Effects of high pressure processing on the physicochemical and microbiological parameters, bioactive compounds, and antioxidant activity of a lemongrass-lime mixed beverage

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Abstract This study determined the optimal pressure and time conditions for the high pressure processing (HPP) of a lemongrass-lime mixed beverage. The physicochemical and microbiological characteristics, bioactive compounds, and antioxidant activity of the beverage treated under the optimal HPP conditions were evaluated immediately after processing and during 8 weeks of storage at 4 °C, compared to untreated (control) and thermally pasteurized beverages. HPP at 250 MPa for 1 min at 25 °C ensured microbiological safety, according to inactivation tests with *Listeria innocua* as the target microorganism, without significant losses of vitamin C and phenolic compounds. Immediately after processing, the HPP treated beverage retained its original bioactive compounds content and showed physicochemical characteristics that were closer to the untreated control compared with the thermally pasteurized beverage. In addition, HPP provided microbiological quality and improved the shelf life of the beverage, demonstrating that it represents a reliable alternative to thermal treatment of lemongrass-lime mixed beverages.

Keywords Nonthermal treatment · Pasteurization · *Cymbopogon citratus* · Vitamin C · Phenolics · Shelf life

Introduction

Lemongrass (*Cymbopogon citratus* Stapf) is an aromatic plant from the Poaceae family originated in India and found mostly in tropical countries (Machado et al. 2015). Lemongrass leaves are widely used to prepare medicinal tea and as a culinary ingredient, especially in Asian cuisine, due to their citrus flavor. Extracts of this plant are rich in phenolic compounds with antioxidant properties and essential oil. The oil contains citral as the major component (65–86%), and chlorogenic acid, limonene, luteolin, menthol, and others as minor components (Figueirinha et al. 2008; Marques and Farah 2009; Silva et al. 2010). Certain medicinal properties are attributed to lemongrass, including analgesic, antipyretic, sedative, antimicrobial, and anti-cancer effects (Machado et al. 2015).

Limes are used in cooking and in the production of beverages and juice concentrates worldwide, mostly because of their refreshing and pleasing flavor (Oliveira et al. 2007). Furthermore, limes are one of the main commercial citrus fruits in Brazil and are produced all year round. In addition, lime juice is also a source of antioxidants such as phenolic acids, flavonoids, and vitamin C (Luzia and Jorge 2009).

A mixed beverage is a kind of nonalcoholic beverage containing at least two characteristic ingredients, which can be a combination of fruit juice, fruit pulp, vegetables, extracts, and essential oils (MAPA 2013). Kieling and Prudencio (2017) formulated a new mixed beverage comprising extracts and essential oil of lemongrass, lime juice, and soymilk, which presented good physicochemical and sensory qualities and was well accepted by potential consumers.

Today, consumers appreciate high quality and healthy beverages. To fulfill this market demand, the beverage

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industry needs to find technologies that ensure microbiological safety and stability during shelf life, preserving freshness, sensorial and nutritional qualities (Martínez-Flores et al. 2015; Lee et al. 2017). Thermal processing of beverages results in a decrease in vitamin (Sancho et al. 1999), nutritional (Martínez-Flores et al. 2015) and antioxidant contents (Patras et al. 2009), in addition to undesirable changes in flavor, taste, and color (Bermúdez-Aguirre and Barbosa-Cánovas 2012). By contrast, non-thermal treatments, such as high pressure processing (HPP), cause minimal nutritional losses and sensory changes (Medina-Meza et al. 2015; Patras et al. 2009; Sancho et al. 1999). HPP also allows better retention of bioactive compounds, such as polyphenols (Marszalek et al. 2017), and antioxidant activity (Medina-Meza et al., 2015). According to Patras et al. (2009), small molecules such as volatile compounds, pigments, and vitamins are not markedly affected by HPP.

HPP can be applied to inactivate vegetative microorganisms, thus ensuring microbial safety and an extended shelf life of food products (Guerrero-Beltrán et al. 2004). The process consists of the application of hydrostatic pressure, ranging from 100 to 800 MPa, to beverages or foods, packaged or not, leading to microorganism inactivation by protein denaturation, cell injury or disruption (Guerrero-Beltrán et al. 2005). The main problem connected with the HPP of fruit and vegetable products is the incomplete inactivation of native enzymes, which can cause changes during storage (Marszalek et al. 2017).

