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Osmosonication parameters affect *Salmonella* Typhimurium and *Lactobacillus rhamnosus* reductions in solutions and fruit juices*

Parámetros de osmosonicación afectan la reducción de *Salmonella* Typhimurium y *Lactobacillus rhamnosus* en soluciones y jugos de frutas

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Abstract

Introduction. Sonication followed by storage in high osmotic pressure, referred to as osmosonication, can significantly reduce microbial loads in food products. Understanding the parameters that influence these reductions is essential. **Objective.** To evaluate the effect of osmosonication parameters on the reduction of *Salmonella* Typhimurium and *Lactobacillus rhamnosus* in model solutions and fruit juices. **Materials and methods.** This study was conducted at the Universidad de Costa Rica, San José, Costa Rica between January and December 2012. Completely randomized experiments were designed for both model solutions and fruit juices, with data analyzed using ANOVA. The parameters evaluated included amplitude (20, 25, 30, 35, and 40 %), sonication time (10, 20, and 30 minutes), percentage of pulsations time (90, 50, 10, and 0), pectin content (0, 0.5, and 1,0 %), and cellulose content (0, 0.15, and 0.30 %), pH (2.5; 3.5; 4.5; 5.5, and 6.5) or natural juice pulp content. **Results.** *L. rhamnosus* demonstrated greater resistant to osmosonication treatments compared to *S.* Typhimurium. Microbial reductions increased with higher sonication times and amplitudes. The percentage of pulsations time did not significantly influence bacterial reductions. Lower pH values resulted in reduced bacterial resistance to osmosonication. Neither pectin nor cellulose content significantly affected the resistance of either microorganism to osmosonication. Similar treatments applied to fruit juices (blackberry, pineapple, apple, and coconut water) showed that microbial reductions for both bacteria were influenced by the type of juice, likely due to varying pH levels. These effects was also bacteria-dependent. Pulp content affected bacterial resistance to osmosonication, with the impact varying by bacterial species. **Conclusions.** Osmosonication parameters such as amplitude, time, pH, and the pulp content influence the reduction of *Salmonella* Typhimurium and *Lactobacillus rhamnosus* in model solutions and fruit juices.

Keywords: ultrasonic treatment, food safety, process control, food quality, pathogens, foods.

Resumen

Introducción. La sonicación, seguida por almacenamiento bajo alta presión osmótica, conocida como osmosonicación, puede reducir significativamente las cargas microbianas en productos alimenticios. Es esencial comprender los parámetros que influyen en estas reducciones. **Objetivo.** Evaluar los efectos de los parámetros de osmosonicación sobre la reducción de *Salmonella* Typhimurium y *Lactobacillus rhamnosus* en soluciones modelo y jugos de frutas. **Materiales y métodos.** Este estudio se llevó a cabo en la Universidad de Costa Rica, San José, Costa Rica, entre enero y diciembre 2012. Se diseñaron experimentos completamente aleatorizados tanto para soluciones modelo como para jugos de frutas, y los datos fueron analizados mediante ANDEVA. Los parámetros evaluados incluyeron amplitud (20, 25, 30, 35 y 40 %), tiempo de sonicación (10, 20 y 30 minutos), porcentaje de tiempo de pulsación (90, 50, 10 y 0), contenido de pectina (0, 0,5 y 1,0 %) y contenido de celulosa (0, 0,15 y 0,30 %), pH (2,5; 3,5; 4,5; 5,5 y 6,5) o contenido de pulpa en jugos naturales. **Resultados.** *L. rhamnosus* mostró mayor resistente a los tratamientos de osmosonicación en comparación con *S.* Typhimurium. Las reducciones microbianas aumentaron con mayores tiempos y amplitudes de sonicación. El porcentaje de tiempo de pulsación no influyó significativamente en las reducciones bacterianas. Los valores más bajos de pH resultaron en menor resistencia bacteriana a la osmosonicación. Ni contenido de pectina ni el de celulosa afectaron significativamente la resistencia de los microrganismos a la osmosonicacion. Tratamientos similares aplicados a jugos de frutas (mora, piña, manzana y agua de pipa) mostraron que las reducciones microbianas en ambas bacterias dependieron del tipo de jugo, lo que se atribuyó a los diferentes valores de pH. El contenido de pulpa influyó en la resistencia de las bacterias a la osmosonicación, y este impacto varió según la especie bacteriana. **Conclusiones.** Los parámetros de osmosonicación como la amplitud, el tiempo, el pH y el contenido de pulpa en las matrices, influyeron significativamente en la reducción de *Salmonella* Typhimurium y *Lactobacillus rhamnosus* en soluciones modelo y jugos de frutas.

