



Effects of high hydrostatic pressure on the overall quality of Pêra-Rio orange juice during shelf life

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Abstract

The effect of high hydrostatic pressure on antioxidant activity, total phenolic compounds, physicochemical characteristics, color, pectin methylesterase activity, and microbiological count were evaluated during the shelf life of Pêra-Rio orange juice. Pressurized (520 MPa, 60 °C, for 360 s), non-processed and pasteurized (95 °C/30 s) orange juice were compared at zero time of storage. Pressurized and pasteurized juices were studied during a refrigerated 90-day shelf life. Pressurization did not cause expressive change in physicochemical characteristics of Pêra-Rio orange juice along shelf life, but significantly reduced pectin methylesterase residual activity to 13% and microbiological counts below detection levels up to 68 days of storage, with small counts (30.0×10 CFU/mL mesophilic aerobic bacteria and 20.7×10 CFU/mL yeast and mold) at 90 days, capable of ensuring the juice's stability along shelf life. Lightness (L^*) and b^* values were significantly reduced by high hydrostatic pressure during shelf life, while a^* values were significantly higher. Ascorbic acid decreased around 80% during shelf life. Antioxidant activity remained stable after processing and during storage.

Keywords

High hydrostatic pressure, orange juice, shelf life, antioxidant activity, physicochemical characteristics, microbiological count

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INTRODUCTION

Brazil is the largest producer and exporter of orange juice worldwide, withholding 61.3% of the global production in the 2015/2016 harvest. Of the nearly 300 million orange boxes, weighing 40.8 kg each, produced in the 2015/2016 harvest, 15% were consumed in natura and 85% were destined to the industry. Brazil produced 531 mil ton of frozen concentrated orange juice (FCOJ) and 240 mil ton of not from concentrate (NFC) FCOJ equivalent orange juice in the 2015/2016 harvest. The country exports 98% of the orange juice it produces, mostly to European countries (70%), the United States (25%), and Japan (3%) Instituto de Economia Agrícola (IEA), 2017; Secretaria de

Comércio Exterior (SECEX), 2017; United States Department of Agriculture (USDA), 2017). The Brazilian citriculture numbers are expressive—out of five glasses of orange juice consumed worldwide, three come from the Brazilian production (CITRUSB, 2017).

In recent years, a new trend has been observed regarding orange juice consumption of NFC rather than FCOJ. This could be explained by the fact that the NFC juice is exposed a shorter time to high temperatures when compared to FCOJ, resulting in less drastic flavor and aroma modifications (Janzantti et al., 2011).

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Thermal treatment is still the most used processing treatment for orange juice due being able to destroy microorganisms and inactivate enzymes. However, the intense time/temperature conditions may reduce natural and characteristic flavor and ascorbic acid (AA), affecting the juice's overall quality (Janzantti et al., 2011).

The fruit juice industry has been looking into innovative technologies, which use minimal heat treatment and are able to produce juice with more natural-like characteristics, in order to preserve flavor and nutritional aspects, complying with the consumption trend that searches for healthy and flavorful products (Deliza et al., 2005). High hydrostatic pressure (HHP) reduces orange juice contaminating microflora and the enzyme pectin methylesterase's (PME) activity (Nienaber and Shellhammer, 2001) by using pressure instead of high temperature, therefore preserving its sensory and nutritional aspects. Several PME isoenzymes are associated with cloud loss, attributed its endogenous activity, which demethoxylates soluble pectins causing calcium pectates precipitation and clarification of the juice (Versteeg et al., 1980). Cloud stability is an important quality aspect of orange juice, since it positively affects turbidity, flavor, and color characteristics of the juice. A low PME residual activity, however, could still preserve cloud stability during the shelf life of the juice (Bisconsin-Junior et al., 2014).

In addition, color, soluble solids, pH, carotenoid content, and bioactive compounds of orange juice are not considerably affected by HHP (Baxter et al., 2005; Bull et al., 2004; Timmermans et al., 2011; Vervoort et al., 2011).

Although HHP is a well researched technology, to the best of our knowledge, the shelf life study of the Pêra-Rio orange juice has not yet been developed. This technology is still not being used by the Brazilian juice industry, which presents itself as a whole range of opportunities. Bull et al. (2004) reported that Valencia orange juice treated at 600 MPa, 20 °C for 60 s did not affect AA significantly, however a different study found 11% reduction in AA of the juice treated at 100 MPa, 60 °C during 300 s (Sánchez-Moreno et al., 2005). Sánchez-Moreno et al. (2005) reported that after 400 MPa, 40 °C for 60 s, orange juice showed no significant reduction on vitamin C, but presented higher extractability of carotenoids (54%) and flavanones (34%), and no significant difference on antioxidant activity.

