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Inactivation of the microbiota and effect on the quality attributes of pineapple juice using a continuous flow ultrasound-assisted supercritical CO_2 system

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Abstract

Supercritical carbon dioxide (SC-CO_2) inactivation technology represents a promising non-thermal processing method, as it causes minimum impact on the nutritional food properties. The aim of this study was to analyze the combined effect of SC-CO_2 and high power ultrasound (HPU) on the inactivation of natural microbiota and the quality attributes of pineapple juice treated in a continuous flow system. Different juice residence times (3.06-4.6 min), at 100 bar and 31.5 °C, were used. The results indicated that the microbiota inactivation was complete and the differences obtained in the quality attributes (2.2 % for pH, 4.8 % for °Brix, 2% for Vitamin C) were minimal. During storage, microorganisms were not able to recover and the vitamin C decrease could be limited to 8.2% after 4 weeks. The results demonstrated that the SC-CO_2-HPU technique could be an excellent alternative for the cold pasteurization of pineapple juice.

Keywords: Non-thermal process; supercritical CO_2; ultrasound; pineapple juice; quality attributes

INTRODUCTION

Pineapple is an important tropical fruit largely consumed in the form of processed products, such as juices (Costa et al., 2013). Pineapple, and consequently its juice, is one of the fruits with the highest content of antioxidant and phenolic compounds. Some of the phenolic compounds existing in pineapple juice are S-sinapyl-L-cysteine, N-γ-L-glutamyl-S-sinapyl-L-cysteine, S-sinapyl glutathione, and p-coumaric acid. Pineapple juice also contains phytosterols, such as ergostanol and stigmastanol (Ng and Hupé, 1998). These phytosterols lower cholesterol by reducing its absorption. Vitamin C, a water-soluble vitamin abundant in pineapple juice, plays an important role in human nutrition due to its high antioxidant activity. Thus, it reduces the risk of heart disease by preventing the oxidation of low-density lipoprotein (LDL) cholesterol. Pineapple juice is appreciated for its very pleasing aroma and flavor. It is generally drinkable in single-strength, reconstituted or concentrated forms, and can be mixed with other juices to develop new flavors for beverages and other products due to its strong acid flavor (De Carvalho et al., 2008). Conventional thermal treatments of foods, such as pasteurization, have been the first-choice method for the purposes of extending the shelf-life of fruit juices. However, heating processes can affect the freshness and quality of food products, which leads to consumer rejection. Non-enzymatic browning reactions and pigment destruction have been found to be major causes of such problems (Rattanathanarerk et al., 2005), in addition, the loss of organic acids, such as vitamin C, and the decrease in phenolic compounds cause a reduction in product quality (Gómez et al., 2011).

The demand for high quality processed foods, which preserve their natural and fresh-like characteristics, has led to the development of non-thermal processing techniques, as an alternative to conventional heat treatments (Char et al., 2010). New technologies applied to foods are first and foremost of concern because of safety implications. Then, as data accumulate and microbial safety can be ensured to a satisfactory level, other concerns are addressed. These include quality attributes, which involve the physical (color, viscosity, particle size, Brix, etc) and chemical (pH, flavor volatiles and composition) properties of processed products. Enzyme activity, nutritional quality and shelf life, including sensory properties, are also addressed (Balaban and Ferrentino, 2012).

Supercritical carbon dioxide (SC-CO_2) processing is a non-thermal process capable of inactivating microorganisms, at relatively moderate pressures (7.3 MPa) and at temperatures (31.1 ºC) low enough to avoid the thermal effects of traditional methods

