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Effect of two pasteurization methods on the content of bioactive compounds and antioxidant capacity of nance (*Byrsonima crassifolia*) pulp and their kinetics of loss during refrigerated storage

Efecto de dos métodos de pasteurización en el contenido de componentes bioactivos y capacidad antioxidante de la pulpa de nanche (*Byrsonima crassifolia*) y sus cinéticas de pérdida durante el almacenamiento en refrigeración

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Abstract

The aim of this work was to evaluate the effect of two pasteurization methods: Low Temperature Long Time (LTLT) carried out at 63 ± 2.0 ºC/30 min and High Temperature Short Time (HTST) performed at 73 ± 2.0 ºC/15 s on the physicochemical parameters, bioactive compounds, and antioxidant activity of the treated pulp during the refrigeration storage (4 °C) of nance pulp. The material processed by LTLT presented the lowest decrement of ascorbic acid, phenolic compounds, and antioxidant activity (DPPH). Rates of losses of ascorbic acid, phenolic compounds, and antioxidant capacity (DPPH and ABTS) were described by a first order kinetics. The rate constants obtained were higher for HTST pulp, except for the antioxidant capacity determined by ABTS which was slightly higher for LTLT pulp. The $t_{1/2}$ values were, consequently, larger for LTLT pulp indicating a longer time for decreasing the concentration of the bioactive and the antioxidant capacity. Five main phenolic compounds were identified by HPLC: gallic, trans-ferulic and caffeic acids, rutin, and quercetin and the observed increment of the intensity of quercetin peak for LTLT treated pulp could be due to the breakage of rutin. Both pasteurization methods reduced microbial loads to recommended standards and LTLT reduced it more pronouncedly. LTLT pasteurization could be recommended the best alternative for the pasteurization of this pulp.

Keywords: nance pulp, pasteurization, bioactive compounds, kinetics.

Resumen

El objetivo de este trabajo fue evaluar el efecto que tienen dos métodos de pasteurización: Baja Temperatura Largo Tiempo (LTLT) a 63 ± 2.0 °C/30 min y Alta Temperatura Corto Tiempo a 73 ± 2.0 °C/15 s en los parámetros fisicoquímicos, compuestos bioactivos y capacidad antioxidante de la pulpa de nanche pasteurizada durante su almacenamiento en refrigeración (4 °C). La pulpa procesada por el método LTLT, presentó el menor decremento de ácido ascórbico, compuestos fenólicos y capacidad antioxidante (DPPH). Las velocidades de pérdida de ácido ascórbico, compuestos fenólicos y de la capacidad antioxidante (DPPH y ABTS) fueron descritos a través de un modelo cinético de primer orden. Las constantes cinéticas de velocidad fueron mayores para la pulpa pasteurizada por HTST, excepto para la capacidad antioxidante que fue un poco más alta para pulpa pasteurizada para la pulpa pasteurizada por Baja Temperatura y Largo Tiempo. Los valores de $t_{1/2}$ fueron, consecuentemente mayores para la pulpa tratada por este último método e indicaron mayores tiempos para disminuir la concentración de los compuestos bioactivos y la capacidad antioxidante (DPPH). Se identificaron por HPLC, cinco compuestos fenólicos principales: ácido gálico, cafeico y tans-ferúlico, rutina y quercetina y los incrementos observados de la intensidad de la señal del pico correspondiente a la quercetina en la pulpa tratada por LTLT pudo haberse debido al rompimiento de rutina. Ambos métodos de pasteurización redujeron las cargas microbianas a estándares recomendados y el proceso LTLT las redujo más pronunciadamente. La pasteurización LTLT puede recomendarse como la mejor alternativa para la pasteurización de esta pulpa.

Palabras clave: Quitosano, membrana, glicerina, reticulación, permeabilidad.

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1 Introduction

Mexico produces a wide variety of fruits and vegetables and some of them like nance (*Byrsonima crassifolia*) are not widely consumed neither as fresh fruit nor processed and only local markets commercialize them. Nance grows in tropical and subtropical regions having warm, semi warm, and temperate climates (Caballero-Roque *et al*., 2012) and is cultivation extends from Mexico to South America and the Caribbean. It is commonly found in the Venezuelan savanna and coast areas in northeast Brazil and i in the Caribbean islands such as Trinidad and Tobago. Barbados, Cuba, Haiti, and Dominican Republic amongst others (Geilfus, 1994; Orwa *et al*., 2009). In Mexico, nance grows from San Luis Potosi to Tabasco and northern Chiapas, including the central depression of the Lacandon Jungle to Campeche, Yucatan, and Quintana Roo in the Gulf of Mexico. In the Pacific coast it grows from Sinaloa to Chiapas (Pennington y Sarukhán, 2005). Nance has different names in America: nanchi, nananché and nantzin in Mexico; nancito or crabo in Honduras; craboo and crapoo in Belice; doncella and maricao in Dominican Republic; maricao cimarrón verde and peralejo in Puerto Rico; perelejo de sabana in Cuba; tapal in Guatemala; chaparro de chinche or sabana, manteco in Venezuela; murici and mirixi in Brazil (Morton, 1987; Santos, 2013). Nance fruits are consumed on a seasonal basis (SIAP, 2015) and are produced in the wild as well as in small orchards and sold in local markets as a fresh fruit or in the form of jam, liquor and jelly on a retail basis during the production season (Medina-Torres *et al*., 2004; Duarte, 2011; Santos, 2013). The total production in Mexico reached 31,180.11 tons in 2015 (SIAP, 2015) and it has been reported that this fruit is an important source of ascorbic acid, phenolic compounds (Caballero-Roque *et al*., 2012), and other phytochemicals such as carotenoids as lutein and zeaxanthins (Mariutti *et al*., 2014; Irías-Mata *et al*., 2015). It also contains sulfur compounds, esters, and alcohols as ethyl butane an ethyl hexane as well as butyric and hexanoic acids that contribute to their characteristic aroma (Duarte, 2011).

Due to the content of the above compounds it results attractive to study the feasibility of applying thermal processing of the nance pulp aiming at preserving it in refrigeration for extended periods of time. Thermal methods of food preservation are amongst the oldest industrial practices and are widespread used to process fruit pulps and juices and in this regard, pasteurization is used to extend the shelf-life of refrigerated foods (Teixeira, 2014) aiming at inactivating pathogenic bacteria as well as certain enzymes as the polyphenol oxidase (PPO) and reducing the microbial load of the products (Silva *et al*., 2014). There are two main types of traditional pasteurization (excluding ultrahigh temperature or UHT method) i.e. low-temperature-long time (LTLT) and high-temperature-short-time (HTST) methods. Despite their usefulness these processes may cause undesirable changes as the loss of vitamins (mainly A and C), some amino acids as well as minerals and can affect the sensorial characteristics of the product (Floros *et al*., 2010; Pérez-Reyes & Sosa-Morales, 2013). The kinetics of thermal inactivation of undesirable enzymes and microorganisms has received a great deal of attention (Peng et at. 2017) and the effects that subsequent storage in refrigeration has on key nutriments also been studied. In this respect, Quiroz-González (2020) reported that during the storage of pitahaya treated by high pressure processing (HPP), the acidity, phenolic compounds, betalains, and antioxidant activity did not change for processed fruits but decreased by 43, 10, 14 and 5% respectively after 60 days of refrigerated storage. Rábago-Panduro *et al*. (2020) reported that time of storage is one of the most significant variable affecting the bioactive compounds of pecan nuts during storage. Also, Thakkar *et al*. (2018) reported on the shelf life under refrigeration of a milk whey-based functional beverage containing orange juice and probiotics, finding that probiotic bacteria were adequately preserved for 28 days storage at refrigeration (7 °C) conditions. In another study, Olivares *et al*. (2019) reported that low pH affected the survival of *L. casei* during the refrigerated storage of pineapple, raspberry, and orange juices even when the microorganisms were microencapsulated and found that acid conditions affected their viability. Plaza *et al*. (2011) reported the effects of minimal processing on some bioactive compounds of orange fruit during refrigeration and found that, at the end of refrigerated storage, some losses of vitamin C were observed. Additionally, Cai *et al*. (2020) reported that phenolic compounds of apple juice were reduced during refrigerated storage of apple juice treated by ultrafiltration.