The HHP conditions applied in this work were chosen through the results of an optimization study (Bisconsin-Junior et al., 2014), which applied the response surface methodology to evaluate the effect of pressure, temperature and time on PME activity and total counts of aerobic microorganisms, yeasts, and molds. A central composite design (CCD) of three independent variables (pressure, time and temperature) with

five levels and 17 experiments was used (Rodrigues and Iemma, 2009). The increase in pressure (100 to 600 MPa), temperature (30 to 60 °C), and time (30 to 360 s) promoted the reduction on the PME residual activity from CCD (15.4% to 107.9%) and aerobic microorganism count of orange juice. The optimal condition (520 MPa, 60 °C, for 360 s) was defined based on its capacity to reduce PME residual activity below 20% and microbial count lower than 2 log CFU/mL. However, these were not necessarily the same conditions for maintaining optimal vitamin C levels and bioactive compounds activity (Bisconsin-Junior et al., 2015). A different study (Torres et al., 2015), which evaluated the effects of pressure, temperature, and time on orange juice with combinations ranging from 250 to 500 MPa, 25 to 65 °C and 1 to 30 min, found that a 6.5 log cycle reduction was obtained at 400 MPa for 3 min at 25 °C. However, in order to inactivate at least 90% of PME activity the ideal conditions were 500 MPa, at 50 °C for 15 min, corroborating with idea that enzyme inactivation requires more severe conditions, as shown by the optimization study.

The aim of this study was to evaluate the effect of HHP treatment on the quality aspects of the Pêra-Rio orange juice during shelf life, thus offering knowledgeable insight to the citrus industry, enabling it to be produced and commercialized both domestically and abroad.

MATERIALS AND METHODS

Chemicals and materials

Citrus pectin, ABTS diammonium salt, 2,4,6-tris(2-pyridyl)-s-triazine, 2,2-diphenyl-1-picrylhydrazyl, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), and gallic acid were obtained from Sigma-Aldrich (St Louis, MO); AA and glucose purchased from Merck (Darmstadt, Alemanha); Folin-Ciocalteu reagent from Imbralab (Ribeirão Preto, SP, Brazil); potassium persulfate from Fluka (Steinheim, Germany); methanol from JT Baker (Philipsburg, PA); sodium carbonate, sodium hydroxyl, potassium sodium tartrate tetrahydrate, cupric sulfate pentahydrate, and potassium phosphate dibasic from Labsynth (Diadema, SP, Brazil); oxalic acid and 2,6-dichloroindophenol sodium salt hydrate from Vetec (Rio de Janeiro, RJ, Brazil); Petrifilm™ used for counting mesophilic aerobic bacteria, thermotolerant and total coliforms, mold and yeast was obtained from 3M™ (St. Paul, MN).

Orange juice

Oranges from the Pêra-Rio variety were provided by a citrus industry from Araraquara, SP, Brazil. The fruit were cultivated in the region of Bauru, SP, Brazil

(22° 25' 59" S; 49° 10' 31" W), during the 2012/2013 harvest. The oranges were processed at JBT FoodTech Citrus System in Araraquara, SP. Before the extraction, the fruit and the equipment were sanitized using water aspersion, and later submerged in a sodium hypochlorite solution (100 mg of chlorine/L) for 10 min. The extraction itself was performed in the JBT 391B extractor, using ideal configuration for obtaining NFC (not from concentrate) juice and premium juice extractor settings with a UFC-35 finisher (0.25 mm sieve).

HHP treatment

For the HHP treatment, the orange juice (100 mL) was packaged in heat sealed, polyethylene (PE) bags (Selovac 200B II, Selovac, São Paulo, Brazil). The juice was pressurized, according to the experimental design, in a Stansted Food Lab 9000 (Stansted Fluid Power, Stansted, UK). The treatment conditions were previously defined in an optimization study (Bisconsin-Junior et al., 2014). The chosen pressurization conditions were 520 MPa, 60 °C, for 360 s, which are capable of producing orange juice with residual enzyme PME activity lower than 20% and aerobic microorganism count lower than 2 log CFU/mL. The HHP equipment has a 500 mL pressure vessel with a maximum nominal operation pressure of 900 MPa and a temperature range from -20 °C to 90 °C. The temperature in the vessel is controlled by liquid circulation in the outer layer connected to a heating-cooling system. The pressure transmitting fluid used was 70% (v/v) ethanol. The compression rate was 3.5 MPa/s and the decompression time was less than 10 s. During the treatment, the temperature and pressure inside the pressure vessel were monitored (Bisconsin-Junior, et al., 2015).

Pasteurization of the orange juice

The orange juice was pasteurized using the Armfield FTD25D SSHE (Armfield, UK) tubular heat exchanger at 95 °C for 30 s (Braddock, 1999), and later cooled to 20 °C. Afterwards, the juice was packaged aseptically in PE flasks (500 mL).

Physicochemical analysis

The physicochemical parameters of the non-processed, pasteurized, and pressurized orange juice were evaluated. Soluble solids (method no 932.12), total titratable acidity (method no 942.15), pH (method no 945.27), total and reducing sugars (method no 925.36) were analyzed according to Association of Official Analytical Chemists (AOAC, 2011), and ratio was calculated (soluble solids/total titratable acidity). All analyses were performed in triplicate.

Pectin methylesterase

The PME activity was evaluated according to Hagerman and Austin (1986), as reported by Bisconsin-Junior et al. (2014). Orange juice and NaCl (8.8%, w/v) were homogenized (4.5:15, w/v) and centrifuged (18,000 g/20 min at 4 °C). The supernatant was collected and used as the enzymatic extract. The substrate was composed of citrus pectin solution (0.5%, w/v), bromothymol blue (0.01%, w/v) in potassium phosphate buffer (0.003 M) and distilled water. The substrate and enzymatic extract were adjusted to pH 7.5. Substrate was added to the enzymatic extract (20 µL) and the pectin hydrolysis reaction was monitored by the absorbance decrease at 620 nm using a spectrophotometer (Evolution 220, Thermo Scientific, USA), and water as blank. PME activity analyses were performed in triplicate, at 25 °C and results expressed as PME residual activity (%).