(Benedito et al., 2015). Beyond the critical point of CO_2, the differences between liquid and gaseous CO_2 no longer exist in the newly formed supercritical fluid phase, in which its viscosity is lower than in the liquid state and its density and dissolving power are higher than in the gaseous state. Therefore, the use of SC-CO_2 for sterilization purposes is considered to be more effective than the use of CO_2 in its subcritical state (Balaban and Duong, 2014; Ortuño et al., 2014). Moreover, CO_2 can be used in the food industry because it is non-toxic, non-flammable, inexpensive, and GRAS status (Balaban and Duong, 2014; Calvo and Torres, 2010). Although SC-CO_2 technology represents an excellent non-thermal processing method, high pressures, high temperatures and long treatment times are required to guarantee the safety and stability of the food, especially in batch systems. In these systems, coupling with other technologies, such as high-power ultrasound (HPU), might be necessary in order to obtain the required lethality at shorter processing times and lower treatment intensities. As a means of improving the efficiency of batch SC-CO_2 treatments, a continuous system was developed by Paniagua et al. (2016), who studied the inactivation of *S. cerevisiae* in apple juice, using the continuous flow SC-CO_2-HPU at different juice residence times (3.06-9.2 min), temperatures (31-41 °C) and pressures (100-300 bars). The results demonstrated that the maximum inactivation achieved by the system was 7.8 log-cycles. However, there is no report in the literature addressing the use of this continuous technique (SC-CO_2-HPU) for other kinds of juices, nor any reference to the effect of the process on the processed product quality and microbiota after the treatment and subsequent refrigerated storage. Therefore, the aim of this study was to determine the effect of the SC-CO_2-HPU treatment in continuous regime on both the inactivation of the microbiota and the quality attributes of pineapple juice both after the treatment and during refrigerated storage.

MATERIALS AND METHODS

Pineapple juice

Pineapples (*Ananas comosus L.*) were purchased from a local market and kept at 4 °C. Pineapple juice was obtained by washing, peeling and extracting the fruit juice (Ultra Juicer, Robot Coupe J80, USA). Juice extraction took place just prior to processing; consequently, an extraction was required for each experiment. Each experiment required about 1.5 L of juice, 1 L was used for processing (SC-CO_2-HPU), and 0.5 L served as control.

SC-CO_2-HPU processing

Laboratory continuous regime equipment was designed and built for high-power ultrasound-assisted supercritical CO_2 treatment (Figure 1) (Paniagua et al., 2016).

The SC-CO_2-HPU process applied to the juice was as follows: first, liquid carbon dioxide was supplied from the tank to the chiller reservoir. The liquid CO_2 was supplied from the bottom of the chiller reservoir (which stores it at −18 °C) to the pump where it was compressed at the targeted pressure. Initially, the equipment was stabilized at the treatment pressure (100 bar) and temperature (31.5ºC) only with SC-CO_2 at a constant flow rate of 5 mL/min. Then, the ultrasound equipment was connected, and, once the process conditions were attained, the sample to be treated was pumped to the mixing point (7, Fig.1) where it mixed with the SC-CO_2. The mixture went into the sonication vessel (8, Fig. 1), where the HPU was applied. For the experiments with HPU, the power applied during the whole experiment was 40 W±5W (I=250 ±10mA; U=220 ±5 V, measured with a Digital Power Meter, Yokogawa, Model WT210). Pressure and temperature were kept constant during the experiment. The mixture of juice/SC-CO_2 exiting the treatment vessel went into the holding tube (14, Fig. 1) and, finally, into the separation vessel (15, Fig. 1), where it was depressurized and the CO_2 separated from the juice and recirculated to the reservoir (3, Fig. 1). Prior to each experiment, the different sections of the equipment that the product flows through were cleaned and sanitized with disinfectant solution (Delladet VS2, Diversey, Spain), and distilled and autoclaved water. To determine the effect of the residence time on the quality parameters and inactivation of the microbiota of pineapple juice, two residence times (3.06 and 4.6 min) were considered. These residence times were chosen according to our previous work with apple juice (Paniagua et al., 2016)., where it was observed that 4.6 min were enough to obtain a 6 logs reduction of S. cerevisiae. Residence time was calculated using Eq. (1).

$$\tau_{ToT} = \frac{V_{SeV} + V_{SoV}}{q + q_{CO_2}} \quad (1)$$

Where (V_{SoV}) was the volume in the sonication vessel (40 mL) and the holding tube volume (V_{SeV}) was 52 mL, the juice flows (q) were 15 and 25 mL/min and the SC-CO_2 flow (q_{CO_2}) was 5 mL/min.

The process conditions: pressure (100 bar), temperature (31.5°C) and total residence time (3.06 and 4.6 min) were selected from previous experiments in order to

attain acceptable microbial inactivation levels (Paniagua et al., 2016). Before and after the treatment, the natural microbiota and the quality attributes of pineapple juice were analyzed.