We have not found published studies reporting the kinetics of the losses of ascorbic acid, phenolics compounds and antioxidant capacity during the refrigerated storage of HTST and LTST treated nance pulps. Given the above, and bearing in mind the

importance of thermal processes and storage methods in the food industry as well as the compositional characteristics of nance, the objective of this work was to evaluate the physicochemical and microbiological characteristics of nance pulp before and after LTLT and HTST pasteurization as well as to study the effect of these methods on the content of bioactive compounds and antioxidant capacity of nance pulp and to evaluate their kinetics of degradation during 3.5 months of refrigerated storage.

2 Materials and methods

2.1 Raw material

Nance fruits from the state of Oaxaca, Mexico were used for the study. An even yellow color of the skin and a size ranging 1.5-2.0 cm in diameter were set as the criteria of commercial maturity (Ramos-Carbajal, *et al*., 2020). Selected fruits were washed with running water and immersed during 3 minutes in a 100 ppm sodium hypochlorite solution followed by rinsing with distilled water (López-Gálvez, *et al*., 2010).

2.2 Obtention and pasteurization of the pulp

Fruits were feed to a continuous pulper machine (POLI, DERE-1, 022.301107.09, México) fitted with a 0.1 mm mesh. A portion of the obtained pulp was immediately used to perform the physicochemical, microbiological, bioactive compounds content and antioxidant activity. The bulk of the pulp was pasteurized by using a jacketed pan (Madipsa, model M080VV) applying the following temperature and time conditions (Pérez-Reyes & Sosa-Morales, 2013): For the Low-Temperature Long-Time (LTLT) process, 63 ± 2.0 °C during 30 min and For the High-Temperature Short-Time process, 73 ± 2.0 °C for 15 seconds. Unpasteurized and pasteurized pulps were bottled in sealed glass containers and stored under refrigeration conditions (4 ± 1 °C) during the 3.5 months during which, the analyses described below were performed.

2.3 Physicochemical analyses

Moisture content was determined by the vacuum oven method (AOAC 20.013); pH was determined with a pH meter (HACH Sens ion 1, model 51700-23, China) as recommended in NMX-F-317-S-1978; soluble solids (SS) were determined by using an Abbe refractometer (Atago, model NAR 1T, Japan) as described in NMX-F-112-NORMEX-2010; acidity (% acidity) was reported as milliequivalents of citric acid and determined by titration with NaOH 0.01 N following the method reported by NMX-FF-011-1982; Color of samples was determined as the total color difference (ΔE) based on parameters for fresh (L^*, a^* and b^*) and pasteurized (L_0^*, a_0^*, b_0^*) pulp by using a tri-stimuli colorimeter (HunterLab ColorFlex EZ, USA) considering a diffuse geometry d/8 and a vision angle of 8° (Domene & Segura, 2014). The following equation was used:

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad (1)$$

Also, the color index (CI) was evaluated through Equation 2 (Domene & Segura, 2014):

$$CI = \frac{1000 x a^*}{L^* x b^*} \quad (2)$$

2.4 Ascorbic acid

The AOAC 967.21 method was used. Briefly, 15 g of pulp with 10 mL of deionized water were centrifuged (13000xg during 15 min) and 1 mL of the supernatant were added to a 10 mL solution of metaphosphoric acid (1.85% w/v):water, at a ratio of (1:12.5 v/v). This solution was titrated by using a of 2,6-dichlorophenolindophenol (2,6-DCPI) solution (0.0025 w/v) until a pale pink color was apparent. The following equation was then applied:

$$\frac{\text{mg ascorbic acid}}{100\ \text{mL}} = \left(\frac{\text{Concentration of colorant} \cdot \text{mL in titration} \cdot 10}{10\ \text{mL sample}}\right) \times 100 \quad (3)$$

in which:

$$\text{Concentration of colorant} = \left(\frac{10\ \text{mg ascorbic acid}}{\text{mL consumed of 2,6-DCPI in titration}}\right) \quad (4)$$

2.5 *Obtention of extracts for the determination of phenolic compounds and antioxidant capacity*

Extracts of the pasteurized (HTST and LTST) and unpasteurized samples were obtained by following the methodology reported by Moo-Huchin *et al*. (2014). Briefly, samples were extracted by using 80% methanol, sonicated for 10 min., and centrifuged at 13000xg. Supernatant was separated and filtered through Whatman 40 paper, kept in amber bottles and then, refrigerated at 4 ± 1 ºC until further use. All determinations were carried out by triplicate.

2.6 *Phenolic compounds*

The Folin-Ciocalteu (García *et al*., 2015; Singleton & Rossi, 1965) method was used. Briefly, 200 μL of the methanolic extract obtained as described in Section 2.5, were mixed with 200 μL of the Folin-Ciocalteu reagent and let to react for 6 min. Then, the reaction was brought to a halt by adding 2.6 mL of a Na_2CO_3 solution (2%). Absorbances were read after 30 min, interpolated in a standard curve and results were expressed as mg gallic acid equivalents/100 g of pulp (mg GAE/100 g of pulp). All determinations were carried out by triplicate.

2.7 *Identification of phenolic compounds by High-Performance Liquid Chromatography (HPLC-DAD)*

The method reported by Gordon *et al*. (2011) was used. Identification was carried out in an Aligent 1260 HPLC equipment (Agilent Technologies 1200 Infinity Series, 1260 Infinity, USA). A column Eclipse XDB-C18 (Agilent Technologies, USA) was used for the different determinations. Briefly, a binary gradient was used formed by Phase A: 1% (v/v) water-acetic acid and Phase B: 1% (v/v) acetonitrile (ACN)-acetic acid. Duration of each run was 45 min and a flow of 1mL/min was set. Thermostat was adjusted at 30 °C and the detector was programmed at 210, 254, 280, 360, 365 nm. A 20 μL of sample was injected in all cases. The extracts (prepared as described in Section 2.5) were filtered through polytetrafluoroethylene filter having a pore diameter of 0.45 μm and then, the sample was injected to the chromatographer. All chemical reagents had a purity ≥ 98% and the solvents used were HPLC grade.

2.8 *Antioxidant capacity*

2.8.1 *DPPH (free radical 2,2-diphenyl-1-picrilhidrazil) method*

The method reported by Chen *et al*. (2013 and 1999) was used. Briefly, 3.9 mL of the DDDP reagent (100 μM) were added to a mixture containing 68 μL of pulp and 32 μL of deionized water. The mixture was stirred and kept in the dark during 30 min., and the absorbance was read at 0 and 30 min. The percentage of inhibition of the free radical was evaluated as follows:

$$\% \text{ inhibition of radical} = \left(\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}}\right) \times 100 \quad (5)$$

in which:

Abs control: Absorbance of Methanol (80 %) treated with DPPH. Abs sample: Absorbance of the sample treated with DPPH.

Absorbances were then interpolated in a Trolox standard curve and results expressed as Trolox equivalent antioxidant capacity (TEAC) (μmol TEAC/g).

2.8.2 *ABTS (2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical method*

The method reported by Pellegrini, *et al*. (2003) and de Souza *et al*. (2012) was used to determine the antioxidant capacity. Briefly, the ABTS radical ($ABTS^{\bullet+}$) was produced by means of the oxidation of the ABTS by potassium persulfate (140 M). Both reagents were reacted during 16 h and the absorbance was adjusted at 0.7 ± 0.05 units of absorbance at 734 nm. Then, 30 μL of sample and 3.0 mL of ABTS were mixed and absorbances were measured at time zero and at 6 min of reaction time. Absorbances were interpolated in a standard curve and results expressed as Trolox equivalent antioxidant capacity (TEAC) (μmol TEAC/g).

2.9 *Kinetic analyses*

Every 15 days, antioxidant capacity (DPPH and ABTS), ascorbic acid and phenolic compounds were evaluated during 3.5 months in pasteurized (HTST and LTST) nance pulps kept at 4 ± 1 °C. A first order

kinetic model (Ordóñez-Santos & Yoshioka-Tamayo, 2012; Luna-Ramírez *et al*., 2017) depicted in Equation 6, was applied to obtained data. This rate equation was selected given that different authors have reported first order kinetics for the degradation of bioactive compounds in fruits (Zanoni *et al*., 2005; Ordóñez-Santos & Yoshioka-Tamayo, 2012; Luna-Ramírez *et al*., 2017).

$$-kt = \ln\left(\frac{C}{C_0}\right) \tag{6}$$

in which:

k: Rate constant (days^{-1})

t: Time (days)

C_0, C: Concentrations at time zero and at any time t (mg/100 mL for ascorbic acid; μmol TEAC/g for antioxidant capacity and mg GAE/100 g for phenolic compounds) respectively.