Juices are required to achieve the pasteurization standard of 5-log pathogen reduction after processing, for public health reasons (FDA 2004). The inactivation of a number of pathogenic microorganisms, such as *Escherichia coli*, *Salmonella*, and *Listeria monocytogenes* by nonthermal treatments of fruit juices, nectar, purées, and other kind of food products and beverages has been reported (Bermúdez-Aguirre and Barbosa-Cánovas 2012; Martínez-Flores et al. 2015; Patras et al. 2009).

Listeria monocytogenes, one of the pathogenic target microorganisms used in food processing, can grow under conditions in which other foodborne bacteria cannot survive and is more heat resistant. *Listeria innocua*, a non-pathogenic form of *Listeria*, possesses the same characteristics as *L. monocytogenes* once they are phenotypically very similar, which enables both to grow under the same environmental conditions. These characteristics of *L. innocua* and its nonpathogenic nature turns it into an alternative target microorganism to the pathogenic strains (Calderón-Miranda et al. 1999; Silva-Angulo et al. 2015).

There is no information about the thermal or nonthermal processing of lemongrass-lime mixed beverage, since this is a new beverage, which is not available in the market yet. In addition, the effects of HPP on bioactive compounds and

physicochemical and microbiological characteristics of a lemongrass-lime-based product are unknown. Therefore, the objective of this study was to determine the optimal HPP conditions for nonthermal treatment of a lemongrass-lime mixed beverage, evaluating its effects on the physicochemical and microbiological parameters, bioactive compounds, and antioxidant activity, immediately after processing (time zero) and during storage at 4 °C, compared to untreated and to traditional thermally pasteurized controls.

Materials and methods

Mixed beverage preparation

The lemongrass-lime mixed beverage was prepared on the day of processing as follows: lemongrass lyophilized extract (0.26 g/L), lemongrass essential oil emulsion (1.875 g/L), lime juice (90 g/L), sucrose (80 g/L), ascorbic acid (196 mg/L) and ultrapure water were mixed together using a blender. Samples of the mixed beverage were subjected to physicochemical and microbiological analysis immediately after preparation.

The lemongrass essential oil emulsion used in the mixed beverage preparation was made with lemongrass essential oil, xanthan gum, and water (1:1:48 g/g/mL) using a high-speed stirrer (IKA overhead stirrer RW 20, Staufen, Germany). The aqueous phase of the xanthan-water was prepared first, according to the method of Mirhosseini et al. (2008). Lemongrass essential oil was added gradually and the mixture was stirred for 5 min to obtain a homogeneous and stable dispersion. Lime juice was obtained as follows: Fresh Tahiti limes were washed and sanitized using a chlorine solution (150 mg/L). The fruit was cut into halves and the juice was extracted using a manual extractor. Then, the solid particles were separated out by filtration through a cheesecloth.

Inoculum preparation

Listeria innocua (ATCC 51742) pure lyophilized strains were obtained from American Type Culture Collection (Manassas, VA, USA). Stock vials of initial culture were prepared as described by Saucedo-Reyes et al. (2009) and stored at − 80 °C until they were used to determine the growth curve and to obtain a subculture of *L. innocua* for the inactivation experiments.

Growth curve

One stock vial of *L. innocua* was inoculated into 100 mL of tryptic soy broth enriched with 0.6% of yeast extract

(TSBYE). This mixture was shaken constantly at 225 rpm and kept at 37 °C. Samples were taken every 30 min, and the absorbance at 600 nm was determined and used to design the growth curve by optical density. The early stationary growth phase was reached within 6 h.

Subculture

An initial culture of *L. innocua* (one stock vial) was grown in 100 mL of TSBYE under constant agitation (225 rpm) at 37 °C for 5 h (exponential phase) to obtain a cell concentration of 3.5×10^8 colony forming units per mL (cfu/mL). Glycerol 20% (100 mL) was added and the suspension was dispensed into falcon tubes (10 mL) and stored at -4 °C.