[insert Figure 1]

Microbiota analysis

The viability of mesophilic viable bacteria (MVC), yeast and *E. coli* in the juices were determined by plate count. Each sample was serially diluted with sterilized distilled water. 100 µL of the appropriate dilution (10^{-1} and 10^{-2}) were plated in triplicate on LB Agar, PCA Agar or YPD Agar plates and incubated for 24 h at 37 °C, 35 °C or 30 °C, for E. coli, MVC and yeast, respectively, before counting. Results were expressed as $-\log(N/N_0)$, where N_0 is the initial number of cells in the control sample and N is the number of cells in the sample after the different treatments. However, in the case of total inactivation, the results were expressed as $\log(N_0)$.

pH and °Brix

The pH of treated (TJ) and control (CJ) pineapple juice samples was measured using a digital pH-meter (pH Crison 25, Spain). Soluble solids were measured using a refractometer (Pocket Digital Refractometer Hand-held, Atago, Japan). Samples were measured in triplicate at room temperature.

Ascorbic acid (Vit. C)

The ascorbic acid content of TJ and CJ samples was measured using the 2, 6 dichloroindophenol titrimetric method (AOAC 967.21). The ascorbic acid reduced the indicator dye, 2, 6 dichloroindophenol, to a colorless solution through oxidation–reduction reactions. The measurements were taken in triplicate.

Storage of treated samples

0.5 L of the control and treated samples were stored in glass vials and refrigerated at 4°C for 4 weeks. The samples were analyzed to examine the characteristics of the juice during the storage and to compare the behavior of the microbiota and vitamin C in the control and treated samples. The analyses were performed at weeks 0, 1, 2, 3 and 4.

Data analysis

Using the statistical package, Statgraphics Centurion XVI, a multifactorial ANOVA was carried out, and Tukey's test (α = 0.05) was performed to calculate the mean differences in order to evaluate the influence of the treatments used.

RESULTS AND DISCUSSION

Microbiota *inactivation after the* SC-CO_2-HPU treatment

The results of the microbiota inactivation in pineapple juice are shown in Table 1. When the SC-CO_2-HPU continuous treatment was applied, the total inactivation of the initial microbial load of MVC, yeast and *E.coli* was obtained at the two residence times employed. According to Garcia-Gonzalez et al. (2007), the fundamental step in microbial inactivation by means of SC-CO_2 is its contact with the cell membrane and the consequent physico-chemical modifications. The mechanisms involved in the microbial inactivation using SC-CO_2 include the solubilization of CO_2 into the medium where the cells are suspended, an intracellular pH decrease, key enzyme inactivation/cellular metabolism inhibition due to intracellular pH lowering, a direct inhibitory effect of molecular CO_2 and HCO_3^- on the microbial metabolism, a disordering of the intracellular electrolyte balance and the removal of vital constituents from cells and cell membranes.

[insert Table 1]

As for the inactivation rate, it has been seen to increase alongside temperature, pressure and exposure time, where the temperature and pressure tend to act synergistically on each other (Erkmen, 2003; Lin et al., 1992). The inactivation rate is dependent on the initial number of cells, the type of bacterial species and the kind of suspended materials. On the other hand, the organic compounds (such as carbohydrates, fats and others) present in the media may increase the resistance of bacteria to SC-CO_2 treatment (Balaban and Duong, 2014; Benedito et al., 2015).

In SC-CO_2-HPU treatments, the acceleration of the solubilization rate of SC-CO_2 into the liquid and the increase in the mass transfer due to the vigorous agitation produced by the ultrasonic field would permit the rapid saturation of CO_2 in the medium, which might accelerate the inactivation mechanisms (Gao et al., 2009). The other possible mechanism is the cavitation produced by HPU in the liquid phase