Palabras clave: tratamiento de ultrasonido, inocuidad de alimentos, control de procesos, patógenos, calidad de alimentos, alimentos.

Introduction

Pasteurization is the most used method to ensure food safety in fruit juices. However, thermal treatments can reduce nutrient content and affect sensory attributes of food products (Petruzzi et al., 2017). For this reason, there has been increasing interest in finding alternative treatments to inactivate microorganisms without the use of heat. One of these treatments is sonication, a process in which ultrasound is applied to a food product and it is recognized as a potential technology to meet the FDA requirement of a 5-log reduction in pertinent microorganisms found in fruit juices (Starek et al., 2021).

The microbial inactivation obtained by applying ultrasound is caused by cavitation, which occurs when ultrasound propagates in a liquid and causes bubbles due to pressure changes that implode causing zones of high temperature, disruption of the cellular membrane, production of free radicals and heat (Abdulstar et al., 2023; Karthikesh & Yang, 2021; Zupanc et al., 2019). It has been proven that ultrasound is more effective when combined with other technologies. Previous studies have shown that the combination of sonication with a subsequent storage in high osmotic pressure can cause significant reductions of microorganism loads (Wong et al., 2010; 2012).

The term “osmosonication” refers to the technology that combines the application of ultrasound to a medium and its subsequent storage in high osmotic pressure. This technology can be used to inactive pathogens and spoilage microorganisms in fruit juices without causing major nutritional or sensory losses (Starek et al., 2021). It has been proven that sonication followed by storage in high osmotic pressure can reduce *Salmonella* spp. populations by more

than 5-log when applied to blackberry and orange juice (Wong et al., 2010; 2012). These authors also showed that the treatment reduced lactic acid bacteria, which, however, were more resistant to osmosonication than pathogens.

Once the efficacy of sonication followed by high osmotic pressure was proven, the authors consider it relevant to investigate the factors that affect its microbial lethality. Some of the factors that have been proven to affect sonication are the amplitude, ultrasonic intensity, sonication time, pH, temperature, and solid content (Gabriel, 2012; Starek et al., 2021). Therefore, it is of interest to investigate how these factors affect microbial reduction using a combined treatment. Besides, sonication can be applied in a continuous way or by pulsations and little research has been done to evaluate the differences between these methods.

Factors related to the sonication equipment, such as wave amplitude and pulsations time were studied. Moreover, intrinsic characteristics of fruit juices were evaluated using model solutions, specifically pH and solid content (soluble and insoluble) and selected conditions of these parameters were applied to fruit juices. The results derived from this study will broaden scientific knowledge on the technology referred to as "osmosonication" and its potential industrial application on the production of safe and high-quality fruit juices. The main objective of the present study was to evaluate the effect of different osmosonication parameters on the reduction of *Salmonella* Typhimurium and *Lactobacillus rhamnosus*.

Materials and methods

This study was performed at Universidad de Costa Rica, Escuela de Tecnología de Alimentos, and Centro Nacional de Ciencia y Tecnología de Alimentos, San Pedro de Montes de Oca, San José, Costa Rica, from January 2012 to December 2012.

Model solutions and fruit juices

A sucrose solution with a total soluble solid content of 110 g/kg and a pH of 3.5 was used as model solution for the experiments. Also, a concentrated sucrose solution with a total soluble solid content of 650 g/kg was used for high osmotic storage. Both solutions were prepared with commercial table sugar (99.5 % minimum sucrose content). The pH value was adjusted with citric acid when required. Cellulose, as insoluble fiber (Vitacel LC 200, ChemSol), and citric pectin as soluble fiber were used to evaluate the effect of solid content.

Fruit juices studied with different pH, soluble solid content and turbidity included: micro-filtered blackberry juice (pH 2.8, 115 g/kg, 147 NTU), depulped blackberry juice (pH 2.8, 75 g/kg, 8474 NTU), micro-filtered pineapple juice (pH 3.7, 125 g/kg, 729 NTU), depulped pineapple juice (pH 3.6, 130 g/kg, 2507 NTU), 100 % natural commercial pasteurized apple juice (pH 3.7, 110 g/kg) and fresh coconut water (pH 5.3, 62.5 g/kg). Total soluble solids were measured with an handheld analog brix refractometer (resolution ± 0.1) and pH with a pH/ion-meter (12-24v, 50/60Hz, resolution ± 0.1). Both procedures were adapted from official AOAC methods.