For the HPP or thermal inactivation experiments, the content of two subculture tubes (20 mL) was added to 50 mL of TSBYE and then it was left for 20 min at 25 °C, to reactivate the *L. innocua* cells. This suspension was then inoculated into 350 mL of lemongrass-lime mixed beverage before HPP or thermal treatment to obtain an initial cell concentration of approximately 6 log cfu/mL.

High pressure processing

Samples of the mixed beverage (50 mL) were dispensed into polypropylene 15 cm × 24 cm packages (Ultravac Solutions, Kansas City, MO, USA) and vacuum-sealed for HPP. Three packages of the sample were placed inside the cylindrical chamber (internal diameter: 10 cm; internal height: 25 cm) of a high hydrostatic pressure unit (Engineered Pressure Systems Inc., Haverhill, MA, USA) for each processing cycle. The unit was operated using a high pressure pump (Hochdruck Systeme GmbH, Pötsching, Austria) and the pressurization fluid was a solution of 10% Hydrolubric 123B soluble oil (Houghton International Inc., Valley Forge, PA, USA) and 90% water. Come-up time was 0.5 min and depressurization time was 0.2 min. Samples were processed at pressures ranging between 200 and 400 MPa, a holding time of 1 and 2 min, at 25 °C. Immediately after treatment, samples were cooled in a water–ice bath.

First, the effects of pressure and time on vitamin C and total phenolic contents, and inactivation of *L. innocua* were studied. The optimal conditions of pressure and time for HPP at 25 °C were determined based on two principles: on the inactivation of *L. innocua*, as the target microorganism, and on the lack of significant losses of bioactive compounds (vitamin C and total phenolics). The optimized conditions for HPP were then applied for the shelf life study.

Thermal pasteurization

Thermal pasteurization conditions were established based on FDA recommendations (2004) and on preliminary tests using *L. innocua* (ATCC 51742) as the target microorganism. Complete inactivation of *L. innocua* initially inoculated (6 log cfu/mL) was verified when samples of the mixed beverage were treated at 71.1 °C for 3 s. For thermal pasteurization, samples of the lemongrass-lime mixed beverage (350 mL) were placed in a double-walled glass cylinder, with hot water circulation. Then, they were maintained under continued agitation with a magnetic stirrer. The temperature was kept constant (± 0.1 °C) using a re-circulating water bath (VWR Scientific, Model 1166, Niles, IL, USA) and monitored using a thermocouple (K type, Omega Engineering, Inc., Stamford, CT, USA) positioned into the core of the liquid volume. Time was recorded when the temperature in the core reached 71.1 °C. Once the treatment was complete, the liquid was cooled immediately in a water–ice bath.

Shelf life study

Samples of untreated (control), treated by HPP and thermally pasteurized lemongrass-lime mixed beverage were aseptically packaged into 25 mL glass bottles and stored at 4 °C for 8 weeks for the shelf life study. Samples (treated and untreated) were analyzed immediately after processing (zero time) and weekly with regard to pH, acidity, Brix, color, ascorbic acid, total phenolic, antioxidant activity, total mesophiles (standard plate count), total coliforms, and molds and yeast.

Physicochemical analyses

Samples were centrifuged (Sorvall Centrifuge RT 6000B, DuPont Company, Newtown, CT, USA) at $2500 \times g$ and 10 °C for 10 min before physicochemical analyses, with the exception of color and pH determination.

pH, acidity, and Brix values

The pH of the lemongrass-lime mixed beverages was measured using a pH meter (Model FE20, Mettler Toledo, Schwerzenbach, Switzerland), previously calibrated with buffer solutions. Total titratable acidity (TA) was determined by titration (AOAC 1995), and expressed as g citric acid per 100 mL. The total soluble solids content (°Brix) was determined using a digital refractometer (Pocket Pal- α , Atago, Tokyo, Japan).