Microbiological analysis

Orange juice (10 mL) was added to 90 mL sterilized buffered peptone water (BPW). After homogenization, aliquots were serially diluted in BPW and 1 mL of each dilution was inoculated onto Petrifilm™ 3MTM plates for thermotolerant and total coliforms, mesophilic aerobic bacteria, yeast, and mold counts. The thermotolerant and total coliforms, and aerobic microorganisms count were performed after incubation at 35 ± 1 °C for 48 ± 3 h and the yeast and mold count after incubation at 25 ± 1 °C for 120 ± 6 h. The minimum level of detection was 10 CFU/mL (AOAC, 2011). The analyses were performed in triplicate.

Color measurement

Konica Minolta CM 600D (Konica Minolta Sensing, Osaka, Japan) spectrophotometer was used (Meléndez-Martínez et al., 2005) to measure color (D_{65} light source, 10° observation angle, 8 mm opening). The orange juice was placed in a quartz cell of 10 mm optic path length (50 × 38 × 10 mm). L^* (lightness/darkness), a^* (redness/greenness), and b^* (yellowness/blueness) parameters were evaluated. Additionally, chroma (color saturation), Hue angle, and total color difference were calculated. The analyses were performed at 25 °C, in quintuplicate.

Assessment of bioactive compounds and antioxidant capacity

Ascorbic acid. Ascorbic acid analysis was based on the reduction of 2,6-dichloroindophenol (method n° 967.21) (AOAC, 2011). The analyses were performed

in triplicate and the results were expressed as mg of AA/100 mL of orange juice.

Extraction of bioactive compounds. The extraction was based on the procedure described by Asami et al. (2003). Orange juice (5.00 mL) and a methanol:water solution (10 mL, 80:20, v/v) were vortexed (1 min), sonicated (15 min), and centrifuged (10,000 g/20 min at 20 °C). The supernatant was collected and the residue was submitted to the extraction procedure once again under the same conditions. Then supernatants were joined and submitted to total phenolic compounds (TPC) and antioxidant activity analyses.

Total phenolic compounds. The TPC were determined as described by Asami et al. (2003) and Singleton et al. (1999). Orange juice extract (0.4 mL) was added to the Folin-Ciocalteu reagent (0.12 mL). After 6 min, a sodium carbonate solution (4 mL, 70 g/L) was added and the volume fixed to 10 mL with water. The absorbance was measured at 730 nm using a spectrophotometer (Evolution 220, Thermo Scientific, EUA) and compared to a gallic acid calibration curve, with concentrations ranging from 72 to 200 mg/L. The analyses were performed in triplicate and results expressed as mg of gallic acid equivalent/100 mL of orange juice.

A correction factor was calculated to discount AA, since it responds to the Folin-Ciocalteu reaction. Ascorbic acid standard solutions were prepared in concentrations that corresponded to the same range found in orange juice, and submitted to Folin-Ciocalteu reaction. The results, expressed as mL of gallic acid equivalent/100 mL juice provided a correction factor of 0.389, which was deducted from the previously obtained TPC values.

Antioxidant activity. The antioxidant activity was evaluated with ABTS⁺, as reported by Rufino et al. (2010). ABTS (5.0 mL, 7 mmol/L) was added to potassium persulfate (88 µL, 140 mmol/L) to form the ABTS radical solution. The solution was stored protected from light for 16 h to ensure the complete formation of a stable radical. The ABTS radical solution was diluted with ethanol up to an absorbance of 0.70 ± 0.05 at 753 nm. Three solutions of orange juice extract:ethanol were prepared (1:3; 2:5 and 1:2 v/v). A 30 µL aliquot of each orange juice extract:ethanol solutions from non-processed, pasteurized and pressurized juice was mixed with 3 mL of the ABTS radical solution. Absorbance was measured at 753 nm after 6 min of reaction in a spectrophotometer (Evolution 220, Thermo Scientific, USA). Calibration curves built with Trolox ethanolic solutions (100–1600 µmol/L) were used. All the antioxidant activity analyses were performed in triplicate and

results were expressed as µmol Trolox/100 mL of orange juice.

Shelf life. The pressurized and pasteurized orange juices were stored under the same refrigerated conditions at zero (after juice characterization), 23, 45, 68 and 90 days, which corresponded to 0, 25, 50, 75 and 100 of the shelf life of NFC (ASTM, 1993). The physicochemical parameters, extraction and quantification of bioactive compounds, and antioxidant activity were evaluated during shelf life. The non-processed juice was also analyzed at zero time (juice characterization).

Statistical analysis

Results were expressed as mean \pm standard deviation of three replicate analyses and submitted to ANOVA and Tukey (zero time) or Student's *t* test (shelf life), both at $p \leq 0.05$, using the SigmaStat 4.0 software (Systat Software Inc., San Jose, CA). Linear regression analyses and correlation were performed for shelf life results using the Origin 8 software (Origin Lab, Northampton, MA).