Microbial strains, inocula preparation and sample inoculation

A strain of *Salmonella* Typhimurium ATCC 14028, representing one of the pathogens that can be found in liquid food matrixes like fruit juices (Muhammad et al., 2018) was used, previously maintained at -40 °C. Cells were activated on Standard Agar to obtain pure cultures by incubating for 16-24 h at 35 °C. To prepare the inoculum, an activated culture was grown in sterile Triptone Soy Broth, by incubating at 35 °C for 18 hours. The initial inoculum concentration was determined by serial dilutions in 0.1 % peptone solution plated on XLD and incubated for 24 h at 35 °C. Typical colonies were then counted and the results expressed as $\log_{10}$ CFU/mL.

A strain of *Lactobacillus casei* subsp. Rhamnosus ATCC 11443 was used. In this case, it was chosen as an example of spoilage bacteria that can deteriorate liquid food matrixes such as fruit juices (Muhammad et al., 2018). The cells were cultivated in sterile MRS Broth and incubated for 24 h at 35 °C. To prepare the inoculum, an activated culture was grown in sterile MRS Broth and incubated at 35 °C for 18 hours.

The starting inoculum concentration was determined by serial dilutions in 0.1 % peptone solution plated on MRS and incubated at 35 °C for 24 h. Typical colonies were then counted, and the results expressed as log10 CFU/mL (Naghili et al., 2013). Model solutions and juices were inoculated with 5 mL of each bacterial inoculum per 150 mL, to obtain an approximate microbial load of 5-7 $\log_{10}$ CFU/mL of each bacterium. Initial bacterial concentration in the sample was calculated considering the inoculum volume, its initial concentration, and the final volume of the inoculated sample.

Sonication treatments

A continuous recirculating system was used to sonicate the model solution or juice sample based on Wong et al. (2010). The system included a thermostatic bath to maintain the sample's temperature at (25-30) ±2 °C. The sample (150 mL) was placed in a beaker from which it was recirculated in a continuous way at a constant flow rate of 3.7 mL s^{-1}, with a peristaltic pump (0.1-600 rpm, 0.006-3400 mL/min) The feed passed through a stainless steel, cylindrical continuous-flow cell with double walls. The cell featured an ultrasonic probe that had a 13-mm diameter and was positioned at the cell's centre.

Ultrasonic treatment was applied at 20 kHz, with an ultrasonic converter that operated in a continuous way. The ultrasonic processor (750-Watt, 115 VAC, 50/60 Hz) featured a controller with automatic tuning and frequency control for delivering constant amplitude of ultrasonic vibration at the probe tip. The sample was sonicated according to the evaluated parameters (amplitude, sonication method and time), which were varied using the ultrasonic controller. Once the sample was sonicated, 1 mL of the sample was mixed with 100 mL of the concentrated solution. The sample was then stored for 48 hours at -11 ± 1 °C.

Microbial reduction determination

After osmosonication, the bacterial concentration was determined. This was done through the application of the method described in the previous section for the determination of the initial inoculum concentration. The difference between the initial and final bacterial concentration was reported as the microbial reduction, expressed as $\log_{10}$ CFU/mL.

Experimental designs and data analyses

Four experiments with model solutions were designed to determine the effects of several parameters in the logarithmic reduction of *Salmonella* Typhimurium and *Lactobacillus rhamnosus* after osmosonication. The first experiment addressed the effects of five levels of amplitude (20, 25, 30, 35, and 40) %, three levels of sonication time (10, 20, and 30) min, and two bacteria (*S.* Typhimurium and *L. rhamnosus)*. The second experiment evaluatedfour levels of percentage of pulsations time (90 % on, 50 % on, 10 % on, and 0 % on) and two bacteria (*S.* Typhimurium y de *L. rhamnosus)*. In this case, all samples were sonicated at 20 % amplitude during a total time of 10 min.

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The third experiment studied the effect offive levels of pH (2.5; 3.5; 4.5; 5.5, and 6.5) and two bacteria (*S.* Typhimurium and *L. rhamnosus)* on the microbial reduction after osmosonication. The samples were sonicated in a continuous way for 10 minutes at 20 % amplitude. The fourth experiment evaluated the effect of three levels of pectin content (0, 0.5, and 1) m/m %, three levels of cellulose content (0, 0.15, and 0.3) m/m %, and two bacteria (*S.* Typhimurium and *L. rhamnosus)*. The samples were sonicated for 10 minutes at 20 % amplitude.

It was of interest to study the application of the selected parameters in a real food matrix, in a more specific way regarding fruit juices. The sonication parameters chosen were: 40 % amplitude, 30 minutes as total sonication time, with a pulsations time of 10 % (three minutes; at a frequency of 27 s off three seconds on). The effects evaluated were: a) four fruit juices with different pH (micro-filtered blackberry, micro-filtered pineapple, apple juice, and coconut water) and two bacteria (*S.* Typhimurium and *L. rhamnosus)* on the logarithmic reduction; b) two fruit juices (pineapple and blackberry), two levels of pulp content (with pulp or without pulp), and two bacteria (*S.* Typhimurium and *L. rhamnosus*) on the logarithmic reduction.