Color

The color of the samples was measured using a Minolta CM-5 spectrophotometer (Konica Minolta Inc., Osaka, Japan) with a standard illuminant D65 and observed at 10°. Color was expressed as L^* (lightness), a^* (red-green) and b^* (yellow-blue). In addition, chroma (C^*) and hue (H°) were calculated using Eqs. (1) and (2), respectively (Medina-Meza et al. 2015).

$$C^* = [(a^*)^2 + (b^*)^2]^{1/2} \quad (1)$$

$$H^\circ = \tan^{-1}(b^*/a^*) \quad (2)$$

Ascorbic acid

The ascorbic acid (vitamin C) content was quantified by the 2,6-dichlorophenol-indophenol titration method (AOAC 1995) using an extracting solution of metaphosphoric acid-acetic acid. Results were expressed as mg of ascorbic acid per 100 mL.

Total phenolic compounds

The total phenolic compounds were determined using the Folin-Ciocalteu method, as described by Costa et al. (2012). Total phenolic content was quantified by comparison with a calibration curve of gallic acid (4–120 µg/mL), and the results expressed as µg of GAE per mL (GAE = gallic acid equivalents).

Antioxidant activity

The antioxidant activity of the lemongrass-lime mixed beverage was determined using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay according to Casagrande et al. (2007). A control DPPH solution was prepared containing all reagent solutions, except the sample. The antioxidant activity against the radical DPPH $^{\cdot}$ was quantified by comparison to a calibration curve (25–200 µg/mL) of Trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), and the results expressed as µg TE per mL (TE = Trolox equivalent).

Microbiological analysis

For the *L. innocua* count, 1 mL of sample was pour-plated in tryptic soy agar enriched with 0.6% yeast extract (TSAYE), and incubated at 37 °C for 48 h (Calderón-Miranda et al. 1999). To evaluate microbiological stability during storage, 1 mL of each sample was pour-plated in plate count agar (PCA) for total mesophiles (Martínez-Flores et al. 2015) and in violet red bile glucose agar

(VRBG) for total coliforms enumeration (Velázquez-Estrada et al. 2012). These plates were incubated at 35 °C for 48 h. For molds and yeast enumeration, 1 mL of each sample was pour-plated in potato dextrose agar and incubated at 21 °C for 7 days (Martínez-Flores et al. 2015). When necessary, samples were serially diluted in 0.1% sterile peptone water. After the incubation time for each microorganism, the colonies were counted and the microbial count was expressed as colony forming units per mL (cfu/mL).

Statistical analysis

All experiments were conducted in triplicate. Differences between treatments (untreated and HPP under different process conditions) were determined by the one-way ANOVA and the Tukey's test ($p \leq 0.05$). Shelf life experimental data were analyzed according to a Split-Plot system, where the main treatment was the process (untreated, HPP at 250 MPa and 25 °C for 1 min and thermal pasteurization) and the secondary treatment was the storage time (weeks). Differences were determined by the Tukey's test ($p \leq 0.05$). Multiple correlation analysis was carried out to identify correlations between physicochemical parameters. Statistical analyses were conducted using the Statistical Analysis System (SAS Institute Inc., Cary, NC) and the Statistica 7.0 software (Statsoft, Tulsa, OK, USA). The results are reported as the mean $\pm$ standard deviation of three observations.

Results and discussion

Establishing the optimal conditions for high pressure processing

Vitamin C and phenolic contents of lemongrass-lime mixed beverages untreated (control) and HPP treated (200, 250, 300 or 400 MPa for 1 or 2 min) were compared in order to evaluate losses of bioactive compounds after processing.

The mixed beverage showed no significant changes in regards to vitamin C content (22.29 ± 0.56 mg ascorbic acid/100 mL, $p > 0.05$) after HPP at different processing conditions compared to the untreated mixed beverage. The beverage submitted to HPP also showed no significant changes in regards to phenolic content (158.49 ± 0.97 µg GAE/mL, $p > 0.05$) at pressures up to 250 MPa, but decreased by 5.52% ($p \leq 0.05$) when compared to the untreated beverage when the pressure was higher than 300 MPa, regardless of time. However, there was no difference in regards to phenolic content (151.02 ± 1.48 µg

GAE/mL, $p > 0.05$) at pressures between 300 and 400 MPa, independent of time.