RESULTS AND DISCUSSION

PME activity

The pressurization conditions were chosen based on its capacity to reduce PME activity below 20%. The initial count for PME activity of the non-processed orange juice was considered 100% of residual activity and under these conditions HHP successfully decreased PME residual activity to 13%, nearing the residual activity foreseen (15%) in a previous optimization study (Bisconsin-Junior et al., 2014). The pasteurization was able to reduce residual PME activity to 4% (Table 1).

Figure 1 shows linear regression of each parameter for HHP and pasteurized orange juice and confidence interval, in order to illustrate trends regarding PME residual activity and color parameters during shelf life. The heat treatment was more effective at reducing PME residual activity than HHP, however both juices remained stable under 20% of residual PME activity during shelf life. The enzyme's activity is responsible for a great part in orange juice's quality loss, causing reduction in viscosity and cloud as well as separation of phases. Orange juice pressurized at 800 MPa at 25 °C for 60 s resulted in 4% residual PME activity, showing stability for over 90 days at 4 °C and 37 °C storage (Nienaber and Shellhammer, 2001). Orange juice processed at 700 MPa for 60 s resulted in 18% PME residual activity, also stable for over 50 days at 4 °C (Goodner et al., 1998).

Table 1. Residual PME activity (%), microbiological count and color parameters of pressurized (HHP), pasteurized and non-processed orange juice at zero time of storage

Parameter	Non-processed	HHP	Pasteurized
Residual PME activity (%)	100.00 ± 6.85	13.19 ± 2.15	4.24 ± 0.29
Mesophilic aerobic (CFU/mL)	1.03 × 10 ² ± 2.1 × 10	ND	ND
Yeast and mold (CFU/mL)	3.6 × 10 ± 1.5 × 10	ND	ND
Total coliform (CFU/mL)	<10	ND	ND
Thermotolerant coliform (CFU/mL)	ND	ND	ND
<i>L</i> *	41.38c ± 0.16	42.47b ± 0.27	45.68a ± 0.07
<i>a</i> *	-1.78a ± 0.03	-1.32b ± 0.11	-1.21b ± 0.05
<i>b</i> *	16.13c ± 0.24	18.35b ± 0.42	22.21a ± 0.12
Chroma	16.22c ± 0.23	18.40b ± 0.41	22.24a ± 0.12
Hue angle	83.70c ± 0.17	85.87b ± 0.43	86.87a ± 0.13
Total color difference	0.00c ± 0.00	2.52b ± 0.77	7.52a ± 0.13

CFU: colony formation units; ND: not detected; Chroma = $\sqrt{a^{*2} + b^{*2}}$; Hue angle = arctangent (b^*/a^*); total color difference = $\sqrt{(L^* - L^*0)^2 + (a^* - a^*0)^2 + (b^* - b^*0)^2}$.

Values are mean ± standard deviation ($n=3$). Different letter in each row indicate significant difference in Tukey test ($p \leq 0.05$).

Microbiological quality of orange juice

Total coliform count was below detection levels for all the juices at characterization and along shelf life (AOAC, 2011). The mesophilic aerobic count in the non-processed juice was 10.2×10^2 CFU/mL, and yeast and mold 3.67×10^2 CFU/mL. After treatment, both pressurized and pasteurized juice effectively reduced microbiological count below detection levels.

At each period of time during shelf life the pasteurized and pressurized juice were plated and microbiological count was performed. Counts were below detection levels up to 68 days of storage for both juices. At 90 days of storage (estimated shelf life), pasteurized juice had mesophilic aerobic bacteria growth of 12.3×10 CFU/mL and 2.3×10 CFU/mL for yeast and mold, while pressurized juice had 30.0×10 CFU/mL and 20.7×10 CFU/mL of mesophilic aerobic bacteria and yeast and mold growth, respectively, but there was no visual indication of microbiological growth in the juices. At 99 days (110% of shelf life) both HHP and pasteurized orange juices showed visible microbiological growth, indicating that they had lost their quality and that the estimated shelf life was of 90 days.

Color

Both HHP and pasteurized orange juices exhibited a slightly lower lightness (L^*) compared to the non-processed juice ($p \leq 0.05$), and values remained stable during shelf life, with only a slight reduction at 90 days for the HHP juice. The a^* values were significantly higher for HHP and pasteurized juice compared to

the non-processed juice. The same was observed for b^* values, meaning an increase in yellow color. HHP and pasteurized juices showed a slight increase in a^* values and a slight decrease in b^* values during shelf life, being significantly different ($p \leq 0.05$) over time (Table 1, Figure 1). Confidence interval could not be showed for a^* parameter because of visual overlapping data. No changes in L^* values for Navel oranges, pressurized at 400 MPa, 40 °C for 60 s were found (Sánchez-Moreno et al., 2005). An increase in yellow color (b^*) after juice pasteurization of Navel oranges was observed (Cortés et al., 2008). The same trend regarding color change during shelf life (slight L^* value decrease, a^* value increase and b^* value decrease) (Figure 1) was also reported by Timmermans et al. (2011) using Valencia, Pêra and Baladi oranges during 68 days of storage at 5 °C. Pomegranate juice treated at 450 MPa for 90, 150 s and 550 MPa showed an increase in a^* values (Varela-Santos et al., 2012), similar to our results.

