Effect of vacuum-thermosonication on the inactivation of *Escherichia coli*, *Staphylococcus aureus*, polyphenol oxidase and the quality parameters of soursop puree

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ABSTRACT

The combined effect of vacuum, heat and ultrasound (vacuum-thermosonication, VTS) on soursop puree was investigated with regard to the viability of *Escherichia coli* and *Staphylococcus aureus*, inactivation of polyphenol oxidase and sensory quality. The VTS conditions were: vacuum (8.46, 11 and 16.93 kPa), heat (40, 45 and 50 °C), 1–3 intermittent vacuum pulses and ultrasound (24 kHz and 0.34 W/g of acoustic energy density) during 10 min. According to response surface methodology, the best conditions to obtain the highest microbial inactivation of ≥ 7 log CFU of inoculated *E. coli* and *S. aureus*, and reduction in polyphenol oxidase activity (94%) were 16.5 kPa vacuum, 50 °C, and three intermittent vacuum pulses for 10 min with ultrasound. The best VTS conditions did not negatively affect the quality parameters, and there were no significant changes in sensory attributes of the puree (a panel of 50 untrained judges). Therefore, we conclude that VTS appears a viable option in the processing of soursop.

1. Introduction

Soursop (*Annona muricata* L.) is a tropical fruit grown in countries such as Angola, Brazil, Colombia, Costa Rica, Cuba, Jamaica, India, Panama, Peru, Puerto Rico, Venezuela and Mexico. It is an aromatic and bittersweet fruit (14–20°Brix and 0.8–1% malic acid), with white flesh and external green colour (Espinosa, Ortiz, Tovar, Mata, & Montalvo, 2013).

The consumption of fruit puree is highly recommended mainly for its nutritional content and health benefits (Pérez-Beltrán et al., 2018). Unfortunately, fruit purees can deteriorate quickly due to microbial growth, enzymatic browning, and chemical and physical changes if they are not adequately processed (Falguera et al., 2012). Thermal pasteurization is the most common heat treatment applied to fruit-based products, particularly fruit purees. It provides excellent microbial

and enzymatic stability and extends the shelf-life of foods (Rabie, Soliman, Diaconeasa, & Constantin, 2014; Saeeduddin et al., 2017). Soursop pulp has been reported to be a source of bioactive compounds (León-Fernández, Obledo-Vázquez, Vivar-Vera, Sáyago-Ayerdi, & Montalvo-González, 2017) and therefore, is considered very healthy. However, it is highly susceptible to spoilage and browning catalyzed by the polyphenol oxidases (PPO), thus negatively affecting its sensory and nutritional quality (Anaya-Esparza, Velázquez-Estrada, et al., 2017; Dias et al., 2015). Besides, it has been reported that thermal processing causes undesirable alterations, reducing the quality and freshness of soursop-based products (Umme, Asbi, Salmah, Junainah, & Jamilah, 1997; Umme, Bambang, Salmah, & Jamilah, 2001; Umme, Salmah, Jamilah, & Asbi, 1999).

Emerging technologies are investigated as complete or partial alternatives to thermal processing, among which ultrasound application

Table 1

Experimental matrix and treatments (VTS1–VTS15), untreated puree (UNP) and physicochemical parameters in soursop purees treated with vacuum-thermo-sonication (VTS).

Treatments	Vacuum (kPa)	Intermittent vacuum pulses	Initial temperature (°C)	Final temperature (°C)	pH	Titratable acidity (g MAE/100 g)	Total soluble solids (°Brix)
UNP					3.63 ± 0.01^a	1.22 ± 0.01^{bcd}	22.50 ± 0.50^{ab}
VTS1	8.46	2	40	40.25 ± 2.72	3.63 ± 0.01^a	1.21 ± 0.01^{bc}	22.83 ± 0.76^{ab}
VTS2	16.93	2	40	40.38 ± 1.84	3.64 ± 0.01^a	1.20 ± 0.01^{bc}	22.50 ± 0.50^{ab}
VTS3	8.46	2	50	50.50 ± 1.08	3.60 ± 0.01^a	1.31 ± 0.02^e	22.50 ± 0.40^{ab}
VTS4	16.93	2	50	50.88 ± 1.89	3.61 ± 0.01^a	1.27 ± 0.02^b	22.16 ± 0.28^{ab}
VTS5	8.46	1	45	45.25 ± 2.10	3.65 ± 0.01^a	1.08 ± 0.01^a	22.66 ± 0.57^{ab}
VTS6	16.93	1	45	45.38 ± 2.32	3.66 ± 0.01^a	1.28 ± 0.01^b	22.00 ± 0.50^{ab}
VTS7	8.46	3	45	45.63 ± 2.43	3.64 ± 0.02^a	1.18 ± 0.03^b	21.50 ± 0.50^a
VTS8	16.93	3	45	45.75 ± 1.55	3.60 ± 0.01^a	1.25 ± 0.07^{bcde}	22.33 ± 0.28^{ab}
VTS9	11	1	40	40.88 ± 1.93	3.63 ± 0.01^a	1.30 ± 0.03^{cde}	21.83 ± 0.28^{ab}
VTS10	11	1	50	50.50 ± 1.08	3.60 ± 0.01^a	1.27 ± 0.03^{de}	22.00 ± 0.28^{ab}
VTS11	11	3	40	50.88 ± 1.31	3.63 ± 0.01^a	1.33 ± 0.02^e	21.50 ± 0.50^a
VTS12	11	3	50	50.13 ± 1.65	3.59 ± 0.01^a	1.20 ± 0.02^{bc}	23.16 ± 0.28^b
VTS13	11	2	45	45.88 ± 0.75	3.60 ± 0.01^a	1.20 ± 0.01^{bc}	23.16 ± 0.28^b
VTS14	11	2	45	45.63 ± 1.03	3.58 ± 0.02^a	1.20 ± 0.01^{bc}	23.16 ± 0.28^b
VTS15	11	2	45	45.63 ± 1.25	3.59 ± 0.01^a	1.20 ± 0.02^{bc}	23.16 ± 0.28^b

Values are the average of triplicate determinations from 3 different experiments ($n = 3$) $\pm$ standard deviation. Means in a column with different letters are significantly different ($p < 0.05$).

stands out, since the ultrasound can be combined with other barrier technologies (i.e., heat, pressure, antimicrobials, and additives) (Chemat, Zill-e-Huma, & Khan, 2011). The combination of high hydrostatic pressure (HHP), heat and ultrasound, is named man-othermosonication (MTS) and its additive effect significantly increases bacterial and enzyme inactivation compared with thermal processing, ultrasound only or thermosonication (ultrasound plus heat) (Anaya-Esparza, Méndez-Robles, et al., 2017; Caminiti, Noci, Morgan, Cronin, & Lyng, 2012). MTS has proved to be an efficient tool to inactivate microorganisms such as *Listeria monocytogenes*, *Salmonella enterica* and *Escherichia coli* in shorter times than thermal pasteurization, ultrasound, or thermosonication (Lee, Zhou, Feng, & Martin, 2009; Lee, Zhou, Liang, Feng, & Martin, 2009; Pagán, Mañas, Raso, & Condón, 1999), satisfying the US Food and Drug Administration (FDA) Hazard Analysis Critical Control Point (FDA, 2004) stipulations to reduce at least 5 log cycles of spoilage and pathogenic microorganisms. In addition, MTS has been used to inactivate peroxidase (López & Burgos, 1995a), lipoxygenase (López & Burgos, 1995b), PPO (López et al., 1994), polygalacturonase (Vercet & López, 2002) and pectin methylesterase (López, Vercet, Sánchez, & Burgos, 1998) in fruit juices. Tomato pectin methylesterase inactivation was reduced 53-fold when MTS was applied compared with thermal pasteurization (Vercet, López, & Burgos, 1999).