ANOVA analysis was conducted for each experiment to determine the significance of simple effects and interactions among factors (when two or more factors were considered). Both amplitude and pH were analyzed as continuous factors. Analyses were performed using JMP 4.0 at a significance level of 5 % ($\alpha = 0.05$). When required for nominal factors (type of juice) a Tukey test was performed to compare means.

Results

Effect of amplitude

Logarithmic reductions for both bacteria increased with increasing amplitude ($p < 0.0001$) (Figures 1, 2, and 3). This occurred for the three sonication times evaluated. Also, the effect of amplitude on the reduction was lower for *L. rhamnosus* than for *S.* Typhimurium ($p = 0.0269$).

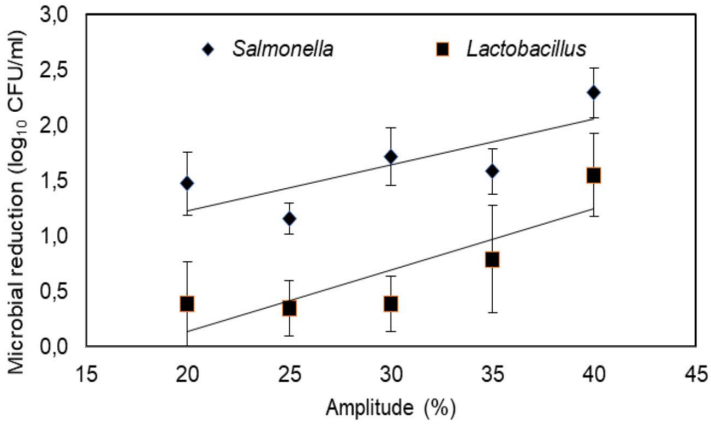

Figure 1. *Salmonella* Typhimurium and *Lactobacillus rhamnosus* reduction after osmosonication according to amplitude for 10-minute sonication. Universidad de Costa Rica. 2012.

Figura 1. Reducción de *Salmonella* Typhimurium y *Lactobacillus rhamnosus* después de la osmosonicación según amplitud para 10 minutos de sonicación. Universidad de Costa Rica. 2012.

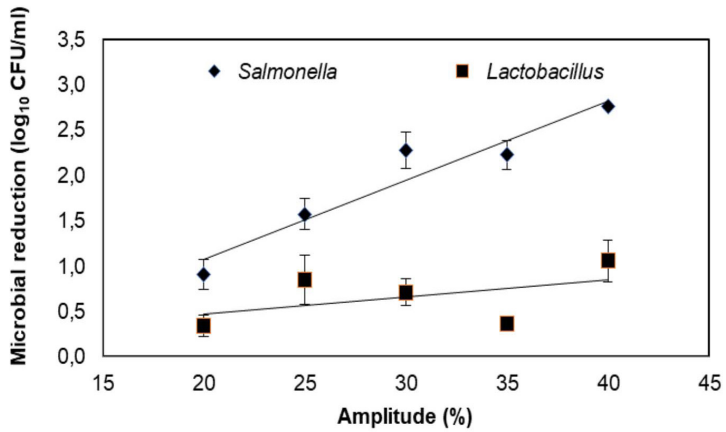

Figure 2. *Salmonella* Typhimurium and *Lactobacillus rhamnosus* reduction after osmosonication according to amplitude for 20-minute sonication. Universidad de Costa Rica. 2012.

Figura 2. Reducción de *Salmonella* Typhimurium y *Lactobacillus rhamnosus* después de la osmosonicación según amplitud para 20 minutos de sonicación. Universidad de Costa Rica. 2012.

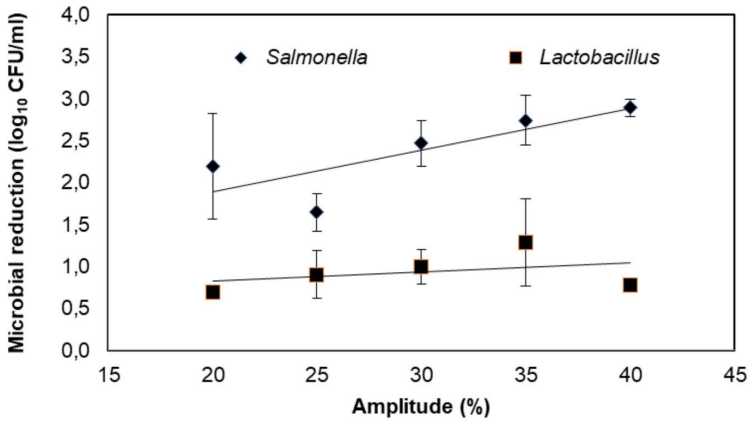

Figure 3. *Salmonella* Typhimurium and *Lactobacillus rhamnosus* reduction after osmosonication according to amplitude for 30-minute sonication. Universidad de Costa Rica. 2012.