Values of k were obtained from the slope of the straight-lines $\ln(C/C_0)$ vs time. Also, the time required to reach half of the minimum concentration or half time ($t_{1/2}$) was evaluated as follows (Luna-Ramírez, *et al*., 2017):

$$t_{1/2} = \frac{-\ln(0.5)}{k} = \frac{0.693}{k} \tag{7}$$

Half times indicate time at which, by following the same kinetic trend (same values of k), the reduction of the concentration would decrease 50%.

2.10 Microbiological analyses

Bacteria, molds, and yeasts counts were determined in the unpasteurized sample as well as in the pasteurized pulps before and after the refrigerated storage. Analyses were carried out according to the Mexican standard NOM-092-SSA1-1994 for aerobic mesophilic bacteria as well as the NOM-092-SSA1-1994 for molds and yeasts. Results were expressed as colony-forming units (CFU)/g of sample.

2.11 Statistical analysis

The statistical analyses were carried out by means of an ANOVA with a significance level $\alpha = 0.05$. Calculations were done by using the Minitab 17.1.0 software and the results were expressed as mean values ± standard deviation.

3 Results and discussion

3.1 Physicochemical analyses

Moisture content, pH, °Bx and acidity as citric acid of the untreated and pasteurized (LTLT and HTST) samples are given in Table 1.

Unpasteurized pulp had a higher moisture content than pasteurized ones for which this parameter decreased by 4.5 and 3.9% for LTLT and HTST samples respectively ($p \leq 0.05$) and these decrements were statistically different ($p \leq 0.05$) among samples. These decrements were probably due to the evaporation of water during pasteurization. A similar value of the initial moisture content was reported by Moo-Huchin *et al*. (2014) (79.50 ± 0.55%). It is also noteworthy that moisture contents did not changed significantly ($p > 0.05$) during refrigerated storage (Table 1). A constant moisture content during the refrigerated storage, would indicate that no water was consumed in degradation reactions such as hydrolysis (mainly of polysaccharides such as pectin) as reported by Massiot *et al*., (1996). This was confirmed by the degrees Brix which were maintained constant during the refrigerated storage.

pH values (Table 1) showed statistically different values ($p \leq 0.05$) amongst the two processed methods and with the untreated sample whereas storage under refrigeration conditions had not an influence on the pH. The slight increment observed in the pH of the pasteurized samples might be due to the loss of ascorbic acid during pasteurization. Similar values of the pH for the unprocessed pulp were reported by Hamacek *et al*. (2014) (3.93 ± 0.02) who worked with Murici, fruit from the Cerrado of Minas Gerais, Brazil. Additionally, pH values of unprocessed and processed pulps, were larger than those reported by Sales & Waughon (2012) who obtained a pH = 3.3 for pasteurized samples of murici from Castanhal-Pará, Brazil. Differences found could be due to different fruit varieties used as well as the regions were fruits were collected and the characteristics of the cultivating methods.

Soluble solids (°Bx) increased from 13.73 to 15.66 and 16.33 after LTLT and HTST pasteurization processes respectively (Table 1), which could be due to evaporation of water during heating. There were significant differences ($p \leq 0.05$) in soluble solids between the two pasteurization methods and with the unpasteurized sample, which confirmed that higher temperatures applied in HTST pasteurization had a

more pronounced effect on this parameter than the long times of treatment in the LTLT process. Obtained figures were larger than those reported by Hamacek *et al*. (2014) when working with Murici, fruit from the Cerrado of Minas Gerais, Brazil who reported a value of 10.73 ± 1.22 °Bx after pasteurization. Differences found could be due to different fruit varieties used as well as differences in the methods of collection of the fruits and, in the cultivating methods.

Acidity significantly ($p \leq 0.05$) decreased after the two pasteurization methods and during the refrigerated storage (Table 1), but no differences were obtained between pulps processed by either type of pasteurization methods. Oxidation of citric acid during pasteurization due to contact with air (Cao *et al*. 2011) as well as residual activity of enzymes could have caused these decrements (Quiroz-González *et al*., 2020). It has been reported that consumer's acceptance is higher when solids are also high and when acidity as citric acid is between 0.08 and 1.95%. Overall, there were statistical differences between both pasteurization methods regarding the physicochemical parameters depicted in Table 1 and final selection of pasteurization method should also consider bioactive compounds content and their stability during storage.

Regarding the color of the samples, the results of the CIELAB parameters for nance pulp before and after pasteurization are presented in Table 2. Obtained luminosity of unpasteurized pulp was similar to that reported by Moo-Huchin *et al*. (2014) who obtained an L^* value of 70.25 for nance pulp from the south-east of Mexico. Additionally, a significant ($p \leq 0.05$) decrease in L^*, a^* and b^* values of both pasteurized pulps with refrigeration were observed (Table 2). Obtained values of ΔE showed how different were the absolute coordinates of the color of pasteurized pulps respect to the unpasteurized materials and constitute an additional indicator of the quality of the pulps in relation to biochemical alterations leading to color changes. Total difference in color (ΔE) of pasteurized pulps was higher for LTLT treated pulp (12.11) than for the HTST one (9.46), representing increments of 71.9 and 57.3% respectively which were a consequence of the temperature-time combinations used. It was noteworthy that longer processing times (30 min for LTLT and 15 s.) for HTST, affected more noticeably the color differences than associated temperatures (63 and 73 ºC respectively). Time-temperature effects on ΔE depend on factors such as pH, moisture content, water activity and the chemical compounds present in the media which influenced ΔE by following their particular color-change kinetics (Casanova *et al*., 2020). For instance, alterations in color of the nance pulps during refrigerated storage could be due to residual polyphenol oxidase activity (Moo-Huchin *et al*., 2014) and formation of melanoid compounds due to ascorbic acid decomposition (Badui, 2016). Both ΔE values obtained for the final products (after the 3.5 months in refrigerated storage) lie within the greatly perceptive range reported by Cserhalmi *et al*. (2006). Both pasteurized pulps after the refrigerated storage period, kept adequate values of luminosity (L^*) far from dark colors ($L^* \sim 20$) (Moo-Huchin *et al*., 2014), and the positive a^* values obtained, indicated a tendency towards red colors while positive b^* values showed tendencies to yellow. Overall, the color of both processed pulps would be appropriate for being used commercially since results of the color indexes varied from yellow-green for control and pasteurized samples at the beginning of the storage to pale-yellow intense-orange at the end of the 3.5 months (Domene & Segura, 2014) which are characteristics of the mature nance pulp. It would be possible to use the color index to aid assessing the quality of the pulp since, in the case of large values of a^*, the color of the pulp will tend to red while small values of b^* will indicate a tendency to yellow tones as given by Equation 2.

3.2 Ascorbic acid

Initial unpasteurized pulp had a content of ascorbic acid of 8.86 mg/100 mL and decreased to 6.29 (loss of 29.0%) and 5.90 mg/100 mL (loss of 33.4%) for LTLT and HTST processes respectively which in turn decreased to 3.28 (LTLT) and 2.87 mg/100 mL (HTST) at the end of the 3.5 months of refrigerated storage. Sales and Waughon (2013), reported ascorbic acid contents of 8.17 and 4.31 mg/mL before and after pasteurization which are within the range of the figures obtained in the present work. Aeration during pasteurization and consequent exposure to oxygen, presence of sugars as well as values of a_w above 0.65 favored the degradation of this compound given that Cu^{+2}, Zn^{+2} and Fe^{+2} can act in the oxidation catalysis of the ascorbic acid when present in water solutions (Herbig & Renard, 2017). Oxygen interference may partially be avoided by vacuum packing and reported kinetics are valid under the experimental conditions of this study.