Although MTS is effective on enzymes and microbial inactivation in model systems, there are no studies related to the effect of the combination of vacuum (instead of HHP), heat and ultrasound. At this combination of technology for food processing, we named vacuum-thermo-sonication (VTS).

Vacuum technology is used for the incorporation of additives in fruit and vegetable tissues because the vacuum can facilitate mass transfer by favoring gas exhaustion from intracellular spaces and enhanced mass transfer inside the tissues (Mújica, Valdez, López, Palou, & Welti, 2003; Tovar, Montalvo, Damián, García, & Mata, 2011). The combination of this technology with heat and ultrasound to allow the design of green sustainable and innovative technology as VTS, it could have great potential benefits in industrial processes for the food preservation as until now it has been reported to ultrasound (Both, Chemat, & Strube, 2013). In the present work, we studied the processing of soursop puree using VTS (a combination of vacuum, heat, and ultrasound) and evaluated the lethal effect on pathogenic bacteria (*Escherichia coli* and *Staphylococcus aureus*), PPO inactivation, and effects on the quality of the puree.

2. Materials and methods

The research was carried out in two stages. The initial stage was conducted to establish the best experimental conditions under VTS to obtain the highest reduction in inactivation rates on *E. coli* ATCC 8739, *S. aureus* ATCC 33862, and PPO from soursop puree. In the second stage, the nutritional composition, physicochemical parameters, and sensory evaluation of the vacuum-thermosonicated soursop puree (VTSP) were evaluated under the best conditions.

2.1. Chemical reagents

All chemicals and solvents were analytical grades. Catechol, Bradford reagent, bovine serum albumin, and phenolphthalein were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA).

2.2. Soursop puree

Mature soursop fruits harvested from an orchard located in the village of El Tonino, near Tepic, Nayarit, Mexico. Fruits were washed with chlorinated water (200 ppm), peeled, and deseeded, and pulp was homogenized with a commercial mixer (Nutribullet NB-101B, Los Angeles, CA, USA). Untreated puree (UNP) was used as a control.

2.3. Vacuum-thermosonication treatments

A Box-Behnken design was used, considering the following factors at different levels: temperature (40 °C, 45 °C or 50 °C), vacuum (8.46, 11 or 16.93 kPa) and intermittent vacuum pulses (IVPs), 1, 2 or 3, administered individually for 5 s each (Table 1). VTS treatments were performed in a discontinuous VTS reactor (batch process), as shown in Fig. 1. The VTS reactor was coupled with an ultrasound equipment (Hielscher UP400S, Hielscher Ultrasonics, Teltow, Germany) at a constant frequency (24 kHz) with a 22-mm diameter tip sonotrode (Model No. H22, Hielscher) and delivering acoustic energy density (AED) of 85 W/cm² or AED of 0.34 W/g according to Eq. (1) as reported by O'Donnell, Tiwari, Bourke, and Cullen (2010). The reactor was coupled with a vacuum pump to generate a vacuum inside the chamber. The applied vacuum with a low degree of vacuum was considered. The temperature inside the reactor was controlled with a recirculating water bath and monitored with a thermometer coupled to the system prototype. The temperature of the purees was measured at the end of
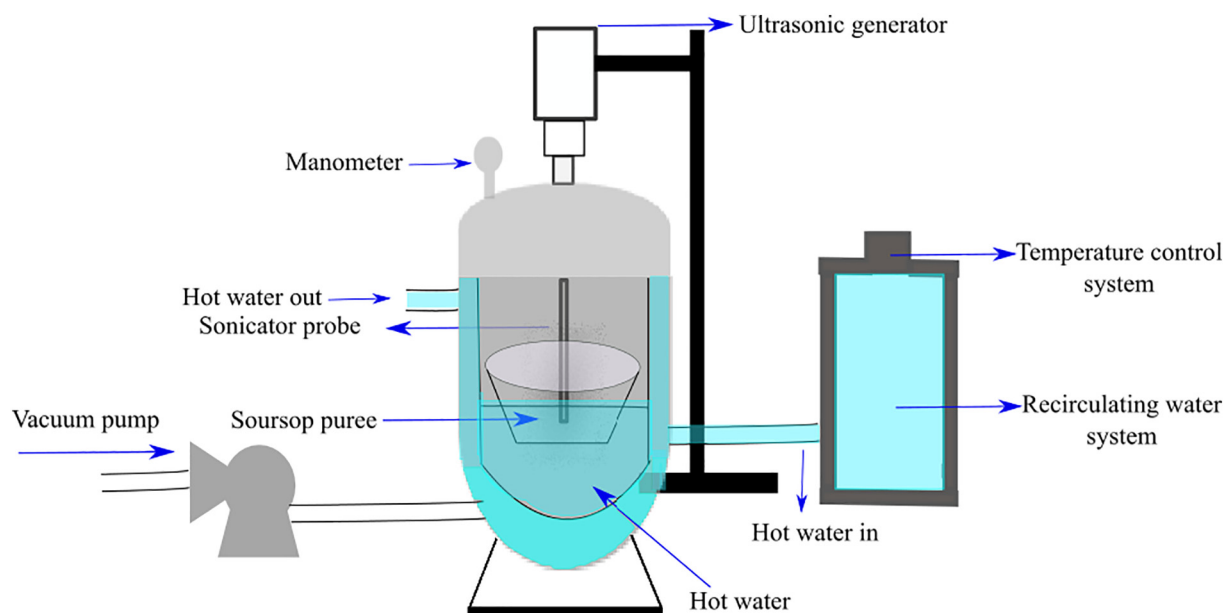
Fig. 1. Laboratory scale vacuum-thermosonication batch system.

the treatment (Table 1). For each treatment, 250 g of soursop puree was placed in the VTS reactor; then the ultrasound, temperature, and vacuum conditions were applied according to the design. All the samples were treated with VTS for 10 min. IVPs were applied as follows: for 1 IVP, the vacuum was applied for 300 s continuously followed by 5 s without vacuum and then 300 s with vacuum. For 2 IVPs, vacuum was applied for 200 s continuously, then 5 s without vacuum, 200 s with vacuum followed by 5 s without vacuum and finally 200 s with vacuum. For 3 IVPs the same methodology was followed using 150 s of constant vacuum for each IVPP of 5 s each.