Figura 3. Reducción de *Salmonella* Typhimurium y *Lactobacillus rhamnosus* después de la osmosonicación según amplitud para 30 minutos de sonicación. Universidad de Costa Rica. 2012.

Effect of percentage of pulsations time

Logarithmic reductions obtained for *L. rhamnosus* (0.3 and 0.6 $\log_{10}$ UFC/mL) were lower ($p < 0.0001$) than those of *S.* Typhimurium (1.5 and 1.8 $\log_{10}$ CFU/mL) (Table 1). This was valid for all pulsations times evaluated. Pulsations time had no effect on the microbial reduction of any of the bacteria evaluated ($p = 0.4645$, $1\text{-}\beta = 0.99$), which implies that equivalent reductions were obtained after continuous and non-continuous sonication.

Table 1. *Salmonella* Typhimurium and *Lactobacillus rhamnosus* reduction in model solutions after osmosonication (sonication at 20 % amplitude for 10 minutes) for different percentage of pulsations time and pulsations frequency. Universidad de Costa Rica. 2012.

Cuadro 1. Reducción de *Salmonella* Typhimurium y *Lactobacillus rhamnosus* después de osmosonicación (sonicación a 20 % de amplitud durante 10 minutos) según el porcentaje de tiempo de pulsaciones y frecuencia de pulsaciones. Universidad de Costa Rica. 2012.

Sonication treatment		Microbial reduction[b] (log_{10} CFU/mL)	
Pulsations time (Tp) (%)[a]	Pulsations frequency (on(s)/off (s))	S. Typhimurium	*L. rhamnosus*
10 (0.535 kJ)	3/27	1.5 ± 0.5	0.4 ± 0.7
50 (3.305 kJ)	15/15	1.5 ± 0.2	0.6 ± 0.3
90 (5.949 kJ)	27/3	1.8 ± 0.4	0.5 ± 0.3
100 (7.108 kJ)	0	1.8 ± 0.2	0.3 ± 0.2

[a]Values in parenthesis correspond to the energy consumption during sonication, registered by the equipment. / [a]Valores en paréntesis corresponden al consumo energético registrado por el equipo durante la sonicación.
[b]Means shown with 95 % confidence interval (n= 3). / [b]Promedios con intervalo de confianza al 95 % (n= 3).

Effect of pH

The effect of pH on microbial reduction was significant ($p = 0.0079$). Reduction decreases with increasing pH for both bacteria (Figure 4). The highest reductions were obtained for a model solution with pH 2.5, as expected. However, for *S*. Typhimurium, the reduction obtained was 2.9 log_{10} CFU/mL, whereas *L. rhamnosus* was reduced in only 1.0 log_{10} CFU/mL.

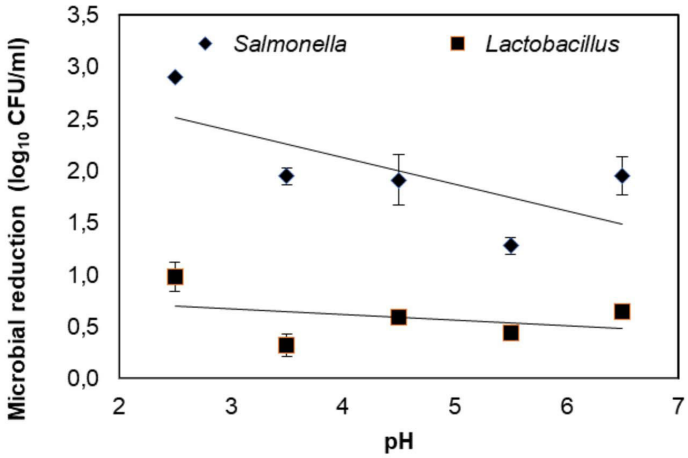

Figure 4. *Salmonella* Typhimurium and *Lactobacillus rhamnosus* reduction after osmosonication according to model solution pH. Universidad de Costa Rica. 2012.

Figura 4. Reducción de *Salmonella* Typhimurium y *Lactobacillus rhamnosus* luego de osmosonicación según pH de la solución modelo. Universidad de Costa Rica. 2012.