(Gogate et al., 2011). Cavitation refers to the formation, growth, and implosion of tiny bubbles of CO_2 or water vapor in a liquid when ultrasounds travels through it. Cavitation has been proven to cause cracked or damaged cell walls, which enhances the penetration of SC-CO_2 inside the cells, changing the cellular equilibrium and facilitating the extraction of intracellular compounds, thus accelerating the death of the microbial cells. Ortuño et al. (2014) observed that after the SC-CO_2-HPU treatment, the cell wall and cell membrane were totally disrupted, thus easing the disintegration of the cytoplasm and the inactivation of cells. The damage caused by the treatment was serious enough to prevent a possible regrowth of cells. In the present research, although the conditions of the treatment were mild (31.5 °C, 100 bar), the inactivation was complete. This is due to the combined effect of both technologies that allows the mechanisms explained above to obtain satisfactory results. To date, there is only one work covering the use of a continuous SC-CO_2-HPU system for microbial inactivation (Paniagua et al., 2016). In this previous work, apple juice was treated and only the inactivation of inoculated *S. Cerevisiae* was considered. However, in the present paper it has been proved that this novel technology can be used to inactivate the juice microbiota, which is the real contamination in food products and its removal is a condition to exploit the technology at industrial level.

Effect of the SC-CO_2-HPU treatment on the quality attributes of pineapple juice

The results obtained for the effect of the treatments on the quality attributes of pineapple juice are shown in Table 2. The treatment caused a significant ($p<0.05$) increase in the pH values between the CJ and TJ (2.22% and 3.61% increase for 3.06 and 4.6 min respectively). In the case of the °Brix, results showed a slight but significant ($p<0.05$) decrease between the CJ and TJ (4.8% for both residence times). As regards the pH, there was a significant effect of the treatment time on the variation produced by the treatment; the longer the residence time, the greater the variation. However, no effect of residence time was observed for the ºBrix. Arreola et al. (1991) measured the pH and ºBrix of Valencia orange juice treated with SC-CO_2 at 7–34 MPa, 35–60°C and for 15–180 min in a batch system. They showed that there was no significant ($p<0.01$) difference between the pH and °Brix of the original juice and the SC-CO_2 treated one. Kincal (2000) used a continuous-flow SC-CO_2 system for orange juice treatment under pressures of 38, 72 and 107 MPa, CO_2 juice ratios of 0.40 to 1.18 and a residence time of 10 min. This author found no significant ($p>0.05$) changes in pH and °Brix after the treatment. Moreover, Fabroni et al. (2010) studied the orange

juice treated with supercritical carbon dioxide, under different treatment conditions (130, 230 bar, 36± 1 °C, 0.385, 0.770 gCO_2/gjuice; residence time was 15 min) and no statistically significant ($p>0.05$) differences were found between the pH and °Brix of the treated and control samples. However, according to the research carried out by Bermúdez-Aguirre and Barbosa-Cánovas (2012), the application of thermo-sonication (24 kHz, 400 W) promoted significant changes ($p<0.05$) in the pH of the three juices tested (pineapple, grape and cranberry). The main changes observed in pH by Bermúdez-Aguirre and Barbosa-Cánovas (2012) were attributed to the formation of certain chemical products (nitrite, hydrogen peroxide and nitrate) during the ultrasonic application. Therefore, it seems that the change in the pH observed in the present study brought about by the continuous SC-CO_2-HPU treatment could mainly be due to the effect of ultrasound, or the combination of both techniques (SC-CO_2-HPU), rather than for the single use of SC-CO_2. However, although a change in pH is observed in the present study, the final values of treated juices are within the range of pH values for natural pineapple juice.

[insert Table 2]

As to vitamin C, the results indicated a moderate, but significant ($p<0.05$), decrease between the CJ and TJ (Table 2). Moreover, a significant ($p<0.05$) effect of the residence time was observed, the vitamin loss only being 1.97% for a residence time of 3.06 min and 5.9% for 4.6 min. Fabroni et al. (2010) observed that the average vitamin C content was reduced by 6.5% after the SC-CO_2 treatment (230 bar, 36±1 °C, 5.08L/h juice flow rate, 0.770 gCO_2/gjuice), but remained unchanged, with respect to the untreated juice, after the treatments at lower pressure, regardless of the amount of CO_2 employed. Ascorbic acid degradation is characterized by simultaneous aerobic and anaerobic reactions, the aerobic degradation being the fastest one (Ahrne et al., 1996). As air content is limited in the SC-CO_2 treatment, the observed vitamin degradation could be due to the effect of ultrasound in the process. Adekunte et al. (2010) observed a degradation of vitamin C (32.5%) using ultrasounds (61 μm for 10 min). Rawson et al. (2011) observed a reduction in ascorbic acid in watermelon juice when using thermosonication. The reduction was variable, but reached 50% when the maximum temperature (45°C), amplitude (61 μm) and time (10 min) were employed. This behavior is mainly due to sonochemical reactions and the extreme physical conditions which occur during sonication. It is known that hydrogen ions (H^+), free radicals (O^-, OH^-, HO_2-) and hydrogen peroxide (H_2O_2) are formed during the sonolysis of the water molecules (Feril & Kondo, 2004; Pétrier et al., 2007) present in juice samples. The ascorbic acid degradation during ultrasonic processing could be related to oxidation reactions, promoted by the interaction with free radicals formed during sonication.