Degradation of ascorbic acid during refrigerated storage was correctly described ($R^2 = 0.92$) by a first order kinetics (Equation 6) and is shown in Figure 1. For HTST pulp a slightly faster decay

was observed than that for LTLT pulp which was reflected in the values of the rate constant (k) that was larger than for the LTLT process (Table 3) as given by Equation 6. Consequently, the half time ($t_{1/2}$) as given by Equation 7, was shorter for the HTST process: 87.7 days in comparison to 93.6 days for the LTLT pasteurization (Table 3). These values agreed with figures of total loss of ascorbic acid depicted in Table 3 which indicated larger total loss of ascorbic acid for the HTST process as compared with the LTST pasteurization. Higher temperatures induced a relatively larger decomposition of ascorbate into dehydroascorbic acid which is very unstable given that the lactone ring is easily hydrolyzed to produce 2,3-diketogulonic acid (Serra & Cafaro, 2007). Zanoni *et al*. (2005) reported that thermal degradation of vitamin C in pasteurized orange juice followed a first order kinetics. Ascorbic acid is frequently used as an indicator of vitamin losses in foods (Sánchez-Chávez *et al*., 2015).

Table 1. Physicochemical characterization of unpasteurized and pasteurized nance pulps.

Storage time (days)	Sample	Moisture (% wb)	pH	°Brix	% Acidity (as citric acid)
0	Unpasteurized pulp	80.13 ± 0.26	4.02 ± 0.01	13.7 ± 0.11	0.31 ± 0.07
0	LTLT	77.63 ± 0.47^{a}	4.06 ± 0.02^{a}	15.9 ± 0.11^{bc}	0.21 ± 0.02^{c}
	HTST	78.01 ± 0.19^{a}	4.04 ± 0.00^{ab}	15.8 ± 0.11^{ab}	0.20 ± 0.02^{d}
15	LTLT	77.14 ± 0.06^{a}	4.06 ± 0.01^{a}	15.8 ± 0.11^{bc}	0.21 ± 0.01^{c}
	HTST	77.65 ± 0.30^{a}	4.05 ± 0.00^{a}	16.0 ± 0.11^{a}	0.26 ± 0.00^{a}
30	LTLT	76.91 ± 0.36^{a}	4.01 ± 0.02^{b}	16.1 ± 0.11^{ab}	0.23 ± 0.01^{bc}
	HTST	77.54 ± 0.25^{a}	3.99 ± 0.01^{cd}	15.8 ± 0.11^{ab}	0.24 ± 0.01^{abc}
45	LTLT	77.13 ± 0.37^{a}	4.02 ± 0.00^{ab}	16.2 ± 0.11^{a}	0.27 ± 0.01^{a}
	HTST	77.74 ± 0.27^{a}	3.90 ± 0.01^{d}	15.7 ± 0.11^{bc}	0.25 ± 0.01^{ab}
60	LTLT	77.52 ± 1.12^{a}	4.03 ± 0.01^{ab}	16.3 ± 0.11^{a}	0.27 ± 0.01^{a}
	HTST	78.14 ± 0.27^{a}	4.00 ± 0.00^{cd}	15.5 ± 0.11^{c}	0.22 ± 0.01^{cd}
75	LTLT	76.95 ± 1.14$_{a}$	4.05 ± 0.01^{ab}	15.6 ± 0.11^{c}	0.25 ± 0.00^{ab}
	HTST	77.96 ± 0.81$_{a}$	4.02 ± 0.01^{abcd}	15.0 ± 0.11^{d}	0.22 ± 0.02^{bcd}
90	LTLT	76.3 ± 0.78$_{a}$	4.04 ± 0.01^{ab}	15.8 ± 0.11^{bc}	0.25 ± 0.01^{ab}
	HTST	77.4 ± 0.92^{a}	4.01 ± 0.01^{bcd}	15.1 ± 0.11^{d}	0.21 ± 0.01^{d}
105	LTLT	76.53 ± 0.86^{a}	4.06 ± 0.02^{ab}	15.6 ± 0.11^{c}	0.25 ± 0.00^{ab}
	HTST	76.97 ± 0.29^{a}	4.02 ± 0.01^{abc}	15.5 ± 0.20^{c}	0.21 ± 0.01^{d}

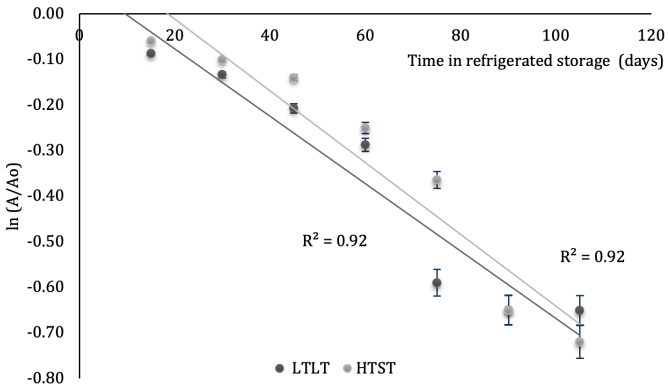

Fig. 1. First order kinetics of ascorbic acid loss in refrigerated nance pulp. Continue lines represent linear fittings. Bars denote standard error.

3.3 Phenolic compounds

Unpasteurized pulp had a phenolic compounds value of 352.72 ± 23.37 mg GAE/100 g, whereas pulps treated by LTLT and HTST processes had larger values of phenolic compounds: 389.62 and 388.72 mg GAE/100 g pulp for LTLT and HTST treatments, respectively and which decreased to 343.81 (11.8% loss) and 335.25 (13.7% loss) mg GAE/100 g pulp respectively after 3.5 months of storage. The larger amounts obtained of phenolic compounds for processed pulp respect to those obtained for the unprocessed one might be due to partial hydrolysis of these compounds that gave place to shorter and reactive phenolic chains which increased their availability (Nisar *et al*., 2015; Verardo *et al*., 2018). Also, partial disruption of cells due to high temperatures may liberate these compounds into the media.

In Figure 2, the kinetics of loss of phenolic compounds are presented for samples subjected at the two different pasteurization processes. Both processes were described by a first order kinetics (Equation 6). HTST process caused, as in the case of ascorbic acid, a higher decay rate in phenolic compounds which was reflected in larger values of rate constant k for the HTST depicted in Table 3. Consequently, the half time ($t_{1/2}$) as given by Equation 7, was shorter for the HTST process in comparison to the LTLT pasteurization. It has been reported that heating provokes alteration of cell walls and therefore favors the liberation of hydrolytic and oxidative residual enzymes that may cause the progressive destruction of phenolic compounds over time of storage (Kips *et al*., 2017). These authors also reported that some of the hydroxycinnamic acids and other flavonoids are not affected by thermal processing while isoquercitrin and rutin are heat-sensitive and also reported that during a 3-months storage period there were not pronounced decrements of phenolic compounds which coincides with findings in our work.

Table 2. CIELAB parameters for nance pulp before and after pasteurization and after refrigerated storage.

Sample	Parameter			ΔE	IC
	L*	a*	b*		
Unpasteurized	71.41±0.68^{a}	4.44±0.38^{a}	41.45±0.90^{a}	-	1.50
LTLT initial	65.79±0.22^{b}	4.06±0.01^{b}	37.32±0.04^{b}	6.98	1.65
HTST initial	67.18±0.11^{c}	3.69±0.06^{c}	38.14±0.31^{c}	5.42	1.44
LTLT final	61.31±0.47^{d}	5.24±0.05^{d}	34.80±0.42^{d}	12.11	2.45
HTST final	62.61±0.71^{d}	5.72±0.22^{e}	38.19±1.24^{e}	9.46	2.39

Different superscript letters in the same column indicate significant differences ($p < 0.05$).
$L*$: Luminosity; $a*$: Green-red; $b*$: Blue-yellow; ΔE: Total difference in color; CI: Color index.