The response variables tested include lethal and sublethal damage of *E. coli* and *S. aureus*; polyphenol oxidase (PPO) activity and residual enzyme activity of PPO (PPO$_{RA}$); titratable acidity, total soluble solids, and pH. The design was repeated three times, and the response variables were measured in triplicate.

$$AED = P/V \qquad (1)$$

where AED is the acoustic energy density (W/g), P is the absolute ultrasound power (W), and V is the mass of the sample (g).

2.4. Lethal and sublethal damage of VTS on E. coli and S. aureus

Cell suspension and inoculation of *E. coli* and *S. aureus* into the purees were performed as recommended by Briñez, Roig-Ságues, Hernández-Herrero, and Guamis-López (2006). Freeze-dried cultures (*E. coli* ATCC 8739 and *S. aureus* ATCC 33862) were rehydrated in tryptone soy broth (DIBICO, Mexico City, Mexico) at 37 °C for 24 h. After incubation, the broth (0.1 mL) was spread using a disposable loop on a petri dish with tryptone soy agar (TSA) (DIBICO, Mexico City, Mexico) and incubated at 37 °C for 24 h. Subsequently, the cell suspension was prepared by adding enough inoculum of bacteria (*E. coli* and *S. aureus*) to sterile peptone water at 0.1% (11 mL) to obtain a concentration of 8.0 and 7.0 log CFU/mL (when the absorbance was 2.0 ± 0.5 at 405 nm) measured using a spectrophotometer (Jenway 6705, Felsted, UK). The puree was inoculated with cell suspension (10 mL/kg) and homogenized before treatment. Lethal and sublethal injury to the bacterial strains was assessed by serial dilution using the pour plate method. For each treatment, 10 g were placed into 90 mL of sterile peptone water and homogenized. Serial dilutions (up to 10^{-8}) were made in peptone water before and after VTS, and samples were plated in TSA by the pour-plate method and incubated at 37 °C for 24 h.

This procedure was repeated for each treatment (VTS1–VTS15). The results were expressed as log CFU/g. Lethality was calculated as the difference between the logarithms of colony counts in TSA before and after VTS treatment (log No – log N). To detect bacterial cell injury, dilutions of VTS samples were pour plated in TSA, 4% sodium chloride was added followed by incubation at 37 °C for 24 h (García, Gómez, Condón, Raso, & Pagán, 2003). The sublethal injury was calculated by the difference obtained from the counts (Supplementary data) between the cultures in TSA (before treatment) and TSA + NaCl (after treatment) for each treatment (VTS1–VTS15) and expressed as a percentage according to the following equation (García et al., 2005):

$$\text{Sublethal injury } (\%)$$
$$= \frac{(\log \text{ CFU mL}^{-1} \text{ in TSA}) - (\log \text{ CFU mL}^{-1} \text{ in TSA + NaCl 4\%})}{\log \text{ CFU mL}^{-1} \text{ in TSA}} \times 100$$

$$(2)$$

2.5. Scanning electron microscopy

Cells (*E. coli* and *S. aureus*) treated under VTS (the best conditions of inactivation) and their respective controls (untreated cells) were visualized by scanning electron microscopy. The dehydration procedure was carried out increasing concentrations of ethanol-water solutions (10, 25, 40, 50, 70, 80, 90 and 95%) as described by Zeraik and Nitschke (2010). The samples were maintained desiccated until gold sputtering and analyzed using a scanning electron microscopy (JOEL model JSM6390LV/LGS) operating at 15 kV (all SEM images were analyzed using INCA® suite 4.08 software).

2.6. Polyphenol oxidase activity and residual enzyme activity

Enzymatic extraction of PPO was done using the method described by Bora, Holschuh, and da Silva (2004). The results are expressed as specific activity of PPO (SAPPO, U/mg protein). Total protein concentration (mg) was determined by the Bradford method (Bradford, 1976). Residual enzyme activity of PPO was calculated and expressed as a percentage of PPO$_{RA}$ (Fonteles et al., 2012).

2.7. Titratable acidity, total soluble solids, and pH

Titratable acidity (TA), total soluble solids (TSS) and pH were

Table 2
Predicted and actual values of lethal and sublethal effects of vacuum-thermosonication on *Escherichia coli* and *Staphylococcus aureus* inoculated in soursop puree.

Treatments	Vacuum (kPa)	Intermittent vacuum pulses	Initial temperature (°C)	Final temperature (°C)	*Escherichia coli*			*Staphylococcus aureus*		
					Experimental lethality (log CFU/g)	Predicted lethality (log CFU/g)	Sublethal damage (%)	Lethality (log CFU/g)	Predicted lethality (log CFU/g)	Sublethal damage (%)
VTS1	8.46	2	40	40.25 ± 2.72	3.44 ± 0.01[a]	3.47	99.99	2.81 ± 0.01[a]	2.81	99.98
VTS2	16.93	2	40	40.38 ± 1.84	4.52 ± 0.01[c]	4.53	99.99	4.26 ± 0.01[f]	4.26	99.95
VTS3	8.46	2	50	50.50 ± 1.08	5.09 ± 0.03[d]	5.21	99.99	5.36 ± 0.01[i]	5.36	99.99
VTS4	16.93	2	50	50.88 ± 1.89	7.18 ± 0.05[j]	7.23	100	6.98 ± 0.01[j]	6.98	100
VTS5	8.46	1	45	45.25 ± 2.10	4.19 ± 0.01[b]	4.55	99.99	3.49 ± 0.01[b]	3.49	99.99
VTS6	16.93	1	45	45.38 ± 1.84	5.47 ± 0.01[f]	5.63	99.99	3.91 ± 0.01[e]	3.91	99.96
VTS7	8.46	3	45	45.63 ± 2.43	5.64 ± 0.11[g]	5.42	100	5.14 ± 0.06[g]	5.14	99.97
VTS8	16.93	3	45	45.75 ± 1.55	7.22 ± 0.02[j]	7.12	100	5.34 ± 0.01[h]	5.34	99.99
VTS9	11	1	40	40.88 ± 1.93	3.37 ± 0.01[a]	3.14	99.99	3.60 ± 0.01[c]	3.60	99.87
VTS10	11	1	50	50.50 ± 1.08	5.77 ± 0.01[h]	5.47	99.99	5.02 ± 0.01[g]	5.02	99.99
VTS11	11	3	40	50.88 ± 1.31	4.22 ± 0.01[b]	4.42	99.99	3.83 ± 0.01[d]	3.83	99.89
VTS12	11	3	50	50.13 ± 1.65	6.03 ± 0.02[i]	6.15	99.99	5.98 ± 0.01[i]	5.98	99.99
VTS13	11	2	45	45.88 ± 0.75	5.38 ± 0.01[f]	5.25	99.99	5.02 ± 0.01[g]	5.02	99.98
VTS14	11	2	45	45.63 ± 1.03	5.26 ± 0.01[e]	5.25	99.99	5.03 ± 0.01[g]	5.02	99.99
VTS15	11	2	45	45.63 ± 1.25	5.33 ± 0.01[ef]	5.25	99.99	5.02 ± 0.02[g]	5.02	99.99

Values are the average of triplicate determinations from 3 different experiments ($n = 3$) ± standard deviation. Means in a column with different letters are significantly different ($p < 0.05$). VTS1–VTS15, the key to the samples numbers is given in Table 1.

determined according to AOAC methods (Horwitz & Latimer, 2005). TA was assessed in 10 g of puree mixed with 25 mL of distilled water and titrated using an automatic titrator (SCHOTT Instruments, Berlin, Germany) with NaOH (0.1 mol/L) and using phenolphthalein as indicator. The results are expressed as equivalent grams of malic acid per kilogram (g MAE/kg) of puree. TSS were determined using a refractometer (Abbe 315RS, Royal Tunbridge Wells, England), and the results are expressed as °Brix. pH was measured using a pH meter (HANNA Instruments, HI 221, Bedford, UK).