Hydroxyl radicals produced by cavitation may be involved in the degradation of ascorbic acid. Sonication can be related to advanced oxidative processes, since both pathways are associated with the production and use of hydroxyl radicals (Adekunte et al., 2010). Although ultrasounds were employed in the present research, the ascorbic acid degradation was minimal due to the short residence times used, the low oxygen content and, possibly, to the lower cavitation energy found in a supercritical medium. Another important factor in this type of juice is the effect of the treatment on its phenolic content. Although previous works have shown no reduction of phenolic compounds when using only SC-CO_2 (Del Pozo et al., 2006), its combination with HPU could affect its content and should be matter of future research.

Microbiota changes during refrigerated storage

Microbial counts were performed every week for a storage period of 4 weeks at 4°C, chosen to emulate the temperature found during retail and household refrigerated storage. The initial microbial load of control juice was 1.4 x 10^4 CFU/mL of MVC, 5.8 x 10^3 CFU/mL of yeast and 6.9 x 10^3 of *E. coli.* After 4 weeks, the counts for the control juice reached values of 9.06 x 10^6 CFU/mL, 2.46 x 10^6 CFU/mL and 1.17 x 10^6 CFU/mL for MVC, yeast and *E. coli*, respectively. In the case of the processed juices, the treatment produced an initial microbial count of zero and after the 4 weeks of storage, no growth was observed. Other authors that have conducted SC-CO_2 inactivation treatments have found that, although just after the treatment no microbial growth is found, bacterial cells are able to recover and growth appears during product storage (Ortuño et al., 2014). However, in the present study, the combined treatment (SC-CO_2-HPU) causes critical damage to the cells, which are not able to recover and, therefore, no growth is observed during refrigerated storage. Although the non-recovery of microorganisms after a SC-CO_2+HPU batch treatment was previously proved for E. coli and S. cerevisiae (Ortuño et al., 2014), this is the first work that demonstrates the non-viability of natural microbiota after a SC-CO_2+HPU continuous treatment.

Stability of vitamin C during refrigerated storage

The results for vitamin C obtained during the 4 weeks of refrigerated storage are shown in Table 3. At the beginning of the storage, the content of vitamin C in the treated juice was 33.4 and 34.8 ppm for 3.06 and 4.6 min of residence time, respectively. This value was slightly lower than that found in control (untreated)