Patras et al. (2009) studied the effect of HPP on the vitamin C and phenolic contents of strawberry and blackberry purées. They verified that the vitamin C content of untreated purées decreased significantly (about 9%) after HPP, independent of the pressure applied (400, 500, or 600 MPa). The phenolic content did not change significantly when samples were processed at 400 MPa and 20 °C for 15 min, but increased at higher pressure (600 MPa) compared with the untreated control. According to the authors, this increase in total phenolic content may be related to the increased extractability of some phenolic compounds by high pressure processing.

However, in addition to the pressure-induced extraction, there is also a degradative effect of phenolic compounds (Medina-Meza et al. 2015). When the extraction induced by HPP is higher than the degradation, an accumulative effect is observed. Otherwise, a decrease is observed. Thus, the decrease in the mixed beverage phenolic content after HPP at pressure higher than 300 MPa can be attributed to a degradative effect rather than extraction.

According to Medina-Meza et al. (2015), there were no significant losses of vitamin C in spinach sauce submitted to HPP at 400 MPa for 5 min; however, they noted a reduction in its content between 20 and 30% at higher time or pressure. In their study, the vitamin C content was time-dependent. The authors suggested that there is a food matrix effect on vitamin C degradation.

Jandhyala et al. (2002) studied the effects of HPP and thermal sterilization on vitamin retention, using standard solutions at different pH (2.2, 4.5 and 6.0). They found that vitamin C retention was higher at pH 2.2 or 4.5 than at pH 6.0 after HPP or thermal treatment. The greater retention of vitamin C at acidic pH reflects the fact that the ascorbic acid is more stable to treatment at acidic pH (Gregory 2010; Jandhyala et al. 2002). In the present study, the mixed beverage retained 100% of the original vitamin C content after HPP, regardless of pressure and time, in the range studied, at a pH of about 2.7.

Listeria monocytogenes is a vegetative bacterial pathogen that may be found in acidic juices (pH 4.6 or less), and can be a target for safety performance tests (FDA 2004). In addition, according to the FDA, a treatment must be capable of achieving at least 5-log pathogen reduction in the juice (minimum requirement). However, in the present study, *L. innocua* (nonpathogenic) was chosen as the target microorganism for the inactivation experiment, as recommended by Calderón-Miranda et al. (1999).

In this experiment, the *L. innocua* counts (cfu/mL) were determined before and after HPP treatment at the studied pressure and time conditions. Results showed that the *L. innocua* count before HPP treatment was 83×10^5 cfu/

mL. Samples treated at 200 MPa for 1 and 2 min showed *L. innocua* counts of 16 and 2 cfu/mL, respectively. These results indicate an inactivation of 5 log cfu/mL of *L. innocua* at 200 MPa/1 min and 6 log cfu/mL at 200 MPa/2 min. Samples processed at pressures higher than 250 MPa for 1 or 2 min showed no detectable counts of *L. innocua*. This indicates complete inactivation of *L. innocua* at 250 MPa/1 min.

According to Wang et al. (2016) pressure, pH value, and temperature are crucial factors that affect HPP with respect to enzymes and microbial inactivation. Velázquez-Estrada et al. (2012) verified that for orange juice, which is known to have low pH (about 3.6), processing at pressures from 200 to 300 MPa and at 20 °C for a short time (30 s) ensured microbial quality and enzyme inactivation. Likewise, the acidic pH of the mixed beverage may have contributed to the inactivation of the target microorganism by HPP under mild processing conditions of pressure, time, and temperature.

According to Farkas (2016), an HPP design should focus on high production rate to conform to the industry's demand (high production per hour). Thereby, the lower the processing time at a given pressure, the greater the number of cycles per hour, enhancing the production rate. Based on this concept, and also considering the microbiological safety of the mixed beverage and the preservation of bioactive compounds, the optimal conditions for high pressure processing of the lemongrass-lime mixed beverage for the shelf life study were determined as 250 MPa for 1 min at 25 °C. Thus, the application of this combination of pressure, time, and temperature successfully inactivated the target microorganism and caused no significant losses of vitamin C and phenolic contents.