2.8. Response surface model validation

All data from the first stage were analyzed with a response surface methodology (RSM) using analysis of variance ($p < 0.05$) with STATISTICA v. 10 (StatSoft, Tulsa, Oklahoma, USA) software, and after obtaining the best experimental conditions (temperature, vacuum and IVPs) for VTS that inhibited *E. coli*, *S. aureus* and PPO activity. The puree (250 g) was treated again under these best conditions (three times) to validate the model. UNP was used as a control. The samples were stored at −20 °C until they were analyzed.

2.9. Evaluation of quality parameters of vacuum-thermosonicated soursop puree

2.9.1. Nutrient composition

Moisture (Method 925.09), protein (Method 920.152), fat (Method 950.54) and ash (Method 940.26) contents were determined following AOAC methods (Horwitz & Latimer, 2005). Soluble sugars were analyzed by the phenol-sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Total dietary fibre (TDF) is the sum of soluble dietary fibre and insoluble dietary fibre. Dietary fibre content was analyzed by the AOAC enzymatic-gravimetric method (Method 991.42) modified by Mañas and Saura-Calixto (1995).

Titratable acidity (TA), pH, and TSS were evaluated as mentioned in Section 2.7. The colour was analyzed using a colourimeter (Konica Minolta, CR300, Osaka, Japan). Total colour difference was determined according to Tiwari, Muthukumarappan, O'Donnell, and Cullen (2008).

2.9.2. Sensory evaluation

The evaluated purees were the VTSP (16.5 kPa, 3 IVPs, and 50 °C) and UNP. Sufficient samples were processed, and preference tests (colour, taste, odour, and consistency) with an unstructured hedonic scale were performed with a panel of 50 untrained judges (Pedrero & Pangborn, 1997).

2.10. Statistical analysis

Data of three different batches ($n = 3$), with experimental analysis realized in triplicate, were statistically analyzed by RSM, in the first stage. In the second stage, the experiment was conducted in experimental uni-factorial design, also using three different batches ($n = 3$). The analyses were performed using an analysis of variance ($p < 0.05$) with STATISTICA v. 10 (StatSoft, Tulsa, Oklahoma, USA) software. Differences between means were compared using the Tukey test ($\alpha = 0.05$). Results were expressed as mean ± standard deviation.

3. Results and discussions

3.1. Effect of VTS on physicochemical parameters

Titratable acidity (TA), pH, and total soluble solids (TSS) values for the vacuum-thermosonicated soursop puree (VTSP) are given in Table 1. VTS processing did not result in significant changes ($p < 0.05$) in pH, TA, and TSS in VTS1–VTS15 samples compared with UNP. It was previously established that emerging technologies such as the VTS should not change the fundamental properties of the treated products after preservation treatment (NACMCF, 2006). Previous reports on apple, cranberry (Palgán et al., 2012), orange juice (Caminiti et al., 2012) and apple cider (Lee, Kim, Cadwallader, Feng, & Martin, 2013) treated by MTS under different experimental conditions had similar results at the VTS conditions used in this experiment. VTS as MTS did not change the physicochemical parameters either.

3.2. Lethal and sublethal effect of VTS on Escherichia coli and Staphylococcus aureus

Table 2 shows that all treatments reduced the plate count after VTS (initial concentration was 8 log CFU/g approximately for both microorganisms). Besides, all VTS treatments (except VTS1, VTS2, VTS5, and VTS9) showed inactivation above the ≥5 logs stipulated by the FDA (2004). Similarly, all treatments led to > 99% sublethal damage for both microorganisms. Sublethal injury is related to the higher sensitivity of survivors to stress conditions after any treatment. According to García et al. (2005), MTS treatment should be correlated with the
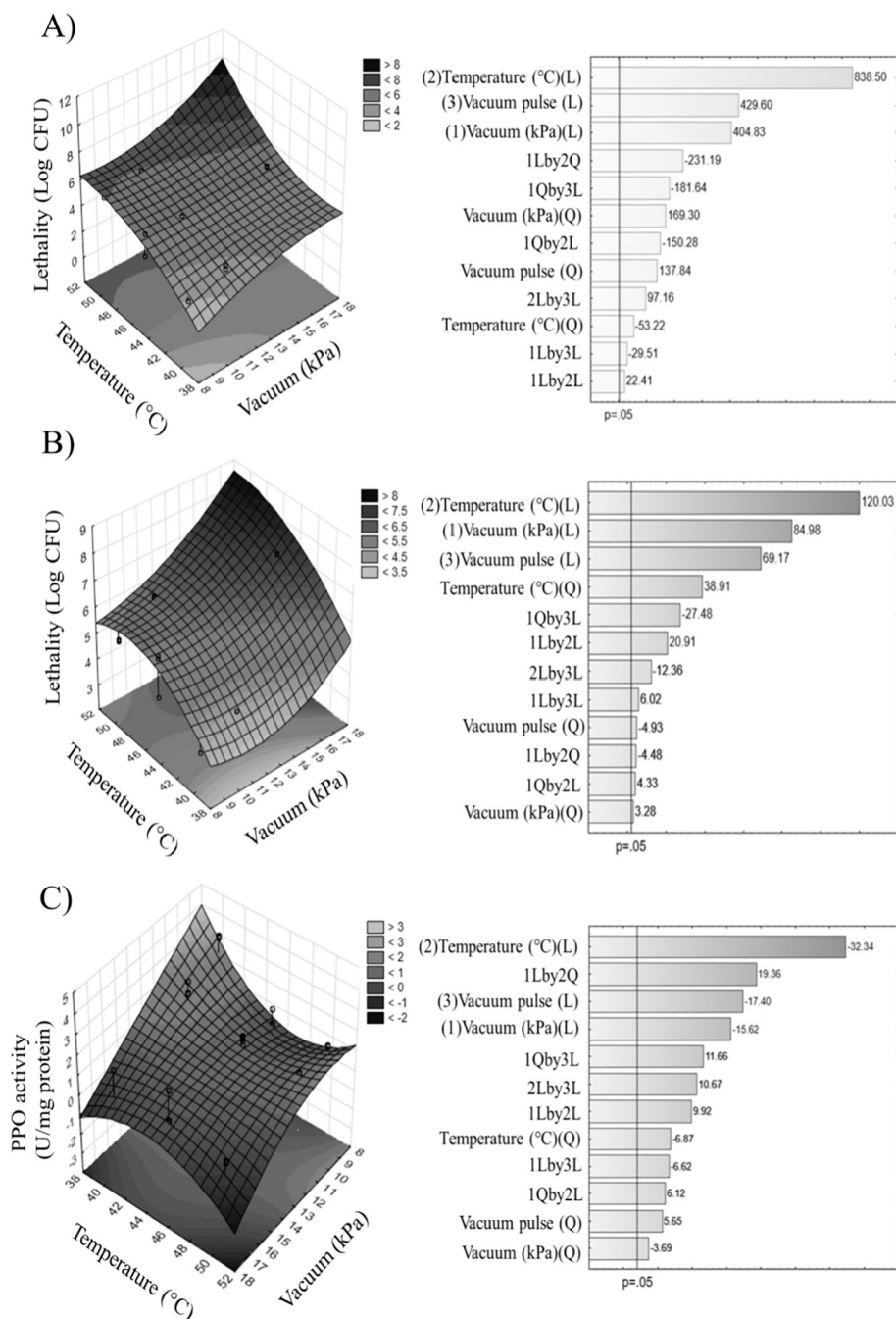
Fig. 2. Response surface plots and Pareto chart of the effect of vacuum-thermosonication on *Staphylococcus aureus* (A) and *Escherichia coli* (B) lethality and polyphenol oxidase activity (PPO) (C).