pineapple juice (35.51 ppm Vit. C / 100 mL). The vitamin C content of the 3 different samples studied significantly ($p<0.05$) decreased during the 4 weeks of storage. The greatest decrease corresponded to the SC-CO_2-HPU treated sample with a residence time of 3.06 min (19.5% variation) and the smallest to the control juice (6%). No change in the vitamin content was observed after 2 weeks of refrigerated storage in the case of the SC-CO_2-HPU treated samples with a residence time of 4.6 min; this shows that, although the initial inactivation is greater for this residence time, it provides better results during storage. Choi et al. (2002), studied ascorbic acid retention in blood orange juice during refrigerated storage. These authors observed that ascorbic acid decreased gradually as storage time progressed; more than 50% was lost within 3 weeks of storage and was completely degraded after 5 weeks of storage in the case of the natural juice. The decrease in vitamin C content during storage was also observed by Klimczak et al. (2007), who found that 19 % of the ascorbic acid in natural orange juice degraded while refrigerated for 6 weeks. Piljac-Zegarac et al. (2009) studied the ascorbic acid degradation of six dark fruit juices (blackcurrant, cranberry, blueberry, pomegranate, strawberry and cherry). The juice samples were separated into two groups, with strawberry, blackcurrant and cherry exhibiting high vitamin C content and cranberry, pomegranate, and blueberry exhibiting moderate-low vitamin C content. During refrigerated storage, the vitamin C content in blueberry juice dropped to zero after 7 days of storage, and reduced to 50% of the initial value within the first 74 h. Vitamin C showed better stability in the other juices; in the cases of pomegranate and cranberry, it dropped to zero after 9 days in refrigerated storage, while it showed a gradual, but steady, decline in cherry and strawberry juices. By day 28, the vitamin C content in strawberry and cherry juices dropped to 58% and 35% of the initial values, respectively. According to the literature, the decrease in the vitamin C content in the juice during storage is dependent on the storage conditions, such as temperature, oxygen and light access. Oxygen is usually mainly responsible for the loss in vitamin C during storage. In this regard, the significant vitamin C reduction in the present study might be due to the presence of oxygen in the head-space of the glass bottle. Vitamin C retention has been used as an indicator of fruit juice shelf-life. It has been accepted that the shelf-life of fruit juice could be determined by a 50% loss or the half-life of the vitamin C (Odriozola-Serrano et al., 2008; Laorko et al., 2013). Therefore, according to Table 3, none of the juices reached their shelf-life after the 4 weeks of refrigerated storage. Moreover, the lower reduction of vitamin C during storage (4 weeks) of the juice treated with a residence time of 4.6 min (8.16% reduction), indicates that this treatment would provide a higher quality of the juice, compared to the juice treated for 3.06 min.

[insert Table 3]

CONCLUSIONS

The results demonstrated the potential of the continuous SC-CO_2-HPU inactivation technique. The microbiota was completely inactivated using mild process conditions. The changes in the different quality attributes provoked by the treatment are minimal and the final values are within normal ranges for natural pineapple juice. On the other hand, the storage results showed that no microbial growth/recovery is observed and that minimal reductions in vitamin C are found. Further studies should consider the effect of the treatment on the sensory attributes, especially flavor, of the processed juice. Thus, the use of mild process conditions could lead to an increase in the quality of the product treated using this technique.

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DECLARATION OF CONFLICTING INTERESTS

The authors declare that there is no conflict of interest.

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Highlights

- Ultrasound-assisted supercritical CO_2 treatment completely inactivated juice microorganisms
- By shortening residence time, juice quality characteristics were improved
- Microorganisms were not able to recover during refrigerated storage
- Changes in vitamin C during storage were minimal
- The innovative technology presented employs mild process conditions

Fig. 1. Supercritical CO_2 continuous treatment system. 1. CO_2 tank; 2. N_2 tank; 3. Chiller reservoir; 4. CO_2 Pump; 5. Liquid reservoir; 6. Liquid Pump; 7. Mixing point; 8.Sonication vessel; 9. Sonotrode; 10. Insulation joint; 11. Ceramics; 12. Power generation unit; 13. Thermostatic bath; 14. Continuous contact tube; 15. Separation vessel; 16. Treated sample; 17. CO_2 Recirculation; 18. Sonication vessel output, 19. Separation vessel output, V. valve; VS. non-return valve; VM. micrometric valve; P. Manometer; T. temperature sensor.