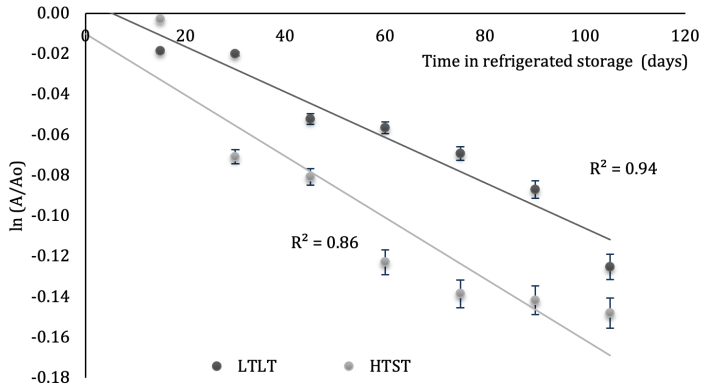

Fig. 2. First order kinetics of phenolic compounds loss in refrigerated nance pulp. Continue lines represent linear fittings. Bars denote standard error.

Table 3. Rate constant (K) and half time ($t_{1/2}$) for degradation kinetics of bioactive compounds and antioxidant capacity for both pasteurization methods applied.

	k x 10^{-3} ($days^{-1}$)		**$t_{1/2}$ (days)**	
	LTLT	**HTST**	**LTLT**	**HTST**
Ascorbic acid	7.4	7.9	93.6	87.72
Phenolic compounds	1.1	1.5	630.0	462.0
Antioxidant capacity (DPPH)	1.7	2.2	407.6	315.0
Antioxidant capacity (ABTS)	1.9	1.6	364.7	433.1

3.4 Identification of phenolic compounds in unpasteurized and pasteurized pulps

Phenolic compounds identified in unpasteurized and pasteurized pulps according to their retention times (Figures 3-5) were: gallic and caffeic acid, rutin, trans-ferulic acid and quercetin. Intensity of the signal of peaks corresponding to gallic and caffeic acids increased for LTLT pulp and remained practically unchanged for pulp processed by HTST with respect to the unprocessed one. Intensity of the signal of peak for rutin increased for both pasteurized pulps but was relatively higher for LTLT pulp. Additionally, intensity of the signals of peaks for trans-ferulic acid and quercetin also increased in LTLT pulp and remained practically unchanged for HTST pulp. Unidentified peaks in retention times of 18.5 and 20.9 min probably corresponded to those reported by Gordon *et al*. (2011) for proanthocyanidin, galotanins and derivatives of gallic acid. The above reported increment of the intensity of quercetin peak for LTLT pulp might be due to the breakage of rutin into quercetin and rutinose (Kapešová *et al*. 2019). Larger losses of ascorbic acid (Section 3.2) than those obtained for the phenolic compounds (Section 3.3) may be due to the protective effect of this acid against the oxidation of phenolic compounds and mainly quercetin (Martínez-Flórez *et al*., 2002).

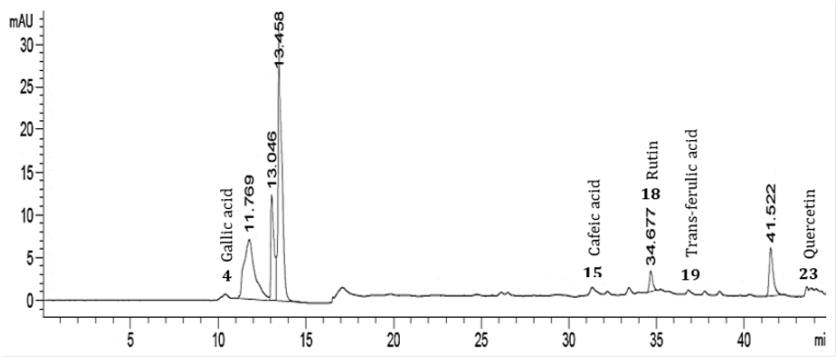

Fig. 3. Chromatogram for the extract of unpasteurized nance pulp (λ=280 nm).

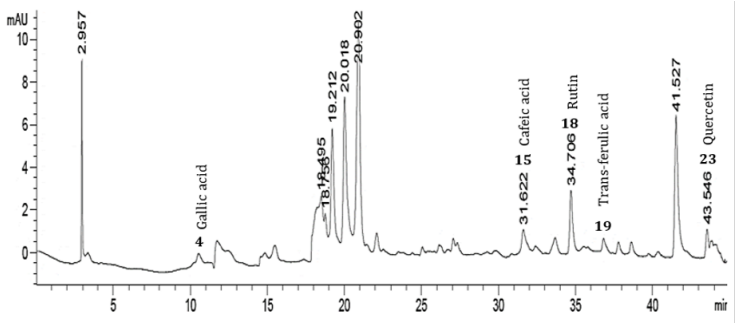

Fig. 4. Chromatogram for the extract of LTLT pasteurized nance pulp (λ=280 nm).

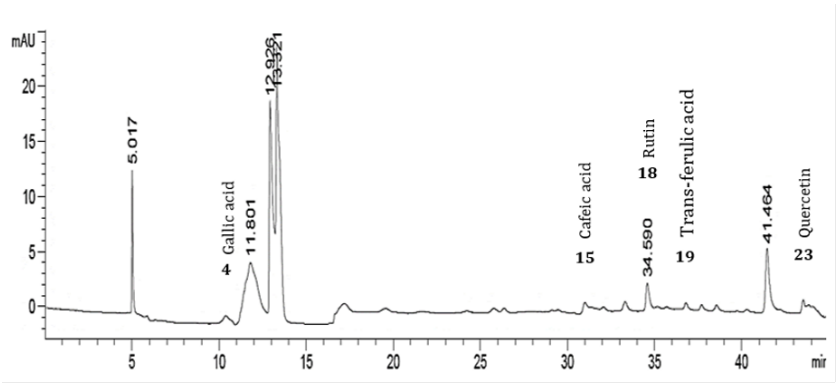

Fig. 5. Chromatogram for the extract of HTST pasteurized nance pulp (λ=280 nm).

3.5 Antioxidant capacity (DPPH)

Unpasteurized pulp had an antioxidant capacity of 10.68 ± 0.00 μmol TEAC/ g which was larger than reported values for other pulps of nance of different varieties than those used in this work. In this respect, Almeida *et al*. (2011) reported 6.46 μmol TEAC/ g and Moo-Huchin *et al*. (2014) obtained a value of 3.72 μmol TEAC/ g. Almeida *et al*., (2011) reported on 11 fruits recollected from the city of Fortaleza, Ceará, Brazil. They used murci fruit from this region having 11.8 mg/100 mL while our nance fruits had an initial value of 8.86 mg/100 g (24.9% less). This lower amount, type of soil and climate amongst other factors could explain differences found. Pasteurized pulp by LTLT had initial and final values of antioxidant capacities at the end of the refrigerated storage of 13.79 and 10.14 μmol TEAC/ g respectively (5% decrement), whereas HTST pulp showed values of 14.19 and 9.36 μmol TEAC/ g (12% decrement). Differences found may be due to different varieties and effects of processing (Vallejo-Castillo *et al*., 2020). Increments of antioxidant activity after pasteurization may be linked to the increment of phenolic compounds (Nisar *et al*., 2015; Verardo *et al*., 2018) and disruption of cell walls that allow the release of these compounds which have a positive influence on the antioxidant capacity. Losses of antioxidant capacity are shown in Figure 6. Both degradations were described by a first order kinetics. HTST process caused, a higher decay rate in antioxidant capacity (Figure 6) which was reflected in larger values of the rate constant k (Equation 6) for this method as depicted in Table 3. Consequently, the half time ($t_{1/2}$) as given by Equation 7, was shorter for the loss of antioxidant capacity due to the HTST process in comparison to the LTLT pasteurization.

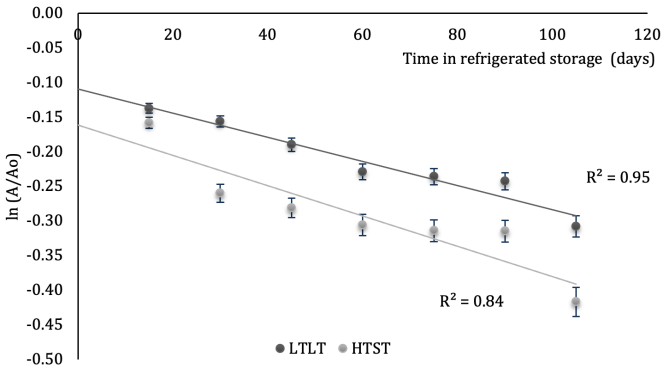

Fig. 6. First order kinetics of antioxidant capacity (DPPH) loss in refrigerated nance pulp. Continue lines represent linear fittings. Bars denote standard error.

