 15 d storage. HHP processing reduced residual PME activity by 59-63 % ($p = 0.0001$), remained approximately constant during 30 d storage, and then decreased even further reaching 16 % residual activity at 60 d (Fig. 2). Probably, HHP process induced tertiary and quaternary conformational changes of the enzyme (Buckow & Terefe, 2017; Morales-de la Peña *et al.*, 2018). Residual PME activity reduction after HHP or storage at 4±1 °C effect was sufficient to retain the juice without alteration of its rheological properties (data not shown).

3.4 Effect of HHP on color parameters

HHP treatments had no effect on juice ΔE ($p > 0.05$); however, this color parameter increased during storage for untreated and HHP-treated samples ($p = 0.0001$). Color stability of HHP-treated juices storage effects have been previously reported. No color changes were observed for strawberry juice treated at 600 MPa (4 min), orange juice treated at 600 MPa (1 min), and mulberry juice treated at 500 MPa (5 min) but during storage for 60, 30 and 28 d at 4 °C the corresponding

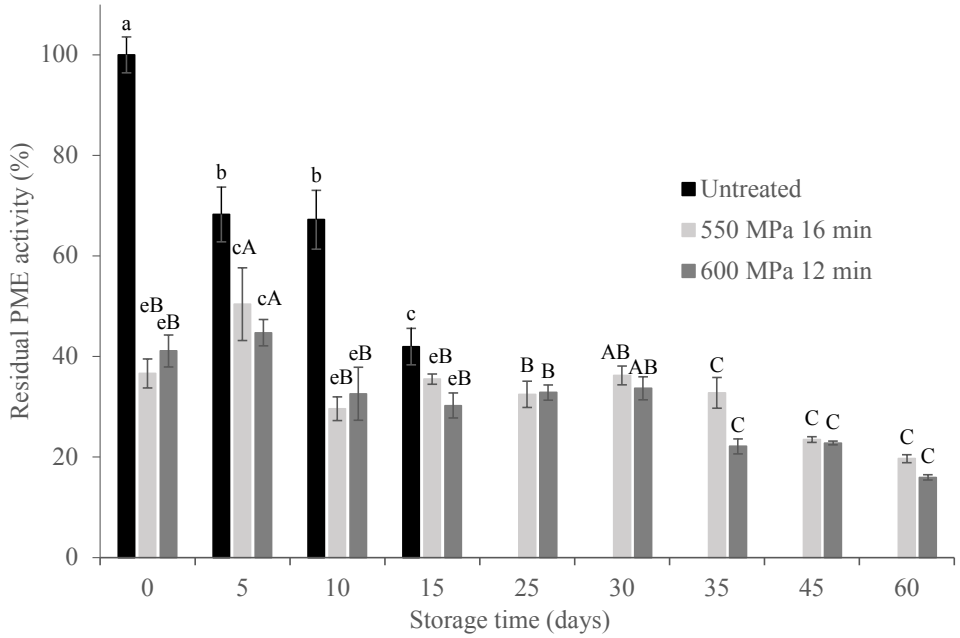

Fig. 2. Changes in residual PME activity in pitaya juice during storage at 4±1 °C). Values sharing same letter indicate no significant differences (Tukey mean comparison, $p < 0.05$). Lowercase and uppercase letters indicate the evaluation of all or only treated juices stored 15 and 60 d, respectively. Vertical bars: standard deviation.