Chroma and Hue angle were higher in HHP and pasteurized juices ($p \leq 0.05$), remaining mostly stable during shelf life, and presenting a slight decrease over time for both juices (Table 1, Figure 1). Total color difference indicates the magnitude of the non-processed juice's color difference in relation to the pressurized and pasteurized juice. Both HHP and pasteurized juices exhibited total color difference value higher than 2, which indicates that it is possible to visually notice the color difference among the juices, as reported by Francis and Clydesdale (1975). Similar results were obtained by Cortés et al. (2008), which observed an increase in chroma after pasteurization of orange

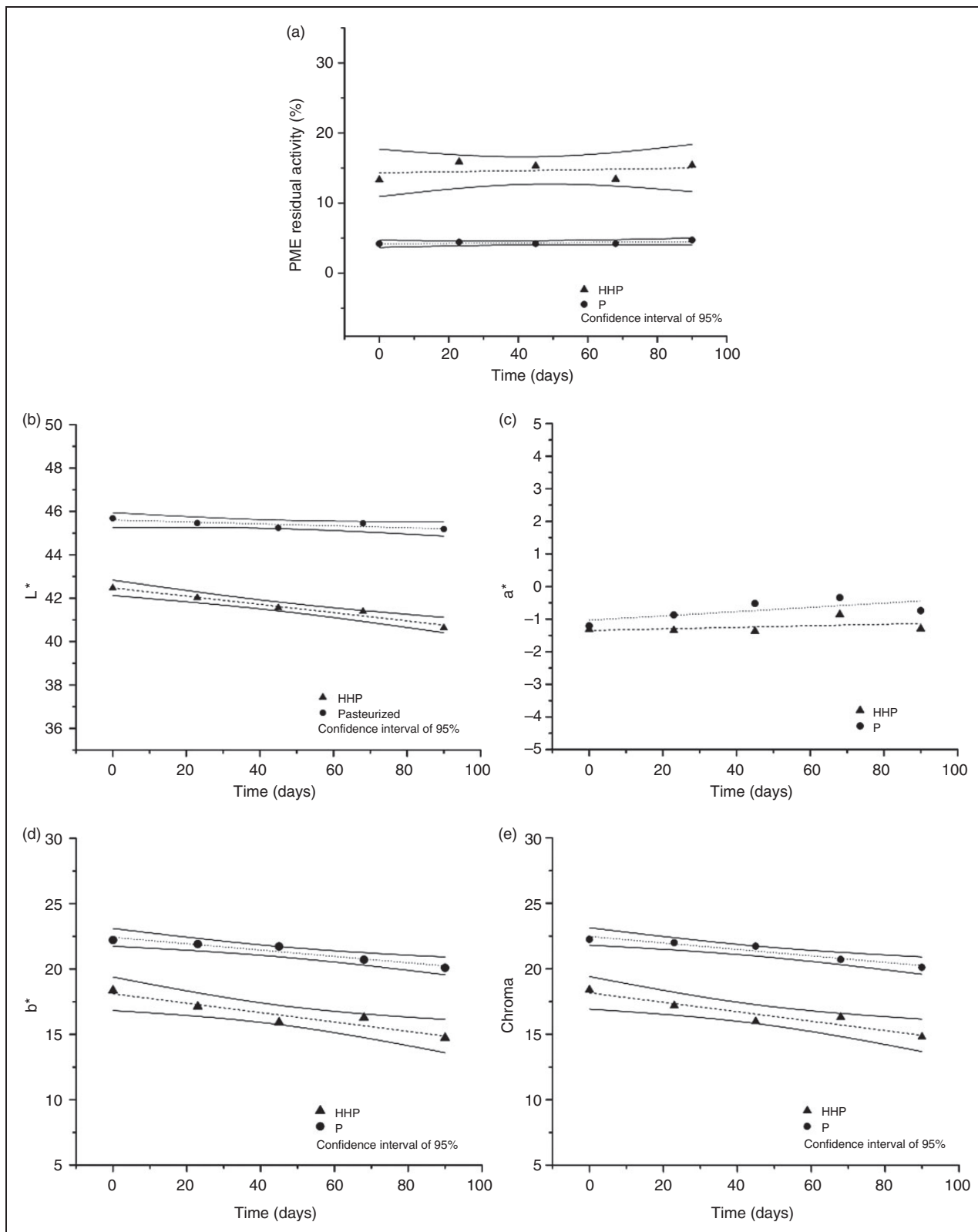


Figure 1. Residual PME activity (%), L^* , a^* , b^* and Chroma parameters for pressurized (HHP) and pasteurized (P) orange juice during shelf life.

juice and high intensity pulsed electric field. An increase in Hue angle was also observed after pasteurization of orange juice (Lee and Coates, 2003). Total color difference was significantly higher in treated pomegranate juice sample at 350, 450 and 550 MPa compared to a control untreated sample, and a decrease was observed during storage (Varela-Santos et al., 2012). Additionally, a study conducted with pumpkin puree treated with HHP found that color was better preserved (total color difference lower than 2) when using lower pressure levels (400 MPa rather than 600 MPa) and treatment time (200 s rather than 600 s) (González-Cebrino et al., 2015).