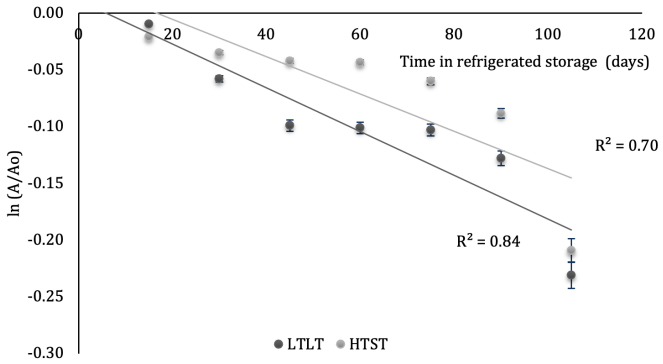

Fig. 7. First order kinetics of antioxidant capacity (ABTS) loss in refrigerated nance pulp. Continue lines represent linear fittings. Bars denote standard error.

3.6 Antioxidant capacity (ABTS)

Unpasteurized pulp had an antioxidant capacity of 7.45 μmol TEAC/ g which was lower than reported values for other pulps of nance of different varieties as reported by Almeida *et al*. (2011) who reported 15.73 μmol TEAC/ g but slightly larger than those obtained by Moo-Huchin *et al*. (2014) of 6.61 μmol TEAC/ g. Almeida *et al*., (2011) reported on 11 fruits recollected from the city of Fortaleza, Ceará, Brazil. They used murci fruit from this region having 11.8 mg/100 mL while our nance fruits had an initial value of 8.86 mg/100 g (24.9% less). This lower amount, type of soil and climate amongst other factors could explain differences found. Pasteurized pulp by LTLT had initial and final values of antioxidant capacity after 3.5 months of refrigerated storage of 9.7 and 7.5 μmol TEAC/ g respectively (20% decrement), whereas HTST pulp showed values of 8.9 and 7.2 μmol TEAC/g (19% decrement) for the pasteurized sample and that at the end of the refrigerated storage respectively. Increments of antioxidant activity of the pasteurized pulps in respect to the control one may, as in the case of antioxidant activity (DPPH), be linked to the increment of phenolic compounds (Nisar *et al*., 2015; Verardo *et al*., 2018) and disruption of cell walls which allows the release of these compounds having a positive influence on the antioxidant capacity. LTLT process caused, a higher decay rate in antioxidant capacity (Figure 7) which was reflected in larger values of rate constant k (Equation 6) for this method as shown in Table 3. Consequently, the half time ($t_{1/2}$) as given by Equation 7, was shorter for the loss of antioxidant capacity due to the LTLT process in comparison to the HTST pasteurization.

Table 4. Results of the microbiological analyses of unpasteurized and pasteurized nance pulp.

Sample	Mesofiles	Molds	Yeasts
Unpasteurized pulp	900 CFU/g	4800 CFU /g	<1 CFU /g
Pulp (LTLT) initial (time zero)	5 CFU /g	ND	ND
Pulp (HTST) initial (time zero)	50 CFU /g	ND	ND
Pulp (LTLT) final (105 days)	Absent	Absent	Absent
Pulp (HTST) final (105 days)	Absent	Absent	Absent
Recommended values (NOM-130-SSA1-1995)	50 CFU/g	<10 CFU/G	<10 CFU/G

CFU: Colony-forming units; ND: No determined.

3.7 Microbiological analyses

In Table 4, the results of the microbiological analyses are presented. As expected, unpasteurized pulp had a higher microbial count than pasteurized pulps obtained by both methods and HTST treated pulp had a larger microbial load than LTLT pulp. However, both pasteurized pulps had microbial counts within the standard (NOM-092-SSA1-1994; NOM-111-SSA1-1994). At the end of the refrigerated storage, both pulps showed microbial loads below detection levels which could be due to inhibition of microorganisms by the refrigeration conditions, biocide effect of phenolic compounds (Ayala-Zavala *et al*. 2005) and the pH. According to the pH values found for these pulps (Table 1), they are classified as acid or medium acid foods ($3.7 < pH < 4.5$) (Ramaswamy & Abbatemarco, 1996), Decrement of aerobic mesophilic count was observed during refrigerated (4 °C) storage of pitaya juice as from the 35th day of the cool storage (Quiroz-González *et al*. 2020; García-Mateos *et al*., 2019).

Conclusions

Pasteurization had a relatively little effect on bioactive compounds profiles of both pasteurized pulps with respect to unpasteurized one. LTLT processed pulp had the lowest effect on such profiles since 88.2% of phenolic compounds and 71.0% of ascorbic acid were preserved during 3.5 months in refrigeration conditions. Also, only a 5% reduction in antioxidant activity (DPPH) was observed for the pulp processed by LTLT. Rates of loss of these bioactive compounds as given by the kinetic constants were also higher for HTST pulp and (except for antioxidant capacity by ABTS) which was slightly higher for LTLT pulp. The $t_{1/2}$ values were, consequently, larger for LTLT pulp which indicated a longer time for deceasing the concentration of bioactive compounds and of the antioxidant capacity. Gallic and caffeic acid, rutin, trans-ferulic acid and quercetin were identified as the main phenolic compounds present in nance pulp and the increment of the intensity of quercetin peak for LTLT pulp could be due to the breakage of rutin. Refrigeration aided in preserving bioactive compounds during storage. Over the 105 days of storage, relatively low amounts of these were lost according to the kinetics parameters determined (k and $t_{1/2}$). Both pasteurization methods reduced microbial load to recommended standards but LTLT reduced it more pronouncedly. Considering losses of the different bioactive compounds and antioxidant capacity and microbiological counts obtained, LTLT pasteurization could be recommended the best alternative for the pasteurization of this pulp.

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Nomenclature

C_0, C: Concentrations at time zero and at any time t.
CFU: Colony-forming units.
CI: Color Index.
ΔE: Total color difference.
$t_{1/2}$: Half time
HPP: High Pressure Processing.
HTST: High Temperature Short Time.
HPLC-DAD: High-Performance Liquid Chromatography.
LTLT: Low Temperature Long Time.
PPO: Polyphenol Oxidase.
k: Rate constant.
SS: Soluble Solids.
TEAC: Trolox equivalent antioxidant capacity.
Wb: Wet basis.

References

Almeida, M. M. B., de Sousa, P. H. M., Arriaga, Â. M. C., do Prado, G. M., de Carvalho Magalhães, C. E., Maia, G. A. & de Lemos, T. L. G. (2011). Bioactive compounds and antioxidant activity of fresh exotic fruits from northeastern Brazil. *Food Research International 44*, 2155-2159. https://doi.org/10.1016/j.foodres.2011.03.051

Ayala-Zavala, J. F., Wang, S. Y., Wang, C. Y. & González-Aguilar, G. A. (2005). Methyl jasmonate in conjunction with ethanol treatment increases antioxidant capacity, volatile compounds and postharvest life of

strawberry fruit. *European Food Research and Technology 221*, 731-738. https://doi.org/10.1007/s00217-005-0069-z

Badui Dergal, S. (2016). *Química de los Alimentos* (6ta edición). Capítulo 6: Vitaminas y nutrimentos inorgánicos. Pearson, México.

Caballero-Roque A., Vela G., Pérez J., Escobar R. & Ballinas J. (2012). Uso de nanche (*Byrsonima crassifolia* (L.) Kunth) en gelatina artesanal para niños. *Etnobiología 10*, 50-55.

Cai, M., Xie, C., Lv, Y., Yang, K. & Sun, P. (2020). Changes in physicochemical profiles and quality of apple juice treated by ultrafiltration and during its storage. *Food Science & Nutrition*. https://doi.org/10.1002/fsn3.1593

Cao, L., Zhou, G., Guo, P. & Li, Y. (2011). Influence of pasteurising intensity on beer flavour stability. *Journal of the Institute of Brewing 117*, 587-592. https://doi.org/10.1002/j.2050-0416.2011.tb00508.x

Casanova, F. C., de Souza, M. A., Fisher, B., Colet, R., Marchesi, M. C., Zeni. J., Dallago, R. M., Paroul, N., Cansian, R. L. & Backes, G. T. (2020). Development of enzymatic - colorimetric time - temperature integrator for smart packaging. *Biointerface Research in Applied Chemistry 11*, 9335-9345. https://doi.org/10.33263/BRIAC112.93359345

Cauvain, S. P. & Young, L. S. (2008). Bakery Food Manufacture and Quality: Water Control and Effects. (2nd edition). John Wiley & Sons, USA.