degree of sublethal injury caused by the treatments applied to the microbial population. Therefore, it is possible that a reduction of almost 7 log in both microorganisms in VTS8 (16.93 kPa, 3 IVPs, 45 °C, 20 kHz and 10 min) for *E. coli* and VTS4 for *S. aureus* (16.93 kPa, 2 IVPs, 50 °C, 20 kHz and 10 min), was achieved with a similar effect like MTS, in the sublethal injury reduction.

The mechanism of action of MTS has been widely reported (Kahraman, Lee, Zhang, & Feng, 2017; Milani, Ramsey, & Silva, 2016; Zhu et al., 2017). At 400 kPa, 20 kHz, 61 °C for 2 min, Lee, Zhou, Feng, et al. (2009) achieved a 5 log reduction of *E. coli* K12 as an indicator in citrate buffer solution (pH 4) after 2 min. Also, in the same study, non-linear inactivation curves were observed with a fast initial reduction, followed by a slow reduction in microbial survival counts. It has also been reported that the complexity of the matrix has a significant impact

on the lethality of MTS (Lee et al., 2013), and MTS (400 kPa, 20 kHz, 59 °C for 2 min) treatment led to broken cell membranes, surface pitting, exposed cytoplasmic content, cell debris and shrinkage of *E. coli* K-12 cells (Lee, Zhou, Liang, et al., 2009). Zhu et al. (2017) reported that MTS deforms bacterial cells, decreases cell volume, ruptures cell membranes, congeals cytoplasmic content to form a clot, and whereby the cell damage caused by MTS on the microbial cell is irreversible. Zhu et al. (2017) also reported a synergistic effect of the factors studied. Kahraman et al. (2017) and Milani et al. (2016) concluded that microorganisms exposed to MTS are subjected to excessive pressure, even during cavitation and at increased temperature.

However, inactivation of microorganisms by VTS using vacuum instead of HHP has not yet been reported. The physical effect of the VTS could be an accumulative effect: the application of heat and vacuum

induced the first effect in the inactivation of pathogenic bacteria and PPO. On the other hand, the ultrasound effect is the cavitation, the generation of intracellular cavitation on bacterial and enzymatic inactivation. The high-energy released by the implosion can cause high cellular sensitivity and more significant disruption of the cell membrane, leading to a cumulative and lethal effect in bacteria, the denaturalization of enzymes or extraction of metabolites in vegetal cells (Chemat et al., 2011; Chemat et al., 2017; Khadhraoui et al., 2018).

In the VTS processing was included the application of IVPs to cause changes from atmospheric pressure to vacuum and vice versa; therefore, we hypothesized that the use of vacuum by pulses could cause a deformation-relaxation phenomenon to the vegetal or bacterial cells. The vacuum would create an accumulation of internal cellular stress, expanding the gas inside the cells (Barat, Fito, & Chiralt, 2001; Fito, 1994; Tovar et al., 2011). Thus, it caused the opening of membrane pores, and a faster degassing (Cheng, Soh, Liew, & Teh, 2007). It is also possible that cytoplasmic material leaves of cells, while the heat and ultrasound microbubbles entered to them when the pressure restored (atmospheric pressure).

According to the RSM, the optimum treatments to guarantee FDA compliance for soursop puree were 16.49 kPa, 3 IVPs, and 47.6 °C for *E. coli* with a desirable lethality of 7.4 log CFU/g. For *S. aureus*, the conditions are 16 kPa, 3 IVPs and 49 °C with a desirable lethality of 6.7 log CFU/g. The regression model and the lethality on both microorganisms in soursop puree can be predicted using the following polynomial equations (Eq. (3): $R^2 = 0.99$; Eq. (4), $R^2 = 0.99$; both with 95% confidence level, respectively). Also, adequate adjustment of the experimental data to the models was observed (lack of fit $p > 0.05$ for both equations).

E. coli lethality (log CFU/mL)

$$= -65.99 + 1.61P + 2.63\,T - 0.03\,T^2 + 6.32\,V + 0.06$$
$$V^2 + 0.0007PT^2 - 0.001P^2T - 0.85PV + 0.03P^2V - 0.03TV \quad (3)$$

S. aureus lethality (log CFU/mL)

$$= -193.19 + 20.10P - 0.33P^2 + 7.13\,T - 0.07\,T^2 + 5.14\,V - 0.28$$
$$V^2 - 0.65PT + 0.006PT^2 + 0.005P^2T - 0.85VP + 0.03VP^2 + 0.04VT \quad (4)$$

where P is the number of IVPs, T is the temperature (°C) and V is vacuum (kPa).

Table 2 shows the predicted and experimental lethality of VTS on *E. coli* and *S. aureus*, and similar values were observed in both cases for both microorganisms. The Pareto charts for *S. aureus* (Fig. 2A) and *E. coli* (Fig. 2B) show the effect of the following independent variables on lethality of bacteria at a confidence level of 95%: temperature, IVPs, vacuum; and interactions such as temperature–vacuum, temperature--IVPs and IVPs–vacuum for both microorganisms were observed. The temperature and IVPs were the most critical factors involved in the lethality for both microorganisms under VTS. Pagán et al. (1999) reported a temperature (40–60 °C) dependence of MTS lethality on *Listeria monocytogenes*, *Streptococcus faecium*, *Salmonella enteritidis*, and *Aeromonas hydrophila* inactivation. Guzel, Arroyo, Condón, Pagán, and Bayindirli (2016) observed a reduction in the treatment time (2.9 min to 0.8 min) with increased temperature (35 °C to 60 °C) on inactivation of *E. coli* (2.8 and 2.9 log CFU/mL, respectively) and when MTS (200 kPa, 110 μm amplitude) was applied to apple and orange juice. The results presented in this study suggest that the incorporation of IVPs in VTS treatments leads to similar lethality rates on pathogenic bacteria to those that occur when MTS is applied at 100–400 kPa HHP (Arroyo, Cebrián, Pagán, & Condón, 2011; Lee et al., 2013; Palgán et al., 2012).