Physicochemical analysis

The physicochemical characteristics for the non-processed, pressurized and pasteurized orange juice at zero time are in Table 2. Soluble solids were similar among the non-processed, pressurized and pasteurized orange juice ($p \leq 0.05$). The non-processed juice exhibited higher total titratable acidity ($p \leq 0.05$) and no difference was found between the pasteurized and pressurized juices ($p > 0.05$). Both pressurized and pasteurized juices ($p > 0.05$) showed higher ratio compared to the non-processed juice ($p \leq 0.05$). There was no significant difference in pH for all juices. Reducing and total sugar exhibited similar values, with significant differences for pressurized juice ($p \leq 0.05$) (Table 2).

As reported by Bull et al. (2004) and Sánchez-Moreno et al. (2005), pasteurization also led to an increase in soluble solids in Navel and Valencia oranges, similar to our results. A reduction in total titratable acidity was also observed after pasteurization using Navel oranges (Sánchez-Moreno et al., 2005).

Figure 2 shows the shelf life results for both pasteurized and pressurized orange juice which exhibited

stability in all parameters, as well as no significant differences between the juices ($p > 0.05$), indicating that both HHP and pasteurization did not impact the juice shelf life. Orange juice submitted to HHP and thermal treatment from Valencia and Navel oranges, stored at 4 and 10 °C, showed no significant difference in physicochemical parameters between both juices and untreated juice, as well as no changes during storage ($p > 0.05$) (Bull et al., 2004). Similarly, orange juice pasteurized at 92 °C for 30 s showed no significant changes during 32 weeks of storage for pH and total titratable acidity (Wibowo et al., 2015).

Bioactive compounds and antioxidant activity

Ascorbic acid. The non-processed orange juice showed higher AA levels ($p \leq 0.05$) compared to the pressurized and pasteurized juice, as expected (Table 2). The pasteurized juice presented higher AA than the pressurized juice ($p \leq 0.05$). Plaza et al. (2006) reported that HHP reduced AA levels of Valencia oranges in 5% and Sánchez-Moreno et al. (2005) reported AA reduction of 8% in Navel oranges submitted to 400 MPa, at 40 °C for 60 s. The higher temperature and time conditions applied in this work (520 MPa, 60 °C, 360 s) resulted in a higher reduction of AA levels (16%). Similarly, pasteurization reduced AA levels in 13%, lower than a 17% decrease as reported by Elez-Martínez et al. (2006), and higher than the reduction of 8% reported by Sánchez-Moreno et al. (2005) after pasteurizing orange juice at 90 °C for 60 s.

It should be noticed that our results for AA levels for non-processed, pressurized and pasteurized orange juice were in the same range of Brazilian (Stella et al., 2011) and Spanish (Meléndez-Martínez et al., 2007) orange juice.

Table 2. Physicochemical characteristics, total phenolic content and antioxidant activity of pressurized (HHP), pasteurized and non-processed orange juice at zero time of storage

Parameter	Non-processed	HHP	Pasteurized
Soluble solids (°Brix)	9.5b ± 0.1	9.5b ± 0.0	9.7a ± 0.1
Total titratable acidity (g citric acid/100 mL)	0.9a ± 0.0	0.6b ± 0.0	0.6b ± 0.0
Ratio	10.9b ± 0.1	16.0a ± 0.1	16.0a ± 0.1
pH	4.06a ± 0.01	4.07a ± 0.04	4.05a ± 0.04
Reducing sugar (g glucose/100 mL)	3.7a ± 0.0	3.5b ± 0.0	3.7a ± 0.0
Total sugar (g glucose/100 mL)	7.1a ± 0.0	6.6b ± 0.0	7.1a ± 0.1
Ascorbic acid (mg/100 mL)	38.3a ± 0.3	32.2c ± 0.2	33.3b ± 0.3
Total phenolic compounds (mg gallic acid equivalent/100 mL)	52.85a ± 2.8	52.76a ± 3.9	52.62a ± 2.2
ABTS (µmol Trolox/100 mL)	302.3a ± 2.0	299.1a ± 2.4	294.7a ± 8.3

Values are mean ± standard deviation ($n = 3$) and same letter in each row did not indicate significant difference in Tukey test ($p \leq 0.05$).