Chen, Y., Wang, M., Rosen, R. T. & Ho, C. T. (1999). 2, 2-Diphenyl-1-picrylhydrazyl radical-scavenging active components from *Polygonum multiflorum* Thunb. *Journal of Agricultural and Food Chemistry 47*, 2226-2228. https://doi.org/10.1021/jf990092f

Chen, Y., Yu, L. J. & Rupasinghe, H. V. (2013). Effect of thermal and non-thermal pasteurisation on the microbial inactivation and phenolic degradation in fruit juice: A mini-review. *Journal of the Science of Food and Agriculture 93*, 981-986. https://doi.org/10.1002/jsfa.5989

Cserhalmi, Z., Sass-Kiss, A., Tóth-Markus, M. & Lechner, N. (2006). Study of pulsed electric field treated citrus juices. *Innovative Food Science & Emerging Technologies 7*, 49-54. https://doi.org/10.1016/j.ifset.2005.07.001

de Souza, V. R., Pereira, P. A. P., Queiroz, F., Borges, S. V. & Carneiro, J. D. D. S. (2012). Determination of bioactive compounds, antioxidant activity and chemical composition of Cerrado Brazilian fruits. *Food Chemistry 134*, 381-386. https://doi.org/10.1016/j.foodchem.2012.02.191

Domene, R. & Segura, M. (2014). Parámetros de calidad externa en la industria agroalimentaria. Fichas de Transferencia. Available at: https://www.cajamar.es/es/agroalimentario/innovacion/investigacion/documentos-y-programas/fichas-de-transferencia/parametros-de-calidad-externa-en-la-industria-agroalimentaria/. Accessed: March 22, 2020.

Duarte, O. (2011). Nance (*Byrsonima crassifolia* (L.) Kunth). In: *Postharvest Biology and Technology of Tropical and Subtropical Fruits*, (Yahia, E.M. ed.), Pp.44-50, 51e-52e. Woodhead Publishing. https://doi.org/10.1533/9780857092618.44

Floros, J. D., Newsome, R., Fisher, W., Barbosa-Cánovas, G. V., Chen, H., Dunne, C. P., ... & Knabel, S. J. (2010). Feeding the world today and tomorrow: the importance of food science and technology: an IFT scientific review. *Comprehensive Reviews in Food Science and Food Safety 9*, 572-599. https://doi.org/10.1111/j.1541-4337.2010.00127.x

García, E., Fernández, I. & Fuentes, A. (2015). *Determinación de Polifenoles Totales por el Método de Folin-Ciocalteu*. Universitat Politécnica de València. Escuela Técnica. Available at: http://hdl.handle.net/10251/52056. Accessed: November 22, 2020.

García-Mateos, M.R, Quiroz-González, B., Corrales-García, J., Ybarra-Moncada, M. C. & Leyva-Ruelas, G. (2019). Ozone-high hydrostatic pressure synergy for the stabilization of refrigerated pitaya (*Stenocereus pruinosus*) juice. *Innovative Food Science & Emerging Technologies 56*, 102187. https://doi.org/10.1016/j.ifset.2019.102187

Geilfus F. (1994). *El árbol al Servicio del Agricultor: Manual de Agroforestería para el Desarrollo Rural. Guía de Especies*. CATIE-ENDA, Turrialba, Costa Rica.

Gordon, A., Jungfer, E., da Silva, B. A., Maia, J. G. S. & Marx, F. (2011). Phenolic constituents and antioxidant capacity of four underutilized fruits from the Amazon region. *Journal of Agricultural and Food Chemistry 59*, 7688-7699. https://doi.org/10.1021/jf201039r

Hamacek, F. R., Martino, H. S. & Pinheiro-Sant'Ana, H. M. (2014). Murici, fruit from the Cerrado of Minas Gerais, Brazil: physical and physicochemical characteristics, and occurrence and concentration of carotenoids and vitamins. *Fruits 69*, 459-472. https://doi.org/10.1051/fruits/2014032

Herbig, A. L. & Renard, C. M. (2017). Factors that impact the stability of vitamin C at intermediate temperatures in a food matrix. *Food Chemistry 220*, 444-451. https://doi.org/10.1016/j.foodchem.2016.10.012

Irías-Mata A, Esquivel P, Jiménez VM, Carle R. & Schweiggert RM. (2015). Nance (*Byrsonima crassifolia*) fruits, a source of lutein and zeaxanthin - macular carotenoids involved in human health. *Universidad de Costa Rica. Hohenheim University*. Available at: https://www.researchgate.net/publication/274072409_Nance_Byrsonima_crassifolia_fruits_a_source_of_lutein_and_zeaxanthin_-_macular_carotenoids_involved_in_human_health. Accessed: December 17, 2020.

Kapešová, J., Petrásková, L., Markošová, K., Rebroš, M., Kotik, M., Bojarová, P. & Křen, V. (2019). Bioproduction of quercetin and rutinose catalyzed by rutinosidase: novel concept of "solid state biocatalysis". *International Journal of Molecular Sciences 20*, 1112. https://doi.org/10.3390/ijms20051112

Kips, L., De Paepe, D., Van Meulebroek, L., Van Poucke, C., Larbat, R., Bernaert, N., Van Pamel, E., De Loose, M., Raes, K. & Van Droogenbroeck, B. (2017). A novel spiral-filter press for tomato juice processing: Fate of phenolic compounds, carotenoids and ascorbic acid content during spiral-filter processing, thermal downstream processing and storage. *Journal of Food Engineering 213*, 27-37. https://doi.org/10.1016/j.jfoodeng.2017.06.010.

López-Gálvez, F., Allende, A., Truchado, P., Martínez-Sánchez, A., Tudela, J.A., Selma, M.V. & Gil, M.I. (2010). Suitability of aqueous chlorine dioxide versus sodium hypochlorite as an effective sanitizer for preserving quality of fresh-cut lettuce while avoiding by-product formation. *Postharvest Biology and Technology 55*, 53-60. https://doi.org/10.1016/j.postharvbio.2009.08.001

Luna-Ramírez, K.Y., Arellano-Cárdenas, S., García-Pinilla, S. & Cornejo-Mazón, M. (2017). Kinetic analysis of the stability of antioxidants in blackberry (*Rubus fruticosus* L.) Liquor. *Revista Mexicana de Ingeniería Química 16*, 121-130. https://rmiq.org/iqfvp/Pdfs/Vol.%2016,%20No.%201/Alim6/Alim6.html

Mariutti, L. R., Rodrigues, E., Chisté, R. C., Fernandes, E. & Mercadante, A. Z. (2014). The Amazonian fruit *Byrsonima crassifolia* effectively scavenges reactive oxygen and nitrogen species and protects human erythrocytes against oxidative damage. *Food Research International 64*, 618-625. https://doi.org/10.1016/j.foodres.2014.07.032

Martínez-Flórez, S., González-Gallego, J., Culebras, J. M. & Tuñón, M. (2002). Los flavonoides: propiedades y acciones antioxidantes. *Nutrición Hospitalaria 17*, 271-278.

Medina-Torres, R., Salazar-García, S. & Gómez-Aguilar, J. R. (2004). Fruit quality indices in eight nance [*Byrsonima crassifolia* (L.) HBK] selections. *HortScience 39*, 1070-1073. https://doi.org/10.21273/HORTSCI.39.5.1070

Moo-Huchin, V. M., Estrada-Mota, I., Estrada-León, R., Cuevas-Glory, L., Ortiz-Vázquez, E., Vargas y Vargas, M. D. L., Betancur-Ancona, D. & Sauri-Duch, E. (2014). Determination of some physicochemical characteristics, bioactive compounds and antioxidant activity of tropical fruits from Yucatan, Mexico. *Food Chemistry 152*, 508-515. https://doi.org/10.1016/j.foodchem.2013.12.013

Morton, JF. (1987). Fruits of Warm Climates. Creative Resource Systems, Inc., USA.