Table 3. Net color change and hue in pitaya juice during storage at 4±1 °C

Time (days)	Net color change (ΔE)			Hue (°)		
	Untreated	550 MPa 16 min	600 MPa 12 min	Untreated	550 MPa 16 min	600 MPa 12 min
0	0.10 ± 0.06^{a}	0.14 ± 0.01^{aA}	0.14 ± 0.04^{aA}	10.0 ± 0.1^{a}	10.1 ± 0.2^{aA}	10.1 ± 0.2^{aA}
5	1.10 ± 0.05^{c}	0.57 ± 0.11^{abA}	0.45 ± 0.18^{abA}	10.8 ± 0.3^{b}	10.6 ± 0.1^{bB}	10.7 ± 0.3^{bB}
10	1.41 ± 0.41^{cd}	0.57 ± 0.23^{abA}	0.37 ± 0.03^{abA}	11.1 ± 0.1^{c}	11.1 ± 0.1^{cB}	11.1 ± 0.1^{cB}
15	1.33 ± 0.04^{cd}	1.67 ± 0.08^{eB}	1.61 ± 0.04^{deB}	11.8 ± 1.4^{bc}	11.8 ± 0.2^{bcB}	11.5 ± 0.1^{bcB}
25	-	1.58 ± 0.23^{B}	1.58 ± 0.11^{B}	-	14.2 ± 0.4^{C}	14.7 ± 0.2^{C}
30	-	1.77 ± 0.29^{B}	1.54 ± 0.08^{B}	-	14.6 ± 0.4^{C}	14.8 ± 0.3^{C}
35	-	2.00 ± 0.39^{BC}	2.13 ± 0.38^{BC}	-	15.1 ± 0.2^{D}	15.5 ± 0.5^{D}
45	-	2.10 ± 0.45^{BC}	1.94 ± 0.29^{BC}	-	15.7 ± 0.2^{D}	15.4 ± 0.2^{D}
60	-	2.24 ± 0.30^{C}	2.07 ± 0.11^{C}	-	16.3 ± 0.3^{E}	16.3 ± 0.1^{E}

Values sharing same letter indicate no significant differences (Tukey mean comparison, $p < 0.05$). Lowercase and uppercase letters indicate the evaluation of all or only treated juices stored 15 and 60 d, respectively. After 15 d, untreated samples showed significant spoilage and were discarded.

ΔE values were 5.52 (Bull *et al*. (2004), 4.8 (Cao *et al*., 2012), and 1.38 (Zou *et al*., 2016). In this study, ΔE reached a 2.24 maximum after 60 d (Table 3). Consistent with the absence of effects on ΔE, *hue*, luminosity and *chroma* parameters were not affected by HHP processing ($p > 0.05$). However, treated and untreated juice samples showed a significant *hue* increase during storage (Table 3), i.e., a loss in red tone suggesting a pigment loss. This may reflect enzyme and/or microbial activity not fully inactivated by the HPP treatment (Oey *et al*., 2008). While untreated juices showed a reduction in luminosity (4.2 to 3.4°) and *chroma* (19.8 to 18.6) at 15 d, in treated juices the changes in luminosity (4.3 to 4.4°) and *chroma* (19.9 to 20.3) during cold storage (60 d) was not significant, i.e., treated juices had a bright color and retained color intensity during storage.

Table 4. Changes in total betalains, and betacyanins content (mg g^{-1} db) in pitaya juice during storage at 4±1°C

Storage time (days)	Betalains			Betacyanins		
	Untreated	550 MPa 16 min	600 MPa 12 min	Untreated	550 MPa 16 min	600 MPa 12 min
0	42.7±2.7^{a}	40.8±1.8^{aA}	40.6±1.0^{aA}	21.5±0.9^{a}	20.7±1.0^{aA}	21.1±0.3^{aA}
5	43.4±2.2^{a}	40.8±1.4^{aA}	39.2±4.1^{aA}	21.9±1.1^{a}	20.6±0.7^{aAB}	19.8±2.0^{aAB}
10	42.1±2.9^{a}	41.7±1.2^{aA}	41.1±1.3^{aA}	21.2±1.4^{a}	20.9±0.6^{aA}	20.7±0.6^{aA}
15	41.3±2.2^{a}	41.9±1.0^{aA}	40.5±0.6^{aA}	20.8±1.1^{a}	21.1±0.6^{aA}	20.9±0.2^{aA}
25	-	39.8±1.0^{A}	40.1±2.0^{A}	-	19.2±0.3^{BC}	19.4±1.0^{BC}
30	-	39.2±1.7^{A}	40.2±2.0^{A}	-	18.9±0.8^{BC}	19.0±1.3^{BC}
35	-	37.2±1.0^{B}	36.2±2.7^{B}	-	17.4±0.1^{CD}	17.3±1.5^{CD}
45	-	36.1±1.5^{B}	35.9±1.5^{B}	-	16.9±0.6^{D}	16.9±1.1^{D}
60	-	35.6±2.5^{B}	35.0±0.6^{B}	-	16.4±1.2^{D}	15.8±0.2^{D}

Values sharing same letter indicate no significant differences (Tukey mean comparison, $p < 0.05$). Lowercase and uppercase letters indicate the evaluation of all or only treated juices stored 15 and 60 d, respectively. After 15 d, untreated samples showed significant spoilage and were discarded.