Effect of solid content (pectin and cellulose)

Logarithmic reductions of *S*. Typhimurium and *L. rhamnosus* are presented in Table 2. Microbial reductions did not change when sonication was applied to model solutions with different pectin content ($p = 0.5402$; $1\text{-}\beta = 1$). The same behaviour was determined for cellulose content ($p = 0.2429$; $1\text{-}\beta = 1$).

Table 2. *Salmonella* Typhimurium and *Lactobacillus rhamnosus* reductions in model solutions after osmosonication (sonication at 20 % amplitude for 10 minutes) according to pectin and cellulose content. Universidad de Costa Rica. 2012.

Cuadro 2. Reducción de *Salmonella* Typhimurium y *Lactobacillus rhamnosus* en soluciones modelo después de osmosonicación (sonicación a 20 % de amplitud durante 10 minutos) según contenido de pectina y celulosa. Universidad de Costa Rica. 2012.

Solid content (%m/v)		Microbial reduction ($\log_{10}$ CFU/mL)[a]	
Pectin	**Cellulose**	***S*. Typhimurium**	***L. rhamnosus***
0	0	1.8 ± 0.1	0.3 ± 0.2
0.5	0	2.2 ± 0.1	0.2 ± 0.1
1.0	0	1.8 ± 0.2	0.5 ± 0.2
0	0.15	2.0 ± 0.4	0.10 ± 0.03
0.5	0.15	1.5 ± 0.4	0.4 ± 0.6
1.0	0.15	1.5 ± 0.1	0.1 ± 0.1
0	0.30	1.7 ± 0.6	0.2 ± 0.1
0.5	0.30	1.8 ± 0.6	0.5 ± 0.3
1.0	0.30	1.7 ± 0.2	0.1 ± 0.2

[a] Means shown with 95 % confidence interval (n= 3). / [a]Promedios con intervalo de confianza al 95 % (n= 3).

Effect of fruit juice pH

The type of fruit juice had a significant effect on the reduction of both bacteria ($p < 0.001$) and the effect is bacteria dependent ($p < 0.001$) (Table 3). There were no significant differences in reductions of *S*. Typhimurium

Table 3. *Salmonella* Typhimurium and *Lactobacillus rhamnosus* reductions in fruit juices with different pH after osmosonication (sonication at 40 % amplitude, 30 minutes, 10 % pulsation time). University of Costa Rica. 2012.

Cuadro 3. Reducciones de *Salmonella* Typhimurium y *Lactobacillus rhamnosus* en jugos de frutas de diferente pH luego de osmosonicación (sonicación a 40 % de amplitud, 30 minutos y 10 % de tiempo de pulsación). Universidad de Costa Rica. 2012.

		Microbial reduction ($\log_{10}$ CFU/mL)[a]	
Fruit	**pH**	**S. Typhimurium**	***L. rhamnosus***
Blackberry	2.8	1.9 ± 0.1 [a]	3.5 ± 0.3 [a]
Apple	3.7	1.6 ± 0.2 [a]	0.0 ± 0.1 [b]
Pineapple	3.7	1.9 ± 0.3 [a]	0.1 ± 0.2 [b]
Coconut water	5.3	1.6 ± 0.6 [a]	0.1 ± 0.3 [b]

[a]Different superindex letters in columns indicate significant differences ($\alpha = 0.05$). Means shown with 95 % confidence interval (n= 3) / Letras superíndice diferentes indican diferencias significativas ($\alpha = 0.05$). Promedios con interval de confianza al 95 % (n= 3).

for blackberry, apple and pineapple juice and coconut water, under the evaluated conditions. However, for *L. rhamnosus* a significant difference between reduction of blackberry juice and the other juices was observed. The reduction for blackberry juice was 3.5 $\log_{10}$ CFU/mL. For pineapple juice and coconut water the inoculated microbial load was reduced in 0.1 $\log_{10}$ CFU/ml and there was no reduction in the case of apple juice.

Effect of juice solid content (with pulp or without pulp)

The types of fruit juice had a significant effect ($p < 0.001$) on the reduction of both bacteria and this effect was dependent on the pulp content ($p < 0.001$) and on the type of bacteria ($p < 0.001$) (Table 4). Reductions for blackberry juice are different from the ones of pineapple juice, differences that vary depending whether the juice is pulpous or clear and, on the bacteria evaluated. Reductions of *S.* Typhimurium and *L. rhamnosus* obtained for micro-filtered pineapple juice do not differ from the same fruit juice containing pulp. *S.* Typhimurium population was reduced in (1.9 ± 0.3) $\log_{10}$ CFU/mL in micro-filtered juice and (1.8 ± 0.3) $\log_{10}$ CFU/mL in pulpous juice. However, *L. rhamnosus* showed no reduction in either case.