Shelf life study

Results obtained from the microbiological analyses of the untreated, HPP treated (250 MPa and 25 °C for 1 min), and thermally pasteurized mixed beverage samples during 8 weeks of storage at 4 °C are shown in Fig. 1.

HPP inactivated 85.71% of the total mesophiles (Fig. 1a) and 89.47% of molds and yeast (Fig. 1b) initially present in the untreated beverage. The beverage submitted to HPP was stable for 5 weeks in relation to total mesophiles (Fig. 1a) and for 4 weeks in relation to molds and yeast (Fig. 1b), with very low counts (< 0.3 log cfu/mL) in the respective period. After 4–5 weeks of storage, these microorganisms started to grow, even though the counts of total mesophiles and molds and yeast in the HPP treated samples were always lower than in the untreated control, from the preparation (time zero) to the end of cold storage, indicating that HPP improved the microbiological quality of the untreated lemongrass-lime mixed beverage. As

Fig. 1 Growth of total mesophiles (a) and molds and yeast (b) in the lemongrass-lime mixed beverage during cold storage. Filled square: untreated; filled diamond: HPP treated; filled triangle: thermally pasteurized

shown in Fig. 1, higher levels of microbial growth were observed in the untreated samples after 5 weeks compared with that in the HPP treated samples.

The thermally pasteurized mixed beverage was stable during 8 weeks of storage at 4 °C in relation to mesophiles (Fig. 1a) and molds and yeast (Fig. 1b), probably because these microorganisms are not thermally resistant, thus becoming effectively inactivated by thermal pasteurization.

Considering the acceptable level of the standard plate count (or total mesophiles) of 10 cfu/mL (FDA 2013), for ready-to-drink nonalcoholic beverages, the processed mixed beverage shelf life was 8 weeks and that of the untreated was 5 weeks. Thus, it is possible to state that HPP increased the shelf life of the mixed beverage by 3 weeks (Fig. 1a). The same effect of HPP on extending the shelf life of fruit products was noted by Varela-Santos et al. (2012).

Coliforms (cfu/mL) in the samples of mixed beverage submitted to HPP and thermal pasteurization remained undetectable from beginning to the end of the storage period, conforming to the microbiological quality standard for ready-to-drink juices (Anvisa 2001). This result indicated that HPP ensures the same microbial safety as traditional thermal pasteurization.

Results from the physicochemical analyses of untreated, HPP treated (250 MPa and 25 °C for 1 min), and the thermally pasteurized mixed beverage samples throughout the storage period at 4 °C are shown in Fig. 2. The beverage treated with HPP presented physicochemical parameters close to the untreated beverage immediately after processing (time zero), while thermally pasteurized samples showed greater differences from the original physicochemical characteristics of the untreated mixed beverage.

The pH of the mixed beverages, processed and untreated, remained between 2.57 and 2.86 (Fig. 2a). At the end of storage (8 weeks) the pH of the beverages was slightly lower than at the beginning (time zero).

Immediately after processing, the beverage treated by HPP had a pH similar to the untreated samples, while the pH of the thermally pasteurized beverage was significantly lower than the others.

TA and pH parameters showed an inverse correlation ($r = -0.38$, $p \leq 0.05$). The TA reflects the total concentration of organic acids in the mixed beverage, mostly the citric acid provided by the lime juice. Thus, the higher the citric acid concentration, the higher the TA and the lower the pH of the mixed beverage. Consequently, the TA of the recently thermally pasteurized beverage was higher than that of the untreated samples. Some acid compounds could have been released and solubilized by thermal pasteurization due to the high temperature used (71.1 °C). Processed beverages (HPP treated and thermally pasteurized) presented TA values that were approximately constant, with no significant changes, during 8 weeks of storage (Fig. 2b). Meanwhile, the TA of the untreated beverage increased during storage, probably due to its high microbial count, as shown in Fig. 1.