Table 5. Changes in total phenols and antioxidant activity in pitaya juice during storage at 4±1 °C.

Storage time (days)	Total phenols (mg gallic acid ·g^{-1} db)			Antioxidant activity (mM Trolox Equivalent·g^{-1} db)		
	Untreated	550 MPa 16 min	600 MPa 12 min	Untreated	550 MPa 16 min	600 MPa 12 min
0	0.40±0.01^{a}	0.40±0.01^{aA}	0.40±0.01^{aA}	2.69±0.06^{a}	2.72±0.03^{aA}	2.67±0.06^{aA}
5	0.40±0.00^{a}	0.40±0.01^{aA}	0.41±0.01^{aA}	2.60±0.12^{a}	2.69±0.06^{aA}	2.68±0.03^{aA}
10	0.40±0.02^{a}	0.40±0.01^{aA}	0.40±0.02^{aAB}	2.59±0.10^{a}	2.68±0.08^{aA}	2.68±0.09^{aA}
15	0.39 ±0.01^{a}	0.41±0.01^{aA}	0.40±0.01^{aA}	2.46±0.03^{c}	2.60±0.06^{bA}	2.66±0.08^{bA}
25	-	0.40±0.01^{A}	0.39±0.01^{A}	-	2.63±0.04^{A}	2.64±0.04^{A}
30	-	0.40±0.01^{A}	0.39±0.02^{A}	-	2.60±0.06^{B}	2.52±0.04^{B}
35	-	0.38±0.02^{AB}	0.37±0.02^{AB}	-	2.54±0.05^{B}	2.54±0.01^{B}
45	-	0.37±0.02^{B}	0.36±0.02^{B}	-	2.55±0.08^{B}	2.56 ±0.06^{B}
60	-	0.36±0.02^{B}	0.37±0.00^{B}	-	2.59±0.09^{B}	2.58±0.06^{B}

Values sharing same letter indicate no significant differences (Tukey mean comparison, $p < 0.05$). Lowercase and uppercase letters indicate the evaluation of all or only treated juices stored 15 and 60 d, respectively. After 15 d, untreated samples showed significant spoilage and were discarded.

3.5 Effect of HHP on betalains, total phenolic concentration, and antioxidant activity

In previously reported juice studies, betacyanins (red) and betaxanthins (yellow) were not affected ($p > 0.05$) by HHP processing (Brockington *et al*., 2015; Khan & Giridhar, 2015). In this study, betaxanthins showed no significant changes during storage (21.1 18.9 mg g^{-1} db, data not shown) while betacyanins showed a 28 % reduction (Table 4). This is consistent with work by Ochoa-Velasco and Guerrero Beltrán (2012) who reported a 25 % decrease in the betacyanins content of UV-C treated pitaya juice after 25 d storage at 4 °C, i.e., a storage effect similar to the one here observed. The betacyanins content reduction, which coincided with the *hue* increase during storage, was likely caused by the remaining activity of pitaya enzymes (Garcia-Palazon *et al*., 2004; Morales-de la Peña, Salinas-Roca *et al*., 2018). Also, Azeredo (2009) reported that during refrigerated storage betacyanins are more susceptible to enzyme degradation than betaxanthins.

The total phenolic compounds content in all juices was not affected by HHP treatments. After 45 d storage, a 10 % decrease ($p < 0.05$) was observed in pressure-treated juice (Table 5), a value comparable to

Table 6. Changes in the global acceptability of pitaya juice during storage at 4±1 °C

Pressure (MPa)	Storage time (days) 0	15	60
Untreated	5.57^{a}	—	—
550 MPa/16 min	5.51^{a}	6.25^{c}	5.76^{ab}
600 MPa/12 min	5.17^{a}	6.33^{c}	5.96^{bc}

Values sharing the same letter indicate no significant difference (MSOD).

the 12 % phenolic compounds reduction reported for UV-C treated pitaya juice (Ochoa-Velasco & Guerrero Beltrán, 2013). Moreover, even higher reductions have been reported for pomegranate and kiwi juice (19 and 30 %, respectively) when treated at 550 MPa (2.5 and 10 min, respectively) and subsequently stored for 35 and 42 d at 4 °C, respectively (Varela-Santos *et al*., 2012; Xu *et al*., 2018).