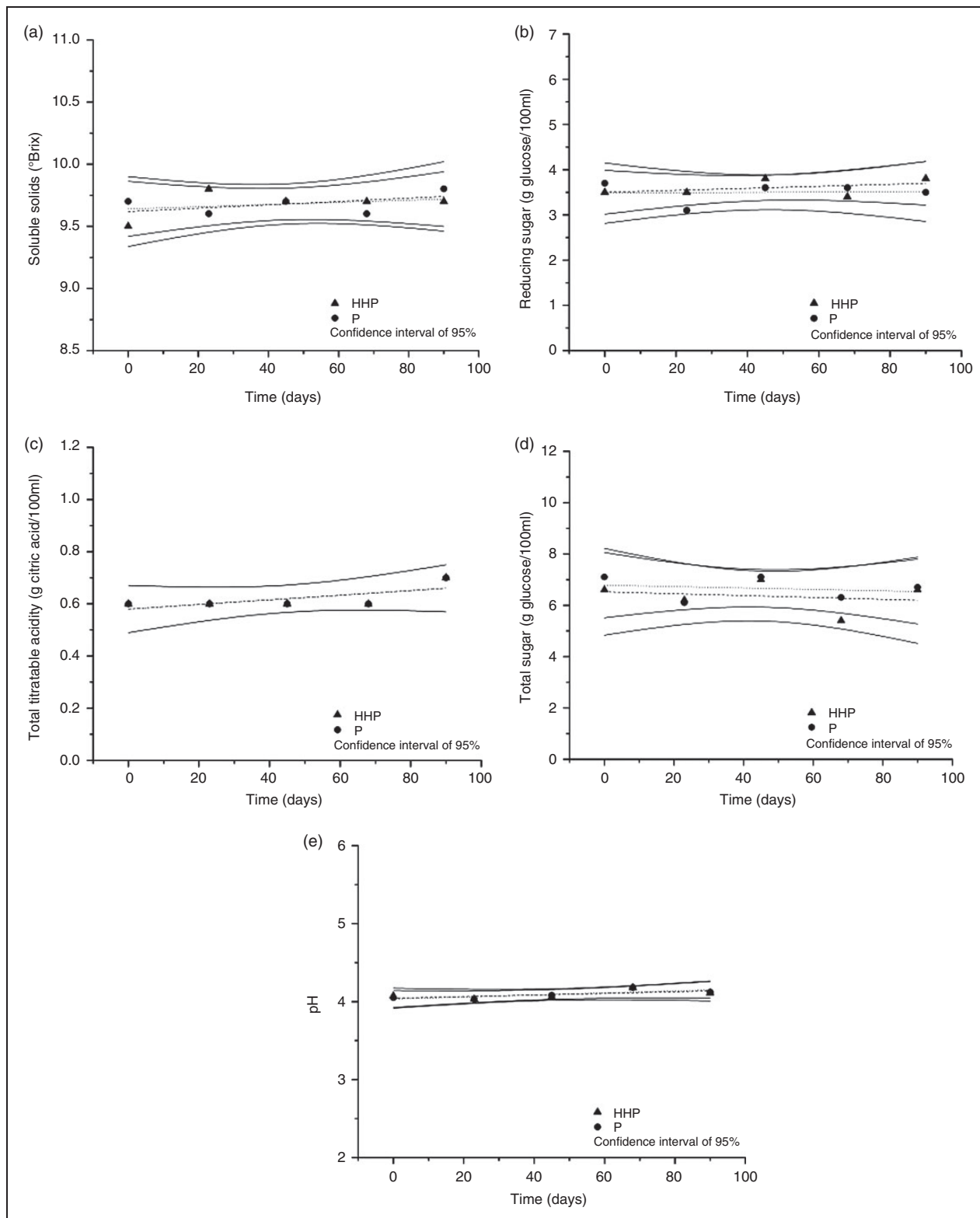


Figure 2. Physicochemical characteristics for pressurized (HHP) and pasteurized (P) orange juice during shelf life.

Figure 3 shows AA levels during storage. Both pressurized and pasteurized juice exhibit the same strong reduction over time ($p \leq 0.05$), reducing around 70–80% of AA levels in 90 days. Ascorbic acid degradation is due to many factors, including type of processing, storage conditions, packaging, oxygen, and light (Shaw and Moshonas, 1991; Teixeira and Monteiro, 2006). The results obtained in our study indicate that a very strong decrease in AA levels occurred during shelf life. Similar results were obtained using Valencia and Navel oranges, which presented a reduction in AA during a three-month storage at 4 °C (Bull et al., 2004). Nienaber and Shellhammer (2001) attributed AA level reduction to its oxidation over time.

Total phenolic compounds. The results for TPC are in Table 2. Non-processed, pasteurized and pressurized orange juice showed no significant difference at zero time, indicating that TPC is resistant to both HHP and thermal treatment. Some studies suggest that TPC may even increase with processing, when antioxidants can be more extracted (Chen et al., 2015; Varela-Santos et al., 2012).

Both pressurized and pasteurized orange juice exhibited the same behavior during shelf life regarding TPC, with a slight decrease over time, and no significant difference between them ($p > 0.05$). Comparative literature on the effects of HHP on TPC for orange juice is scarce; however, a few studies have showed this influence on different fruit juices. A study using papaya beverage treated with HHP and pasteurization, stored at 4 °C showed similar results, with comparable small decrease rate over time, although HHP retained TPC better than pasteurization (Chen et al., 2015). Similarly, a study with mulberry juice, compared thermal treatment (85 °C, 15 min) with HHP (500 MPa, 10 min), and found that HHP was able to retain more TPC (Wang et al., 2016). Varela-Santos et al. (2012) found that pomegranate juice processed with HHP showed slight decrease during storage of 35 days at 4 °C.