Nisar, R., Baba, W. N. & Masoodi, F. A. (2015). Effect of chemical and thermal treatments on quality parameters and antioxidant activity of apple (pulp) grown in high Himalayan regions. *Cogent Food & Agriculture 1*, 1063797. https://doi.org/10.1080/23311932.2015.1063797

NMX-F-112-NORMEX-2010. Norma Mexicana. *Alimentos - Determinación de sólidos solubles - Método refractométrico - Método de ensayo (prueba)*.

NMX-FF-011-1982. Norma Mexicana. *Productos alimenticios no industrializados, para uso humano. Fruta fresca. Determinación de acidez titulable. Método de titulación*.

NOM-092-SSA1-1994, Bienes y Servicios. *Método para la cuenta de bacterias aerobias en placa.* NOM-111-SSA1-1994. Bienes y Servicios. *Método para la cuenta de mohos y levaduras en alimentos.*

Olivares, A., Soto, C., Caballero, E. & Altamirano, C. (2019). Survival of microencapsulated Lactobacillus casei (prepared by vibration technology) in fruit juice during cold storage. *Electronic Journal of Biotechnology, 42*, 42-48. https://doi.org/10.1016/j.ejbt.2019.10.002

Ordóñez-Santos, L. E. & Yoshioka-Tamayo, L. S. (2012). Cinética de degradación térmica de vitamina C en pulpa de mango (*Mangifera indica* L). Vitae, 19(1), S81-S83.

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R. & Anthony, S. (2009). *Byrsonima crassifolia*. Available at: http://www.worldagroforestry.org/treedb/AFTPDFS/Byrsonima_crassifolia.PDF. Accessed: April 23, 2020.

Osorio, C., Franco, M. S., Castaño, M. P., González-Miret, M. L., Heredia, F. J. & Morales, A. L. (2007). Colour and flavour changes during osmotic dehydration of fruits. Innovative *Food Science and Emerging Technologies 3*, 353-359. https://doi.org/10.1016/j.ifset.2007.03.009

Pellegrini, N., Del Rio, D., Colombi, B., Bianchi, M. & Brighenti, F. (2003). Application of the 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation assay to a flow injection system for the evaluation of antioxidant activity of some pure compounds and beverages. *Journal of Agricultural and Food Chemistry 51*, 260-4. https://doi.org/10.1021/jf020657z.

Peng, J., Tang, J., Barrett, D. M., Sablani, S. S., Anderson, N. & Powers, J. R. (2017). Thermal pasteurization of ready-to-eat foods and vegetables: Critical factors for process design and effects on quality. *Critical Reviews in Food Science and Nutrition 57*, 2970-2995. https://doi.org/10.1080/10408398.2015.1082126

Pennington, T. D. & Sarukán, J. (2005). *Árboles Tropicales de México: Manual para la Identificación de las Principales Especies*, 3ª edición. UNAM-Fondo de Cultura Económica. Mexico.

Pérez-Reyes, M. E. & Sosa-Morales, M. E. (2013). Mecanismos de transferencia de calor que ocurren en tratamientos térmicos de alimentos. *Temas selectos de Ingeniería de alimentos 7*, 37-47.

Plaza, L., Crespo, I., de Pascual-Teresa, S., de Ancos, B., Sánchez-Moreno, C., Muñoz, M. & Cano, M. P. (2011). Impact of minimal processing on orange bioactive compounds during refrigerated storage. *Food Chemistry 124*, 646-651. https://doi.org/10.1016/j.foodchem.2010.06.089

Quiroz-González, B., Ybarra-Moncada, M. C., Rodriguez-Martinez, V. S., Welti-Chanes, J. S., García-Mateos, M. R., Corrales-García, J., Ibarra, Moncada Ma. C., Leyva-Ruelas, G. & Torres, J. A. (2020). Refrigerated storage of high hydrostatic pressure treated pitaya (*Stenocereus pruinosus*) Juice. *Revista Mexicana de Ingeniería Química 19*, 387-399. https://doi.org/10.24275/rmiq/Alim588

Rábago-Panduro, L.M., Martin-Belloso. O., Welti-Chanes, J. & Morales-de la Peña, M (2020). Changes in bioactive compounds content and antioxidant capacity of pecan nuts [*Carya*

illinoinensis (Wangenh. K. Koch)] during storage. *Revista Mexicana de Ingeniería Química 19*, 1439-1452. https://doi.org/10.24275/rmiq/Alim1149

Ramaswamy, H. S. & Abbatemarco, C. (1996). Thermal processing of fruits. In: *Processing Fruits: Science and Technology*, (L. P. Somogyi, H.S. Ramaswamy & H.Y. Hui, eds.), Pp. 25-65. Technomic Publishing Co., Inc., USA.

Ramos-Carbajal, E., Pérez-Vázquez J. C., Vázquez-Núñez, J., Hernández-Cuello, G. & González-Mejía, O. (2020). Comparación de las propiedades fisicoquímicas de dos fenotipos de nanche (*Byrsonima crassifolia* L.). *Revista Ciencias Técnicas Agropecuarias 29*, 64-73.

Sales, A. & Waughon, T. G. M. (2013). Influência do processamento no teor de compostos bioativos em frutos de murici e cajá. *Agrarian 6*, 7-15.

Sánchez-Chávez, W., Cortez-Arredondo, J., Solano-Cornejo, M. & Vidaurre-Ruiz, J. (2015). Cinética de degradación térmica de betacianinas, betaxantinas y vitamina C en una bebida a base de jugo de remolacha (*Beta vulgaris* L.) y miel de abeja. *Scientia Agropecuaria 6*, 111-118. http://dx.doi.org/10.17268/sci.agropecu.2015.02.03

Santos, A. (2013). *El nanche Byrsonima crassifolia una alternativa de producción frutícola para el municipio de Actopan, Veracruz*. Trabajo de experiencia recepcional, Universidad Veracruzana, México.

Serra, H. M. & Cafaro, T. A. (2007). Ácido ascórbico: desde la química hasta su crucial función protectiva en ojo. *Acta Bioquímica Clínica Latinoamericana 41*, 525-532.

Silva, F.V.M., Gibbs, P.A., Nuñez H., Almonacid, S. & Simpson, R. (2014). Thermal Processes/Pasteurization. In: *Encyclopedia of Food Microbiology*, (C.A. Batt and M.L. Tortorello, eds.), (2nd edition, Vol. 3), Pp. 577-595. Academic Press, USA. http://dx.doi.org/10.1016/B978-0-12-384730-0.00404-3

Singleton, V. L. & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture 16*, 144-158.

Servicio de Información Agroalimentaria y Pesquera (SIAP)(2017). *Sistema de Información Agroalimentaria de Consulta*. Available at: http://www.siap.gob.mx/. Accessed: June 1, 2020.

Teixeira, A. A. (2014). Thermal food preservation techniques (pasteurization, sterilization, canning and blanching). In: *Conventional and Advanced Food Processing Technologies* (Suvendu Bhattacharya, ed) Pp. 115-128. https://doi.org/10.1002/9781118406281.ch6

Thakkar, P., Vaghela, B., Patel, A., Modi, H. A. & Prajapati, J. B. (2018). Formulation and shelf life study of a whey-based functional beverage containing orange juice and probiotic organisms. *International Food Research Journal 25*, 1675-1681

Vallejo-Castillo, V., Muñoz-Mera, J., Pérez-Bustos, M.F. & Rodriguez-Stouvenel, A. (2020). Recovery of antioxidants from papaya (*Carica papaya* L.) peel and pulp by microwave-assisted extraction. *Revista Mexicana de Ingeniería Química 19*, 85-89. https://doi.org/10.24275/rmiq/Alim593

Verardo, V., Glicerina, V., Cocci, E., Frenich, A. G., Romani, S. & Caboni, M. F. (2018). Determination of free and bound phenolic compounds and their antioxidant activity in buckwheat bread loaf, crust and crumb. *LWT 87*, 217-224. https://doi.org/10.1016/j.lwt.2017.08.063

Zanoni, B., Pagliarini, E., Galli, A. & Laureati, M. (2005). Shelf-life prediction of fresh blood orange juice. *Journal of Food Engineering 70*, 512-517. https://doi.org/10.1016/j.jfoodeng.2004.10.019