Antioxidant activity was not significantly affected by HHP juice processing. During storage, untreated juices showed a significant reduction (9 %) after 15 d, while in HHP-treated juices the decrease reached 6 % after 30 d (Table 5). Cao, Bi *et al*. (2012) and Juarez-Enriquez, Salmeron-Ochoa *et al*. (2015) reported a decrease of 8 and 6 % of antioxidant activity in strawberry juice treated by 600 MPa for 4 min and in apple juice treated at 430 MPa for 7 min when stored for 60 and 34 d at 4 °C, respectively. The antioxidant activity loss observed could be due to a reduction in betalains and phenolic compounds content, which was significant after 35 and 45 d of storage, respectively. Although the synergic effect of organic acids as malic and citric acid should also be considered (Ramírez-Ramos *et al*., 2015; García-Cruz *et al*., 2017), the latter was not evaluated in this study.

3.6 *Effect of HHP on the overall sensory acceptability*

The overall sensory acceptability of juices showed hedonic scale values in the 5.5 and 6.3 range (Table 6). In general, higher acceptability scores corresponded to stored samples. Probably, acceptability was influenced by their acidity, and consequently the panelists noticed a higher sweetness (Mezey & Mezeyová, 2018). This is consistent with a previous report showing that an elevated TSS/TA ratio is indicative of a pitaya fruit with good flavor (Rosas-Benítez *et al*., 2016) and observed also in other fruit juices (Macías-Ojeda *et al*., 2019). In this study, juices stored 15-60 d had a TSS/TA ratio of 125 while fresh juices had 66-71 TSS/TA ratio, which would explain their lower hedonic scale value.

Conclusions

Juices treated at 550 and 600 MPa for 16 and 12 min, respectively, and subsequently stored under refrigeration for 60 d had microbial load below 2 log CFU mL^{-1}. TSS, luminosity, *chroma*, and antioxidant activity were not affected by HHP nor by storage. A residual PME activity decrease was observed when juices were treated by HHP. Acidity, phenolic compounds, and betalains content were not affected by HHP. Sensorial acceptability was not affected by HHP, but it was favored by storage, probably reflecting a lower acidity affecting the perceived sweetness. High pressure hydrostatic is thus a promising alternative offer consumers non-acidified pitaya juice with long shelf life and high quality.

Acknowledgement

Authors acknowledge the support from Tecnologico de Monterrey (Research chair funds GEE1A01001 and CDB081), Universidad Autónoma Chapingo (Conventional project 17076-C 2017), and the doctoral scholarship (265456) from the Mexican National Council of Science and Technology (CONACYT) granted to author Quiroz-González.

Acronyms

HHP	high hydrostatic pressure
PME	pectin methylesterase
TSS	total soluble solids
CUT	come-up time
MSOD	minimum significant order difference
AOX	antioxidant activity
TA	titratable acidity
db	dry basis
Greek symbol	
ΔE	Net color change

References

Akgün, M.P., Ünlütürk, S. (2017). Effects of ultraviolet light emitting diodes (leds)

on microbial and enzyme inactivation of apple juice. *International Journal of Food Microbiology 260*, 65-74.

Alpas, H., Kalchayanand, N., Bozoglu, F., Ray, B. (2000). Interactions of high hydrostatic pressure, pressurization temperature and ph on death and injury of pressure-resistant and pressure-sensitive strains of foodborne pathogens. *International Journal of Food Microbiology 60*, 33-42.

AOAC (1995). *Official Methods of Analysis* (15th ed.). Association of Official Analytical Chemists, Gaithesburg, MD.

Arnaud, V.R., Santiago, G.P., Bautista, P.B. (1997) Agroindustria de algunos frutos. En: *Suculentas Mexicanas Cactáceas*, (P.L. Rodríguez,ed.), Pp. 78-85. CVS Publishers, Ciudad de México, México.

Azeredo, H.M.C. (2009). Betalains: Properties, sources, applications, and stability - a review. *International Journal of Food Science and Technology 44*, 2365-2376.

Bevilacqua, A., Petruzzi, L., Perricone, M., Speranza, B., Campaniello, D., Sinigaglia, M., Corbo, M.A. (2018). Nonthermal technologies for fruit and vegetable juices and beverages: Overview and advances. *Comprehensive Reviews in Food Science and Food Safety 17*, 1-62.