The HPP treated beverage had total soluble solids content, °Brix (%), similar to that of the untreated beverage and lower than that of the thermally pasteurized beverage immediately after processing (Fig. 3c). The high temperature of thermal pasteurization (71.1 °C), combined with the acidic pH, might have induced sucrose hydrolysis (Zurita 2008), increasing sugar solubility and the °Brix of the mixed beverage (Cheftel 1992). °Brix (%) values varied slightly during cold storage; however, at the end of the storage period, no differences were found between the untreated, HPP treated, and thermally pasteurized beverages.

With regard to vitamin C, HPP caused no significant content change; i.e., the mixed beverage retained 100% of its vitamin C content compared with the untreated beverage immediately after HPP (time zero) (Fig. 2d). Conversely, the thermally pasteurized beverage retained only 79% of the original vitamin C content. However, the vitamin C content of the three beverages (untreated, HPP treated and

Fig. 2 Physicochemical parameters of the lemongrass-lime mixed beverage during storage. Different letters (lowercase for time and uppercase for treatments) indicate a statistical difference ($p \leq 0.05$)

thermally pasteurized) decreased drastically during storage. After 3 weeks, the vitamin C content of the HPP treated beverage decreased by 80.6%. After 5 weeks, all beverages (processed and untreated) had lost 87% of their vitamin C

by Tukey's means test. Open square: untreated; filled diamond: HPP treated; open triangle: thermally pasteurized

compared with the fresh beverage (untreated at time zero), indicating that the effect of other factors (such as the presence of residual oxygen, exposure to light and enzymes) can overcome the effect of treatment. This

Fig. 3 Color parameters of the lemongrass-lime mixed beverage after processing and during storage. Different letters (lowercase for time and uppercase for treatments) indicate a statistical difference

emphasizes that the HPP cannot ensure vitamin C retention in the beverage without measures to avoid chemical degradation during storage.

Medina-Meza et al. (2015) also observed greater vitamin C retention in HPP treated (70–80%) than in thermally pasteurized (30%) spinach sauce. They verified significant vitamin C losses only during the first week of storage for HPP and higher vitamin C retention when lower pressure

($p \leq 0.05$) by Tukey's means test. Open square: untreated; filled diamond: HPP treated; open triangle: thermally pasteurized

was applied (80% of initial value at the end of the storage period of 21 days or 3 weeks). Martínez-Flores et al. (2015) found 100% of vitamin C retention in carrot juice treated by ultrasound at 58 °C, packaged in sealed air free plastic bags and stored during 20 days at 4 °C, without exposure to direct light.

According to Gregory (2010), vitamin C is susceptible to oxidation and its stability depends highly on food

composition and storage conditions. In this context, the degradation rate is determined by light, temperature, oxygen concentration, metal ions, and the pH. Oey et al. (2008) confirmed that oxygen plays an important role in the degradation of vitamin C in high pressure processed food products during subsequent storage. According to the authors, vitamin C degradation can be limited by decreasing the initial oxygen concentration; i.e., the stability of vitamin C after HPP depends on the molar ratio of the vitamin and oxygen concentrations.

Immediately after processing, the phenolic content of the HPP treated beverage was similar to that of the untreated samples and higher than that in the thermally pasteurized beverage (Fig. 2e). The phenolic content of the HPP treated beverage decreased quickly in the first week of storage (18.65%), and continued to decrease slowly until 70% of the original quantity and stabilized at 4 weeks of storage. From this point, the phenolic content of the HPP treated beverage was consistently higher than that of the thermally pasteurized and untreated samples until 8 weeks of storage. The phenolic content of both thermally pasteurized and untreated beverages decreased after 3 weeks of storage to the same level (61% of the original quantity), which was lower than that of the HPP treated beverage. The phenolic content of the thermally pasteurized beverage stabilized at this level and was maintained until the end of the storage period, whereas that of the untreated beverage continued to decrease.

Earlier, Patras et al. (2009), ascorbic acid and phenolic contents of strawberry purées were also reported to be significantly higher for HPP treated samples compared to thermally pasteurized samples. Total phenolics, as well as ascorbic acid, remained practically stable in carrot juice sonicated at 58 °C during 20 days of storage at 4 °C (Martínez-Flores et al. 2015). These reports reinforce that, in addition to nonthermal treatment, other measures, such as air-free, packaging and protection against light can contribute to better retention of bioactive compounds.