Additionally, the effect of VTS treatment (16.5 kPa, 3 IVPs, and 50 °C) on the morphological structure of both *E. coli* and *S. aureus* cells were observed by SEM (Fig. 3A–D). Untreated *E. coli* (Fig. 3A) and *S. aureus* (Fig. 3C) cells exhibited their typical forms (Liao et al., 2018),

while treated cells of both bacteria showed morphological changes (Fig. 3B and D); which resulted in bacterial damage, moreover, the cell debris were interspersed among bacterial clusters in the visual field. Similar morphological changes on *E. coli* and *S. aureus* were reported by Chantapakul et al. (2019) after MTS (14 W/mL, 400 kPa, 50 °C, and 5 min) treatment. Also, our images are similar to those reported by Li et al. (2017) who informed that combination of ultrasound (600 W) and mild heat (55 °C) during 15 min have a great impact on the morphological appearance of *S. aureus*, and mentioned that agglomeration on the SEM images are caused by the cells disintegrate and cell fragments. Dong, Chen, Wang, Peng, and Yu (2013) reported morphological changes on *S. epidermis* after submitted to ultrasound (5 min, 300 kHz, 0.5 W/cm^2) combined with vancomycin (4% v/v). Zhu et al. (2017) proposed that possible mechanism of microbial disintegration and cell destruction by MTS is caused by the degeneration of internal cellular proteins and changes in the permeability of the cell membrane followed by the disruption of the cell membrane and cell shrinkage, which kills the cell. We showed that using vacuum instead of high pressure in combination with heat and ultrasound, there is cellular damage on *E. coli* and *S. aureus*.

3.3. The specific activity of polyphenol oxidase and residual enzyme activity in soursop puree after VTS

PPO activity plays an essential role in enzymatic browning, particularly in fruits such as soursop (Bora et al., 2004). The SAPPO in VTS samples is presented in Table 3. A significant effect ($p < 0.05$) on SAPPO was observed after VTS compared with the untreated sample. VTS4 (4%), VTS7 (8%), and VTS10 (6%) showed the lowest residual activity of PPO (PPO$_{RA}$) after treatment. Although there was a reduction of SAPPO in all vacuum-thermosonicated samples, the effect of VTS on enzyme activity was dependent on the experimental conditions (ultrasound, temperature, vacuum, and IPPVs). There are no reports on the effect of VTS on PPO activity in fruit purees. However, the effect of MTS on juices has been studied. Zhu et al. (2017) reported a PPO$_{RA}$ of 10.91% after MTS treatment (350 MPa and 560 W, 40 °C, 5 min) compared with controls in blueberry juice. López et al. (1994) reported a PPO$_{RA}$ of 10% in mushrooms after MTS (390 kPa, 40 °C, 20 kHz, and 145 μm amplitude) treatment in phosphate buffer (20 mM, pH 6.5). In addition, similar enzyme inactivation rates for peroxidase and lipoxygenase were observed in the same study.

PPO inactivation by MTS has been attributed to a synergistic effect of pressure, cavitation (chemical, mechanical and physical effects) and heat, causing protein denaturation, affecting catalytic function (López et al., 1998; López & Burgos, 1995a, 1995b; Vercet et al., 1999; Vercet & López, 2002). Also, the authors mentioned that the intensity of bubble implosion (cavitation effect) might be influenced by pressure and temperature, increasing the enzyme inactivation rates. The effect of VTS on PPO activity can also be attributed to the accumulative effect of vacuum, which could cause a deformation-relaxation phenomenon, entrance of heat, and ultrasound (Barat et al., 2001; Fito, 1994). It is possible that exposition of PPO to heat and ultrasound results in inactivation by denaturation, although more studies are still needed to confirm this fact.

According to the RSM, the best conditions to inactivate PPO, were 15.9 kPa, 50 °C and 3 IVPs with a desirable PPO$_{RA}$ of 0.20% (0.009 U/mg protein). These experimental conditions lead to the highest lethality of *E. coli* and *S. aureus*. The regression model for PPO inactivation in soursop puree can be predicted using the following polynomial Eq. (5) ($R^2 = 0.98$ with 95% confidence level). In addition, adequate adjustment of the experimental data to the models was observed (lack of fit $p > 0.05$).
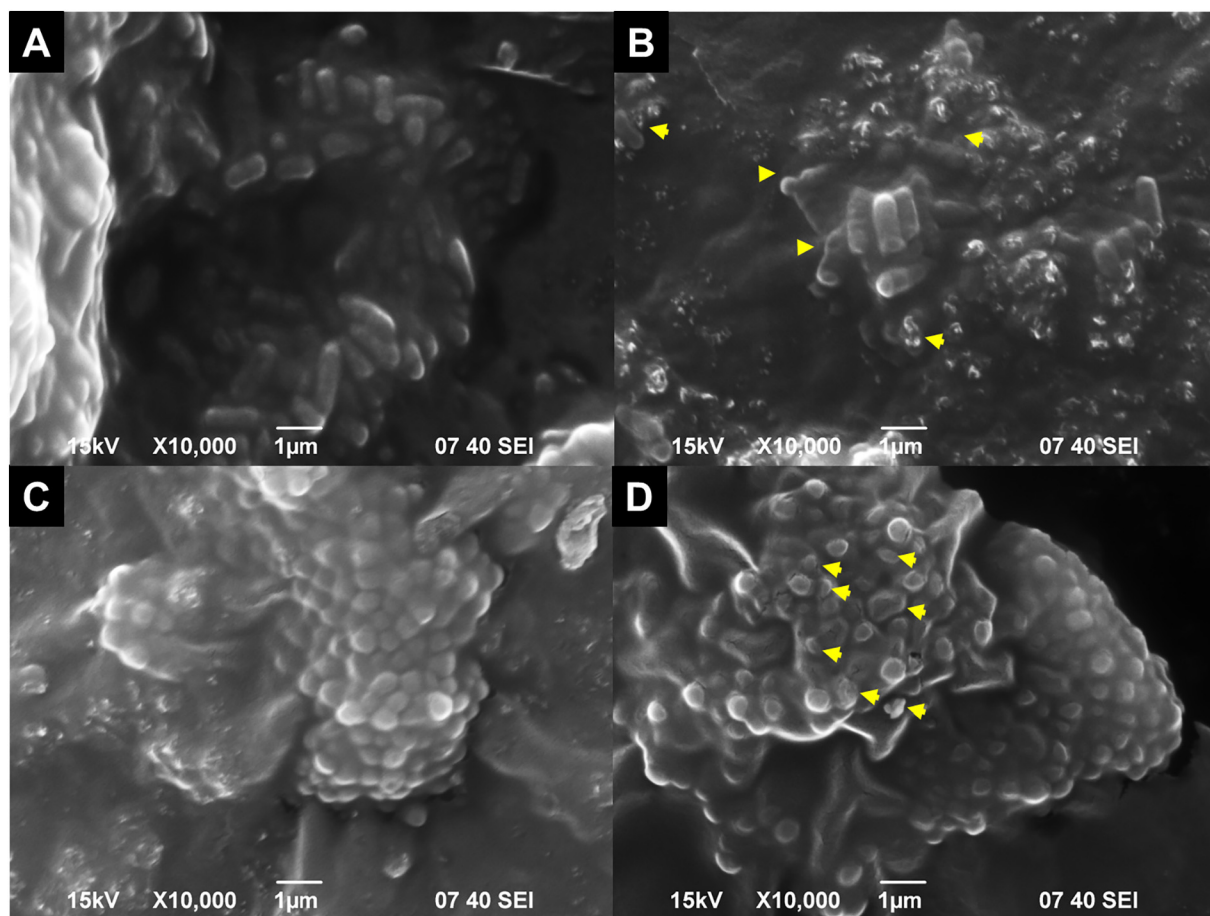
Fig. 3. Scanning electron microscopy images of *Escherichia coli* (A–B) and *Staphylococcus aureus* (C–D) before (A–C) and after (B–D) vacuum-thermosonication (16.5 kPa, 3 IVPs, and 50 °C).