Bravo, H.H., Sánchez-Mejorada, H. (1991). *Las Cactáceas de México* (3rd ed.). Universidad Nacional Autónoma de México, Ciudad de México, México.

Brockington, S.F., Yang, Y., Gandia?Herrero, F., Covshoff, S., Hibberd, J.M., Sage, R.F., Wong, G.K., Moore, M.J., Smith, S.A. (2015). Lineage?specific gene radiations underlie the evolution of novel betalain pigmentation in *caryophyllales. New Phytology 207*, 1170-1180.

Buckow, R., Terefe, N.S. (2017) High-pressure processing effects on endogenous enzymes in fruits and vegetables. In: *High-pressure processing of Fruit and Vegetable Products*, Pp. 39-62. CRC Press, Boca Raton, FL.

Bull, M.K., Zerdin, K., Howe, E., Goicoechea, D., Paramanandhan, P., Stockman, R., Sellahewa, J., Szabo, E.A., Johnson, R.L., Stewart, C.M. (2004). The effect of high pressure processing on the microbial, physical and chemical properties of valencia and navel orange juice. *Innovative Food Science & Emerging Technologies 5*, 135-149

Buxbaum, F. (1961). Die entwicklungslinien der tribes *pachycereeae* f. Buxb. (*cactaceae-cereoideae*). *Botanical Studies 12*, 1-107.

Cao, X., Bi, X., Huang, W., Wu, J., Hu, X., Liao, X. (2012). Changes of quality of high hydrostatic pressure processed cloudy and clear strawberry juices during storage. *Innovative Food Science and Emerging Technologies 16*, 181-190.

Cheftel, J.C. (1995). Review: High-pressure, microbial inactivation and food preservation. *Food Science and Technology International 1*, 75-90.

García-Cruz, L., Dueñas, M., Santos-Buelgas, C., Valle-Guadarrama, S., Salinas-Moreno, Y. (2017). Betalains and phenolic compounds profiling and antioxidant capacity of pitaya (*Stenocereus* spp) fruit from two species (*S. pruinosus* and *S. Stellatus*). *Food Chemistry 234*, 111-118.

García-Cruz, L., Valle-Guadarrama, S., Salinas-Moreno, Y., Luna-Morales, C.d.C. (2016). Postharvest quality, soluble phenols, betalains content, and antioxidant activity of *Stenocereus pruinosus* and *Stenocereus* stellatus fruit. *Postharvest Biology and Technology 111*, 69-76.

Garcia-Palazon, A., Suthanthangjai, W., Kajda, P., Zabetakis, I. (2004). The effects of high hydrostatic pressure on β-glucosidase, peroxidase and polyphenoloxidase in red raspberry (*rubus idaeus*) and strawberry (*fragaria x ananassa*). *Food Chemistry 88*, 7-10.

Garcia, E.F., de Oliveira Araújo, A., Luciano, W.A., de Albuquerque, T.M.R., de Oliveira Arcanjo, N.M., Madruga, M.S., dos Santos Lima, M., Magnani, M., Saarela, M., de Souza, E.L. (2018). The performance of five fruit?derived and freeze?dried potentially probiotic lactobacillus strains in apple, orange, and grape juices. *Journal of the Science of Food and Agriculture 98*, 5000-5010.

González-Agüero, M., Tejerina Pardo, L., Zamudio, M., Contreras, C., Undurraga, P., Defilippi, B. (2016). The unusual acid-accumulating behavior during ripening of cherimoya (*annona cherimola* mill.) is linked to changes in transcription and enzyme activity related to citric and malic acid metabolism. *Molecules 21*, 1-16.

Guerrero-Beltrán, J.Á., Barbosa-Cánovas, G.V., Welti-Chanes, J. (2011). High hydrostatic pressure effect on natural microflora, *Saccharomyces cerevisiae*, escherichia coli, and *Listeria innocua* in navel orange juice. *International Journal of Food Engineering 7*, 1-16.

Hernández-Montes, A. (2007). *Evaluación Sensorial de Productos Agroalimentarios*. Universidad Autónoma Chapingo, Estado de México, México.

Huang, H.W., Lung, H.M., Yang, B.B., Wang, C.Y. (2014). Responses of microorganisms to high hydrostatic pressure processing. *Food Control 40*, 250-259.