Table 4. *S.* Typhimurium and *L. rhamnosus* reductions in fruit juices, with and without pulp, after osmosonication (sonication at 40 % amplitude, 30 minutes, 10 % pulsation time). University of Costa Rica. 2012.

Cuadro 4. Reducciones de *Salmonella* Typhimurium y *Lactobacillus rhamnosus* en jugos de frutas, con y sin pulpa, luego de osmosonicación (sonicación a 40 % de amplitud, 30 minutos y 10 % de tiempo de pulsación). Universidad de Costa Rica. 2012.

Juice characteristics			Microbial reduction[a] ($\log_{10}$ CFU/mL)	
Fruit	**Pulp content**	**Turbidity (NTU)**	***S.* Typhimurium**	***L. rhamnosus***
Blackberry	Pulpless	147	1.9 ± 0.1	3.5 ± 0.3
	With Pulp	8474	2.8 ± 0.2	2.7 ± 0.2
Pineapple	Pulpless	729	1.9 ± 0.3	0.1 ± 0.2
	With Pulp	2507	1.8 ± 0.3	0 ± 0

[a]Means shown with 95 % confidence interval (n= 3). / [a]Promedios con intervalo de confianza al 95 % (n= 3).

Different results were obtained for blackberry juice. Differences between reductions in clear and pulpous juice were found and the behavior depended on each bacteria evaluated. In the case of *L. rhamnosus* the reduction (2.7 ± 0.2 $\log_{10}$ CFU/mL) was lower in the pulpous juice than in the micro-filtered juice (3.5 ± 0.3 $\log_{10}$ CFU/mL). *S.* Typhimurium showed a different behavior: in the micro-filtered juice the microbial load was reduced by 1.9 ± 0.1 $\log_{10}$ CFU/mL, whereas the reduction for the pulpous blackberry juice was 2.8 ± 0.2 $\log_{10}$ CFU/mL. Therefore, the efficacy of the treatment on reducing *S.* Typhimurium was better for the juice containing pulp.

Discussion

Previous studies demonstrated that sonication followed by storage at high osmotic pressure provides a treatment to achieve microbial reductions in liquid matrixes (Wong et al., 2010; 2012). These studies included treatment controls (samples with no sonication, samples with no osmotic pressure, samples with sonication but no osmotic pressure, and samples with osmotic pressure and no sonication) to reach such conclusion. This

observational study aimed to vary several factors of the process (sonication amplitude and time, and percentage of pulsations time) as well as intrinsic factors of the matrixes (pH, solid content) to observe their effect on the achieved microbial reductions.

Results obtained by varying amplitude levels during sonication were similar to those reported in other studies for different microorganisms (Alighourchi et al., 2014; Margean et al., 2020; Starek et al., 2021). An increase in microbial reductions with an increase in amplitude can be explained in terms of the number of bubbles imploding during cavitation, which also increases and causes further disruption of the cellular structure of microorganisms (Zupanc et al., 2019). An optimized combination of time and amplitude could imply energy savings for industrial application of osmosonication. Energetic consumption increases with higher amplitude levels and longer sonication times. Therefore, it is important to optimize and achieve the highest microbial reductions using the lowest possible amplitude levels and time.

Regarding the higher resistance of *L. rhamnosus* to the treatments, similar results were obtained in previous studies (Wong et al., 2010). The better resistance of lactobacilli could be explained in terms of the resistance of its cellular wall, which is higher in Gram positive bacteria when compared to Gram negative bacteria like *Salmonella* (Zupanc et al., 2019). Therefore, it is important to validate the osmosonication process not only in terms of amplitude and time but also considering the pathogens and spoilage microorganisms of relevance in the matrix of interest.

It is very advantageous that the same microbial reductions can be obtained by applying non-continuous sonication compared to pulsed sonication. This could lead to significant energy and economic savings during industrial application. The energy consumption was much lower when pulsations were active for only 10 % of the total sonication time. In this case, the ultrasonic waves are off 90 % of the total sonication time, which means that there is no energy waste during this time. In addition, the temperature rise in the sample is lower when non-continuous sonication is applied.

Ultrasonic energy transferred to a liquid is transformed into heat (Vieira Nunes et al., 2022) and the action of cavitation is an energy-generating process, as mechanical energy causes molecular motion, which causes a temperature rise in the sample (Zupanc et al., 2019). Therefore, when cavitation occurs only for short periods of time, the energy generated and the temperature increase are diminished. It is common for cooling systems to be used to maintain a constant temperature in the sample, and this involves energetic expenditure. The results obtained in the present investigation could represent an important economic savings in the costs of refrigeration systems in industrial applications of osmosonication.