Antioxidant activity. Results of antioxidant activity are given in Table 2. Antioxidant activity of pressurized, non-processed and pasteurized orange juice using ABTS radical assay ranged from 294.7 to 302.3 $\mu\text{mol Trolox}/100\text{ mL}$, with no significant difference between pressurized and pasteurized orange juice ($p > 0.05$). The antioxidant activity levels obtained with ABTS were in the range of those reported by Stella et al. (2011) for Brazilian orange juice. Other studies showed that antioxidant activity was not significantly affected after HHP at 440 MPa, 40 °C for 60 s and pasteurization (Gil-Izquierdo et al., 2002; Sánchez-Moreno et al., 2005). González-Cebrino et al. (2015) were able to show that HHP, while using different pressures and

holding times, was effective in maintaining total antioxidant activity levels in pumpkin puree.

ABTS shows a slight increase during shelf life and overall antioxidant activity for both juices maintained high values along shelf life (Figure 3). It has been reported that HHP can either increase or maintain

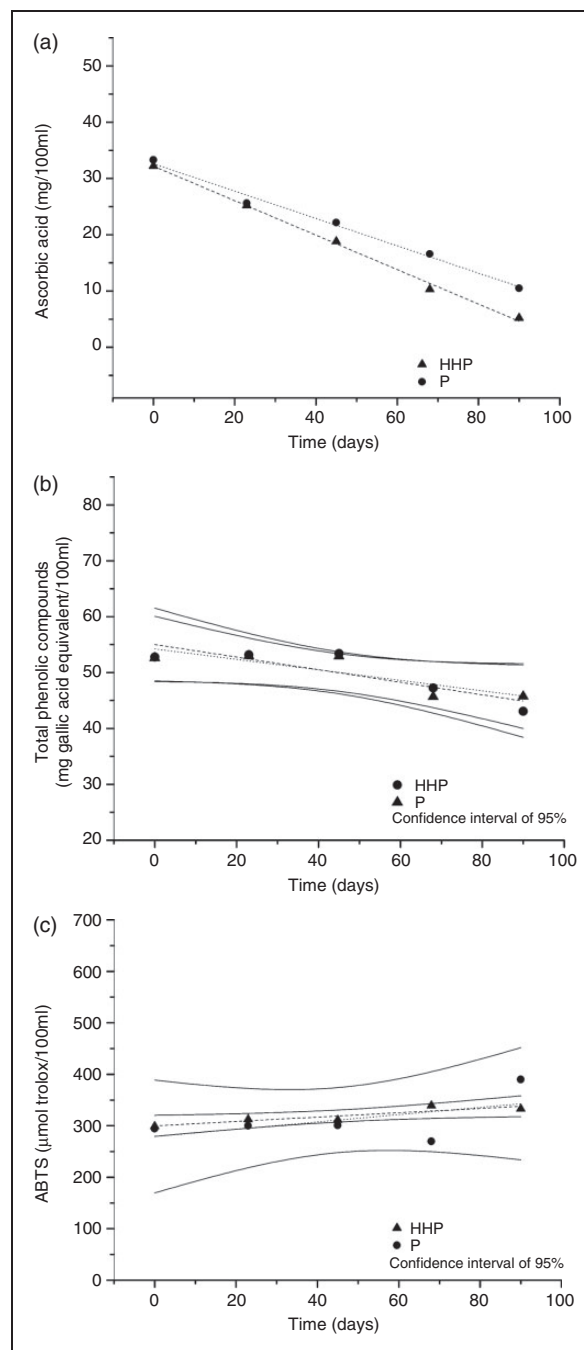


Figure 3. Ascorbic acid (mg/100 mL), total phenolic compounds (mg gallic acid equivalent/100 mL), and antioxidant activity ($\mu\text{mol Trolox}/100\text{ mL}$) in pressurized (HHP) and pasteurized (P) orange juice during shelf life.

antioxidant levels, depending of pressure and time conditions (Casquete et al., 2015).

Antioxidant activity, TPC, and AA correlation. Correlation among antioxidant activity, TPC, and AA was determined for HHP and pasteurized orange juice during shelf life. The correlation coefficient between TPC and AA was positive and strong for both juices ($r=0.8693$ and $r=0.8324$, respectively for HHP and pasteurized). A positive and strong correlation was obtained between TPC and ABTS for HHP juice ($r=0.8322$), and a positive weak ($r=0.3651$) for pasteurized juice. ABTS and AA presented a very strong and positive correlation for HHP juice ($r=0.9249$) and moderate and positive correlation for pasteurized juice ($r=0.5716$).

In a general way, the results indicate that HHP preserved the characteristics of Pêra-Rio orange juice during shelf life, as well as pasteurization, as it did not affect greatly the juice's overall quality and microbiological and enzymatic stability. However, further research is still required. The research group's next step will be an olfactometric and sensorial analysis, in order to explore consumer acceptance of HHP on the juice's aroma.

CONCLUSION

Pressurization did not cause expressive change in physicochemical characteristics of Pêra-Rio orange juice during a refrigerated 90-day shelf life period, but did significantly reduce PME activity and microbiological count, capable of ensuring the juice's stability along shelf life. The orange juice color was slightly affected by HHP over time. Ascorbic acid strongly decreased during shelf life. Antioxidant activity remained mostly stable after processing and during storage. HHP of Pêra-Rio orange juice, maintained the juice's overall quality and ensured microbiological safety and enzymatic stability, rendering it a promising technology for the citrus industry.

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