HPP treated and untreated beverages with higher vitamin C and phenolic contents at time zero had greater antioxidant activity than the thermally pasteurized samples (Fig. 3f). The initial antioxidant activity dropped drastically (74%) after 3 weeks of storage and decreased slightly towards the end of the storage period. Antioxidant activity (DPPH) correlated positively with both the vitamin C ($r = 0.98$, $p \leq 0.01$) and phenolic ($r = 0.93$, $p \leq 0.01$) contents. This indicated that the antioxidant activity of the lemongrass-lime mixed beverage results from a combined effect of vitamin C and phenolic compounds. As phenolic compounds and ascorbic acid are natural antioxidants capable of scavenging free radicals (Martínez-Flores et al. 2015), it would be expected that a reduction in these

components would decrease the antioxidant properties of the mixed beverage.

Figure 3 shows the color parameters for the mixed beverages during cold storage. HPP did not cause significant color change in the mixed beverage; the values of the color parameters, L^* , a^* , and b^* were similar to the untreated samples, immediately after processing and during cold storage (Fig. 3a–c). After thermal pasteurization, the L^* component decreased (Fig. 3a) and the yellow component (b^*) became more intense compared to the untreated mixed beverage (Fig. 3c), indicating that the beverage color changed from light yellow to dark yellow.

Patras et al. (2009) reported better color retention in strawberry and blackberry purées submitted to HPP than in those that were thermally pasteurized. Medina-Meza et al. (2015) observed a spinach sauce darkening after both thermal pasteurization and high pressure processing. However, in general, the spinach sauce processed by high pressure presented smaller color differences than the thermally pasteurized sauce, compared to the untreated.

All mixed beverages (untreated, HPP treated, and thermally pasteurized) presented an increase in the a^* and a decrease in the b^* components after 6 weeks of cold storage, indicating a loss of green and yellow color, respectively (Fig. 3b, c). Color intensity (C^*) also decreased after the same period of storage (Fig. 3d), confirming that color degradation could have started after 6 weeks (42 days) of storage. Hue values (H°) for the mixed beverages remained close to yellow (90°) (Fig. 3e).

HPP at low temperatures is known to have a limited effect on pigments, causing minimal alteration in the natural color of food and beverages. However, changes in color may occur during storage due to incomplete inactivation of enzymes and microorganisms. The degradation of pigments observed in several fruit systems may have been catalyzed by the presence of oxidase enzymes during or after processing (Marszałek et al. 2017; Medina-Meza et al. 2015; Patras et al. 2009).

The green color of the mixed beverage is due to the chlorophyll content from lemongrass leaves; thus, the loss of the green color observed can be attribute to chlorophyll decomposition (Martinazzo et al. 2010). According to Ghuman et al. (2017), chlorophyll, the major component of wheatgrass, acts as antioxidant scavenger. The authors observed in their study that the high radical scavenging capacity of wheatgrass juice is due to the high chlorophyll content as well as to the high phenolic content. Therefore, chlorophyll decomposition as well as phenolic and vitamin C reduction may have contributed to the decrease in the antioxidant activity of the lemongrass-lime mixed beverage.

Conclusion

This study demonstrated clearly that HPP at pressures up to 250 MPa and at 25 °C for a short period of time (1–2 min) had no effect on the vitamin C and phenolic contents of the lemongrass-lime mixed beverage. Nonthermal treatment by high pressure resulted in better retention of the original content of bioactive compounds and the physicochemical quality characteristics of the beverage compared with thermal pasteurization. HPP at 250 MPa and 25 °C for 1 min ensured microbiological safety and improved the microbiological quality and shelf life of the lemongrass-lime mixed beverage. Other measures related to preservation should be combined with HPP to improve stability and ensure the retention of bioactive compounds and the antioxidant activity of the lemongrass-lime mixed beverage until the end of its shelf life. HPP represents a reliable alternative to thermal treatment of lemongrass-lime mixed beverages.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

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