$\text{PPO}_{\text{Reduction}}$

$$= 289.18 - 23.60\,V + 0.25\,V^2 - 11.30\,T + 0.11\,T^2 - 7.44P - 0.19$$
$$P^2 + 0.85VT - 0.008VT^2 - 0.003V^2T + 0.87VP - 0.03V^2P + 0.06TP \tag{5}$$

where P is the numbers of IVPs, T is the temperature (°C) and V is vacuum (kPa).

The predicted PPO and experimental reductions under VTS are presented in Table 3, and similar values were observed in both cases. The fitted function showed that reduction of PPO is mainly dependent on vacuum (V), temperature (T), IVPs (P) and their interactions ($V \times T^2$, $V^2 \times T$, $V \times P$, $V^2 \times P$, and $T \times P$). Furthermore, the Pareto chart (Fig. 2C) shows the effect of the independent variables on PPO, where all linear and quadratic parameters had a statistically significant

Table 3

Experimental and predicted values of the specific activity of polyphenol oxidase (SAPPO) and residual enzymatic activity (PPO_{RA}) after vacuum-thermosonication on soursop puree and untreated puree (UNP).

Treatments	Vacuum (kPa)	Intermittent vacuum pulses	Initial temperature (°C)	Final temperature (°C)	Experimental SAPPO (U/mg protein)	PPO_{RA} (%)	Predicted SAPPO (U/mg protein)
UNP					4.39 ± 0.16^k	100	
VTS1	8.46	2	40	40.25 ± 2.72	3.25 ± 0.06^j	74.03	3.25
VTS2	16.93	2	40	40.38 ± 1.84	1.29 ± 0.01^f	29.38	1.29
VTS3	8.46	2	50	50.50 ± 1.08	0.87 ± 0.01^d	19.81	0.87
VTS4	16.93	2	50	50.88 ± 1.89	0.18 ± 0.05^a	4.10	0.18
VTS5	8.46	1	45	45.25 ± 2.10	1.15 ± 0.01^{ef}	26.19	1.15
VTS6	16.93	1	45	45.38 ± 2.32	1.99 ± 0.01^h	45.33	1.99
VTS7	8.46	3	45	45.63 ± 2.43	0.37 ± 0.05^b	8.24	0.37
VTS8	16.93	3	45	45.75 ± 1.55	0.36 ± 0.06^b	8.20	0.36
VTS9	11	1	40	40.88 ± 1.93	2.37 ± 0.01^i	53.98	2.37
VTS10	11	1	50	50.50 ± 1.08	0.26 ± 0.01^{ab}	5.92	0.26
VTS11	11	3	40	50.88 ± 1.31	1.74 ± 0.02^g	39.63	1.74
VTS12	11	3	50	50.13 ± 1.65	0.99 ± 0.01^{de}	22.55	0.99
VTS13	11	2	45	45.88 ± 0.75	0.96 ± 0.01^{de}	21.86	0.95
VTS14	11	2	45	45.63 ± 1.03	0.94 ± 0.04^c	21.41	0.95
VTS15	11	2	45	45.63 ± 1.25	0.97 ± 0.03^{ef}	22.09	0.95

Values are the average of triplicate determinations from 3 different experiments ($n = 3$) $\pm$ standard deviation. Means in a column with different letters are significantly different ($p < 0.05$). VTS1–VTS15, the key to the samples numbers is given in Table 1.

effect ($p < 0.05$) on the reduction of PPO activity. Caminiti et al. (2012) mentioned that in some cases, the effectiveness of MTS treatment for enzyme inactivation depends on the temperature used in the treatment. A significant increase in PPO reduction was observed when MTS treatment was applied at a temperature of $\geq 50\,°C$ (Zhu et al., 2017).

3.4. Response surface model validation

According to RSM, the optimal conditions to inhibit *E. coli*, *S. aureus*, and PPO activity were similar (16.5 kPa, 3 IVPs, 50 °C for 10 min) and used to verify the reliability of the models and experiments were performed. The experimental values for the lethality of VTS on *E. coli* (7.58 log CFU), *S. aureus* (7.35 log CFU) and SAPPO (0.007 U/mg protein) were similar to those obtained with the predicted values (7.77 log CFU, 7.39 log CFU and 0.26 U/mg protein, respectively) under the optimal conditions. Furthermore, the error rates between the experimental and predicted values of the models using conditions at 16.5 kPa, 3 IVPs, and 50 °C are < 6%, which indicates that the models obtained by RSM are adequate for VTS proposes (Aydar, Bagdathiglu, & Koseoglu, 2017). Furthermore, small differences between the experimental and predicted values may be influenced by the thermal resistance of the food system, which the mathematical model does not consider, as described by Ávila-Sosa, Gastélum, López-Malo, and Palou (2010).

3.5. Nutrient composition and physicochemical parameters

Most of the studies on the effect of MTS focus on bacterial reduction (Lee, Zhou, Feng, et al., 2009; Lee, Zhou, Liang, et al., 2009), enzyme inactivation (López et al., 1998; Vercet & López, 2002) or changes in physicochemical (pH, TA, TSS and colour) parameters (Caminiti et al., 2012; Palgán et al., 2012) of food products. However, reports on the effects of VTS on nutrient content (proteins, fats, carbohydrates, dietary fibre, moisture, and ash) of food products are not available.

The nutritional composition of UNP and VTS purees is presented in Table 4. No differences ($p > 0.05$) in the moisture (80.66 and 79.15 g/ 100 g), protein (1.01 and 1.07 g/100 g), fat (0.41 and 0.37 g/100 g), TDF (3.34 and 3.05 g/100 g) and ash (0.11 and 0.12 g/100 g) contents were detected between UNP and VTS purees, respectively. The values for UNP were similar to those reported for fresh pulp and soursop puree (Fasolin & Cunha, 2012; Moreno-Hernández, Sáyago-Ayerdi, García-Galindo, Mata-Montes De Oca, & Montalvo-González, 2014). On the other hand, an increase ($p < 0.05$) in soluble sugar content was observed with VTS (11.84 g/100 g) compared with UNP (9.06 g/100 g), and therefore VTS promoted an increase in the energy content (54.97 kcal) of VTSP compared with UNP (43.97 kcal). Khadhraoui et al. (2018) demonstrated that ultrasound-assisted extraction promotes the structural changes in vegetal tissues reporting a significant release of valuable metabolites. Furthermore, differences ($p < 0.05$) were observed in the soluble (1.06 and 0.5 g/100 g) and insoluble (2.28 and 2.55 g/100 g) dietary fibre content between UNP and VTSP, respectively. However, there was no difference in TDF content between the samples. Dhingra, Michael, Rajput, and Patil (2012) demonstrated that heating generally changes the ratio of soluble and insoluble fibre.