Juarez-Enriquez, E., Salmeron-Ochoa, I., Gutierrez-Mendez, N., Ramaswamy, H.S., Ortega-Rivas, E. (2015). Shelf life studies on apple juice pasteurised by ultrahigh hydrostatic pressure. *LWT - Food Science and Technology 62*, 915-919.

Khan, M.I., Giridhar, P. (2015). Plant betalains: Chemistry and biochemistry. *Phytochemistry 117*, 267-295.

Lado, B.H., Yousef, A.E. (2002). Alternative food-preservation technologies: Efficacy and mechanisms. *Microbes and Infection 4*, 433-440.

Leyva-Daniel, D.E., Escobedo-Avellaneda, Z., Villalobos-Castillejos, F., Alamilla-Beltrán, L., Welti-Chanes, J. (2017). Effect of high hydrostatic pressure applied to a mexican honey to increase its microbiological and functional quality. *Food and Bioproducts Processing 102*, 299-306.

Liu, Y., Zhao, X.Y., Zou, L., Hu, X.S. (2012). Effect of high hydrostatic pressure on overall quality parameters of watermelon juice. *Food Science and Technology International 19*, 197-207.

Macías-Ojeda, D.A., Cervantes-Arista, C., Pelayo-Zaldívar, C., Escalona-Buendía, H.B., Alia-Tejacal, I., Mendoza-Espinoza, J.A., Rodríguez Verástegui, L.L., Díaz de León-Sánchez, F. (2019). Quality and novel indicators of commercial life in valencia oranges (*citrus sinensis* osbeck). *Revista Mexicana de Ingeniería Química 18*, 979-994.

Mezey, J., Mezeyová, I. (2018). Changes in the levels of selected organic acids and sugars in apple juice after cold storage. *Czech Journal of Food Sciences 36*, 2-7.

Morales-de la Peña, M., Salinas-Roca, B., Escobedo-Avellaneda, Z., Martín-Belloso, O., Welti-Chanes, J. (2018). Effect of high hydrostatic pressure and temperature on enzymatic activity and quality attributes in mango puree varieties (cv. Tommy atkins and manila). *Food and Bioprocess Technology 11*, 1211-1221.

Ochoa-Velasco, C.E., Guerrero Beltrán, J.A. (2012). Ultraviolet-c light effect on pitaya (*Stenocereus* griseus) juice. *Journal of Food Research 1*, 60-70.

Ochoa-Velasco, C.E., Guerrero Beltrán, J.A. (2013). Short-wave ultraviolet-c light effect on pitaya (*Stenocereus griseus*) juice inoculated with *zygosaccharomyces bailii. Journal of Food Engineering 117*, 34-41.

Oey, I., Lille, M., van Loey, A., Hendrickx, M. (2008). Effect of high pressure processing on colour, texture and flavour of fruit and vegetable-based food products: A review. *Trends in Food Science & Technology 19*, 320-328.

Pérez-Grijalba, B., García-Zebadúa, J.C., Ruíz-Pérez, V.M., Téllez-Medina, D.I., García-Pinilla, S., Guzmán-Gerónimo, R.I., Mora-Escobedo, R. (2018). Biofunctionality, colorimetric coefficients and microbiological stability of blackberry (*rubus fructicosus* var. Himalaya) juice under microwave/ultrasound processing. *Revista Mexicana de Ingeniería Química 17*, 13-28.

Pérez-Loredo, M.G., García-Ochoa, F., Barragán-Huerta, B.E. (2016). Comparative analysis of betalain content in *Stenocereus stellatus* fruits and other cactus fruits using principal

component analysis. *International Journal of Food Properties 19*, 326-338.

Pimienta-Barrios, E., Nobel, P.S. (1994). Pitaya (*Stenocereus* spp., *cactaceae*): An ancient and modern fruit crop of Mexico. *Economic Botany 48*, 76-83.

Quiroz-González, B., García-Mateos, M.d.R., Corrales-García, J., Colinas-León, M.T.B. (2018). Pitaya (*Stenocereus* spp.): An under-utilized fruit. *Journal of the Professional Association for Cactus Development 20*, 82-100.

Quiroz-González, B., Rodríguez-Martínez, V., García-Mateos, M.d.R., Torres, J.A., Welti-Chanes, J. (2018). High hydrostatic pressure inactivation and recovery study of *Listeria innocua* and *Saccharomyces cerevisiae* in pitaya (*Stenocereus pruinosus*) juice. Pitaya (*Stenocereus* spp.): An under-utilized fruit. *Innovative Food Science & Emerging Technologies 50*, 169-173.