Few authors have studied the effect of continuous and pulsed thermosonication on the cellular structure of microorganisms. A study showed more damaged *Saccharomyces cerevisiae* cells after continuous thermosonication of apple juice compared to the non-continuous method (Marx et al., 2011). However, in both cases the inactivation of the yeast was complete. Similar results were obtained in the present research, in which no differences in microbial reduction between both methods was observed. This result, in conjunction with the ones reported by Hawrylik (2019) in sonicated wastewater, could mean that although damages to the cell structure are higher when the continuous method is applied, the effect of these damages might not be significant to cell death.

A study reported a higher inactivation of *Saccharomyces cerevisiae* in three fruit juices with a continuous sonication treatment (Bermúdez-Aguirre & Barbosa-Cánovas, 2012). This contradictory result might be explained by the intrinsic characteristics of the sample juices used and their effect on the microbial inactivation mechanisms (Zinoviadou et al., 2015). More specifically, Bagher Hashemi & Roohi (2021) reported that the difference in microbial inactivation using continuous or pulsed sonication is dependent on the amplitude. Their results indicate that for 40 % and 100 % amplitudes, it is preferable to use the pulsed ultrasound, while for a 70 % amplitude, the continuous ultrasound is the best option for microbial inactivation.

The effect of pH on microbial reduction was expected since ultrasound makes microorganisms more sensitive to pH and is an important parameter that affects sonication efficiency (Vilkulin & Vikulina, 2020; Wong et al.,

2010). The reason could be related to the fact that when the concentration of H^+ ions increases, microbial cells respond with defense mechanisms to avoid the increase in intracellular pH (Han et al., 2017). It is therefore important to consider the pH of the food matrix when validating an osmosonication application. The higher resistance of *L. rhamnosus* to the matrix pH can be explained by the higher resistance of its cell wall compared to *S.* Typhimurium (Icer et al., 2023).

The microbial reductions found for the model solutions followed a pattern that was not confirmed with fruit juices of different pH. It is observed that when the treatment was applied to the model solutions, the reductions increased as pH decreased. However, fruit juices did not show this behavior. The differences in the results obtained for fruit juices and for model solutions could indicate an effect of factors intrinsic to each juice, such as the type and amount of acid, and the presence of other compounds. This would need to be studied further to understand the contribution of other factors in a more complex matrix such as fruit juice.

It was determined that similar microbial reductions can be achieved for different solid contents and viscosity when applying osmosonication. This result contradicts previous studies that reported solid content as a critical factor during sonication processes (Salleh-Mack & Roberts, 2007; Gabriel, 2012). Specifically, the presence of D glucose had a protective effect on *E. coli*, increasing the sonication time required for some reduction (Salleh-Mack & Roberts, 2007). The differences obtained between the present investigation and the results these authors could be related to evaluated solid and the lower concentrations used in the present study. This could indicate that at low concentrations (< 1 %), which is the normal concentration found in natural juices, the effect of soluble solid is not significant.

In general, as observed with model solutions, similar microbial reductions can be expected in juices that differ in their cellulose and pectin content at concentrations below 1 %, which facilitates the standardization of osmosonication and its industrial application. However, the results observed with fruit juices were not so clear. In some cases, such as those with blackberry juice, it was easier to inactivate the microorganism such as *Salmonella*, in the juice with pulp than in the juice without pulp. In other cases, the contrary was observed, with *Lactobacillus*.

It is important to mention that, in general, the results obtained after applying sonication followed by high osmotic pressure in fruit juices do not agree with what was expected based on the experiments performed with model solutions. As mentioned before, pH was not significant on the microbial reduction in fruit juices, but it was when the treatment was applied in model solutions. On the other hand, pulp content in juices influenced microbial reduction, but it was determined that cellulose and pectin content did not influence the efficacy of the treatment in model solutions.

However, the results obtained in the present study open many research opportunities in the topic of the effects of intrinsic parameters of fruit juices in the efficacy of osmosonication, as well as their interactions. Knowledge generated towards these effects could help to understand this novel technology deeper and contribute to its industrialization to produce safe and high-quality fruit juices.

Conclusions

Osmosonication parameters, such as amplitude, time, pH, and pulp content of matrixes affect the reduction of *Salmonella* Typhimurium and *Lactobacillus rhamnosus* in model solutions and fruit juices. Survival of *Lactobacillus rhamnosus* after osmosonication was higher than that observed for *Salmonella* Typhimurium. In general, an increase in amplitude, time and pulp content and a decrease in pH result in higher microbial reductions. Osmosonication parameters should be considered to determine the effectiveness of this process in ensuring the food safety of the products.

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Interests conflict

Authors declare no conflict of interest related to this study.

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