TA, pH, TSS, and colour were maintained in puree using the optimal VTS conditions and with similar values to UNP (Table 4). These results comply with the criteria for emerging technologies, i.e., retaining or enhancing the original properties of the food after treatment (NACMCF, 2006).

In general, these results suggest that the optimal conditions for VTS did not promote significant changes in the nutritional and physico-chemical components of soursop puree. Also, soursop puree is considered as a good source of dietary fibre (Olagnero et al., 2007), with low calories (44–55 kcal/100 g of puree) content according to Badrie and Schauss (2010).

The optimal VTS conditions also maintained low SAPPO (0.25 U/mg protein), and their effect is notable concerning the SAPPO (4.26 U/mg protein) with UNP. The VTS experimental conditions applied in this study (16.5 kPa, 3 IVPs, and 50 °C) are adequate to avoid enzymatic darkening of soursop puree. In particular, López et al. (*1998*) and Zhu et al. (*2017*) described a synergistic effect of pressure, heat, and cavitation on PPO inactivation under MTS. PPO inactivation by technologies such as MTS is related to a change in the global conformation of the enzyme (Baltacioglu, Bayindirli, & Severcan, 2017), affecting its specific activity. VTS also could cause a synergic effect, as indicated above.

3.6. Sensory evaluation

The VTS soursop puree received scores for all attributes evaluated (odour, taste, colour, and consistency) statistically equal to values for UNP (Table 5). Similar trends were previously reported by Caminiti et al. (2012) who carried out a sensory evaluation (colour, odour, flavour, sweetness, acidity, overall acceptability and likelihood to buy) in MTS (400 kPa, 35 °C, 20 kHz, 2.2 min) carrot–orange (1:1) juice. VTS did not promote changes in the sensory attributes of soursop puree.

3.7. Potential applications and perspectives

Based on the optimized results, VTS process is a viable alternative for soursop puree preservation. Therefore, this innovative technology exhibits a great potential to apply in viscous food such as fruit purees or similar, a limiting of ultrasound alone (Lee & Feng, 2011). Also, it could use in liquids foods such as milk and its derivates, fruit based-beverages (juice and nectar), and for the extraction of bioactive compounds from

Table 4

Nutritional composition (g/100 g of puree) and physicochemical parameters of untreated puree (UNP) and vacuum-thermosonicated soursop puree (VTSP) at optimal conditions.

Parameter	UNP	VTSP
Energy (kcal)	43.97	54.97
Moisture	80.66 ± 0.57[a]	79.15 ± 0.49[a]
Protein	1.01 ± 0.20[a]	1.07 ± 0.21[a]
Fat	0.41 ± 0.04[a]	0.37 ± 0.09[a]
Soluble sugars	9.06 ± 0.70[a]	11.84 ± 0.47[b]
Total dietary fibre	3.34 ± 0.08[a]	3.05 ± 0.17[a]
Soluble dietary fibre	1.06 ± 0.05[a]	0.503 ± 0.03[b]
Insoluble dietary fibre	2.28 ± 0.04[a]	2.55 ± 0.07[b]
Ash	0.11 ± 0.01[a]	0.12 ± 0.01[a]
Luminosity (L^*)	69.72 ± 0.08[a]	69.59 ± 0.11[a]
a^*	−1.53 ± 0.03[a]	−1.93 ± 0.03[b]
b^*	7.45 ± 0.18[a]	7.56 ± 0.03[a]
pH	3.64 ± 0.06[a]	3.61 ± 0.02[a]
Titratable acidity (mg MAE/g)	1.23 ± 0.01[a]	1.20 ± 0.02[a]
Total soluble solids (°Brix)	22.48 ± 0.06[a]	23.47 ± 0.45[a]
Polyphenol oxidase activity (U/mg protein)	4.26 ± 0.22[a]	0.25 ± 0.0[b]

Experimental conditions for VTS: 16.5 kPa at 50 °C and 3 intermittent vacuum pulses for 10 min. Values are the average of triplicate determinations from 3 different experiments ($n = 3$) ± standard deviation. Different lowercase letters within a row indicate significant differences between treatments ($p < 0.05$).

Table 5

Sensory evaluations of untreated puree (UNP) and vacuum-thermosonicated (VTSP) purees at optimal conditions.

Treatment	Odour	Taste	Colour	Consistency
UNP	8.96 ± 0.23[a]	8.52 ± 0.23[a]	8.64 ± 0.18[a]	8.54 ± 0.16[a]
VTSP*	8.86 ± 0.20[a]	8.84 ± 0.19[a]	8.88 ± 0.15[a]	8.96 ± 0.15[a]

* Experimental conditions for VTS: 16.5 kPa at 50 °C and 3 intermittent vacuum pulses for 10 min. Values are the average of 50 panelists ($n = 50$) ± standard deviation. Different lowercase letters in a column indicate significant differences between treatments ($p < 0.05$).

different vegetal tissues, among others. VTS equipment can be scalable to industrial applications because it is cheaper than MTS. Nowadays, industrial vacuum equipment with temperature control for sale, and this can be adapted with a sonic ultrasound generator. Furthermore, the VTS as well as the ultrasound (Chemat et al., 2017; Pingret, Fabiano-Tixier & Chemat, 2013), can be recognized as a "green" and sustainable alternative due to the reduction of energy consumption unlike MTS, elimination of waste-water, decrease in processing time and costs, insurance of safety and high-quality products.

However, additional researches are needed to generate more knowledge on the effect of the VTS technology on different food matrix to maximize its potential industrial application, since VTS is higher potential than the ultrasound. Likewise, studies on the structural effect of VTS on food systems may be conducted in the future to understand the impact of the interactions between vacuum, heat, and ultrasound on microstructural changes of food systems, pathogenic microorganisms, and enzymes. The challenge scaling up laboratory VTS equipment for commercial production also represents an excellent alternative.

4. Conclusions

The VTS treatment (16.5 kPa of vacuum, 3 IVPs and 50 °C for 10 min) applied in this work was found to be a viable technology for processing soursop puree in compliance with regulatory agencies with regard to inactive pathogenic bacteria and PPO activity, without significant changes in nutrient composition, physicochemical parameters and sensory attributes. The soursop puree has good dietary fibre content and low-calorie content. We demonstrated the effect of VTS on the pathogenic bacteria and PPO inactivation, using vacuum instead of HHP on a fruit puree. However, further studies are needed to evaluate the potential scaling up of VTS technology for industrial applications.

Declaration of competing interest

The authors have declared that no conflict of interest exists.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ifset.2019.102255.

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