Ramírez-Ramos, M., García-Mateos, M., Corrales-García, J., Ybarra-Moncada, C., Castillo-González, A.M. (2015). Antioxidant compounds in prickly pear (*opuntia* sp.) pigmented varieties. *Revista Fitotecnia Mexicana 38*, 349 - 357.

Rosas-Benítez, A., Trujillo-Cárdenas, L., Valle-Guadarrama, S., Salinas-Moreno, Y., García-Cruz, L. (2016). Quality attributes of pitaya (*Stenocereus pruinosus*) fruit handled in postharvest with and without thorns under refrigerated storage. *Revista Chapingo, Serie Horticultura XXII*, 191-207.

Sánchez-Rangel, J.C., Benavides, J., Heredia, J.B., Cisneros-Zevallos, L., Jacobo-Velázquez, D.A. (2013). The folin-ciocalteu assay revisited: Improvement of its specificity for total phenolic content determination. *Analytical Methods 5*, 5990-5999.

Sandate-Flores, L., Rostro-Alanis, M.D.J., Mancera-Andrade, E.I., Esquivel-Hernandez, D.A., Brambila-Paz, C., Parra-Saldívar, R., Welti-Chanes, J., Escobedo-Avellaneda, Z., Rodríguez-Rodríguez, J. (2017). Using high hydrostatic pressures to retain the antioxidant compounds and to reduce the enzymatic activity of a pitaya?pineapple (*Stenocereus* sp.-*fragaria ananassa*) beverage. *Journal of Food Science and Technology 54*, 611-619.

Sokołowska, B., Skapska, S., Niezgoda, J., Rutkowska, M., Dekowska, A., Rzoska, S.J. (2014). Inactivation and sublethal injury of escherichia coli and *Listeria innocua* by high hydrostatic pressure in model suspensions and beetroot juice. *High Pressure Research 34*, 147-155.

Tay, A., Shellhammer, T.H., Yousef, A.E., Chism, G.W. (2003). Pressure death and tailing behavior of *Listeria monocytogenes* strains having different barotolerances. *Journal of Food Protection 66*, 2057-2061.

Valdivia-Nájar, C.G., Martín-Belloso, O., Soliva-Fortuny, R. (2018). Impact of pulsed light treatments and storage time on the texture quality of fresh-cut tomatoes. *Innovative Food Science and Emerging Technologies 45*, 29-35.

Varela-Santos, E., Ochoa-Martinez, A., Tabilo-Munizaga, G., Reyes, J.E., Pérez-Won, M., Briones-Labarca, V., Morales-Castro, J. (2012). Effect of high hydrostatic pressure (hhp) processing on physicochemical properties, bioactive compounds and shelf-life of pomegranate juice. *Innovative Food Science & Emerging Technologies 13*, 13-22.

Villarreal-Lozoya, J.E., Lombardini, L., Cisneros-Zevallos, L. (2007). Phytochemical constituents and antioxidant capacity of different pecan [*carya illinoinensis* (wangenh.) k. Koch] cultivars. *Food Chemistry 102*, 1241-1249.

Wang, Z., Goonewardene, L.A. (2004). The use of mixed models in the analysis of animal experiments with repeated measures data. *Canadian Journal of Animal Science 84*, 1-11.

Xu, X., Deng, J., Luo, D., Bao, Y., Liao, X., Gao, H., Wu, J. (2018). Comparative study of high hydrostatic pressure and high temperature short time processing on quality of clear and cloudy se-enriched kiwifruit juices. *Innovative Food Science and Emerging Technologies 49*, 1-12.

Yáñez-López, L., Domínguez, J., Fajardo, M.C., Malpica, F., Soriano, J., Pelayo, M.A., Armella, F., Diaz-de-León, F. (2005). Quality attributes of different types of cactus pitaya fruits

(*Stenocereus griseus*). *Acta Horticulturae 682*, 645-650.

Zou, H., Lin, T., Bi, X., Zhao, L., Wang, Y., Liao, X. (2016). Comparison of high hydrostatic pressure, high-pressure carbon dioxide and high-temperature short-time processing on quality of mulberry juice. *Food and Bioprocess Technology 9*, 217-231.