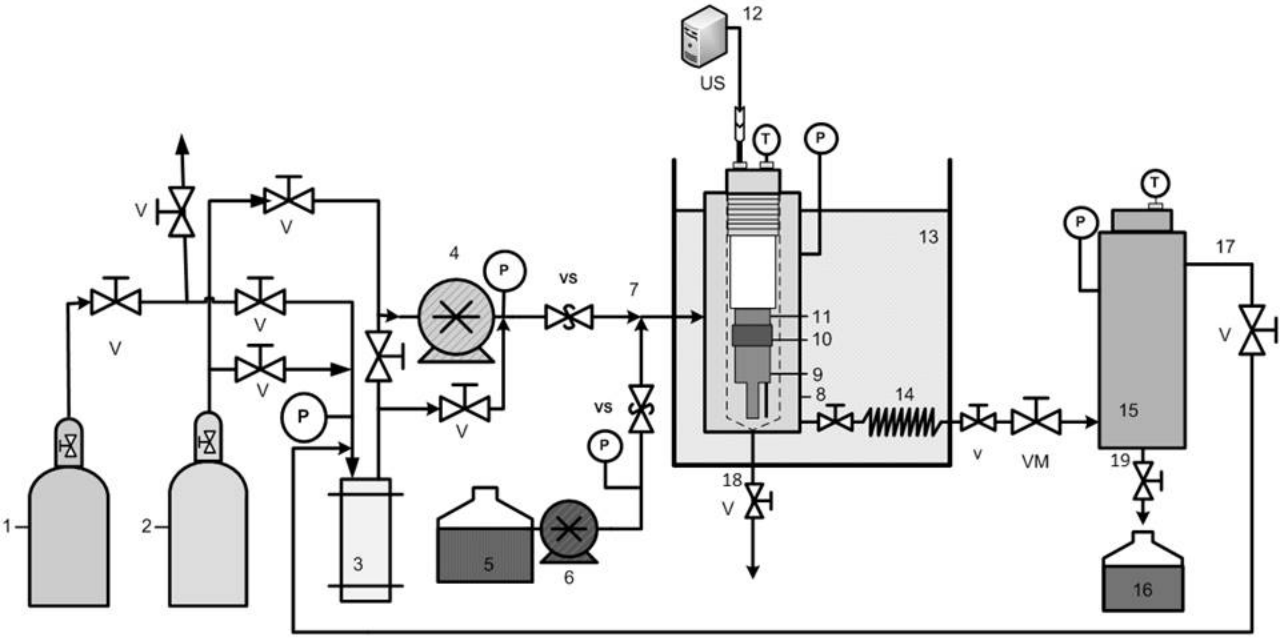
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Table 1. Inactivation of the microbiota in pineapple juice at different residence times (3.06 and 4.6 min)

		Treatment/Conditions	
		100 bar, 31 ºC 3.06 min	100 bar, 31 ºC 4.6 min
MVC	N_0 (CFU/mL)	$1.40x10^4$	$1.40x10^4$
	N (CFU/mL)	0	0
	log (N_0)	4.15	4.15
Yeast	N_0 (CFU/mL)	$5.80x10^3$	$5.80x10^3$
	N (CFU/mL)	0	0
	log (N_0)	3.76	3.76
E. coli	N_0 (CFU/mL)	$6.90x10^3$	$6.90x10^3$
	N (CFU/mL)	0	0
	log (N_0)	3.84	3.84

MVC: Mesophilic viable bacteria

Table 2. Effect of the SC-CO_2-HPU continuous treatment on pH, ºBrix, and Vit. C for different juice residence times (3.06 and 4.6 min).

		Treatment/Conditions	
		100 bar, 31 ºC 3.06 min	100 bar, 31 ºC 4.6 min
pH	Control (CJ)	3.6±0.01[c]	3.6±0.01[c]
	Treated (TJ)	3.68±0.01[a]	3.73±0.01[b]
	Variation	2.22%	3.61%
º Brix	Control (CJ)	12.5±0.11[c]	12.5±0.11[c]
	Treated (TJ)	11.90±0.10[a]	11.93±0.09[a]
	Variation	-4.80%	-4.80%
Vit. C (ppm)	Control (CJ)	35.50±0.18[c]	35.5±0.18[c]
	Treated (TJ)	34.82±0.07[a]	33.45±0.13[b]
	Variation	-1.97%	-5.90%

Different letters indicate significant differences ($p < 0.05$)

Table 3. Analysis of vitamin C during refrigerated storage (4 weeks) of SC-CO_2-HPU treated (at two different residence times) and control pineapple juices

Week	Control		Treated (residence time 3.06 min)		Treated (residence time 4.6 min)	
	ppm Vit. C	% of Variation	ppm Vit. C	% of Variation	ppm Vit.C	% of Variation
0	35.51± 0.01[a]		33.4±0.10[a]		34.8±0.22[a]	
1	35.51± 0.01[a]	0	31.81±0.08[b]	-4.76	34.8±0.15[a]	0
2	34.8± 0.09[b]	-1.99	30.2±0.12[c]	-9.58	34.8±0.08[a]	0
3	34.51±0.05[c]	-2.81	29.5±0.17[d]	-11.67	33.38±0.05[b]	-4.08
4	33.38±0.04[d]	-5.99	26.9±1.02[e]	-19.46	31.96±0.09[c]	-8.16

Different letters within a column indicate significant differences ($p < 0.05$)