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Fermentation of Lulo Juice with *Lactobacillus reuteri* CECT 925. Properties and Effect of High Homogenization Pressures on Resistance to In Vitro Gastrointestinal Digestion

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Abstract: The aim of this study was to evaluate the use of lulo juice as substrate for producing a potentially probiotic beverage with *Lactobacillus reuteri* CECT 925. Lulo juices at two pH levels and two levels of HPH treatment have been considered to evaluate the effect of these variables on *Lactobacillus reuteri* CECT 925 growth, physicochemical and antioxidant properties, and the resistance of microbial cells to gastrointestinal digestion in vitro. Regarding the growth of *Lactobacillus reuteri* CECT 925, it was mainly affected by the pH of the medium, the rectified juice at pH 5.5 being the most appropriated one. The growth of *Lactobacillus reuteri* CECT 925 mainly increased the antiradical capacity of the juices. In general, *Lactobacillus reuteri* CECT 925 showed good resistance to in vitro gastrointestinal digestion conditions, reaching levels above 10^7 CFU/mL in all cases. The highest resistance was observed in the juice treated at 150 MPa followed by the juice homogenized at 100 MPa.

Keywords: non-thermal treatment; tropical fruit juices; probiotics; functional food

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1. Introduction

A positive consumer attitude towards a healthy and balanced diet has been growing lately all over the world. Therefore, the demand for foods that promote health and well-being, such as functional foods containing phytochemicals and probiotics has increased [1]. The latest revised and accepted definition of probiotics states that they are "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" [2]. Although traditional matrices used for the incorporation of bacterial species with beneficial properties are dairy matrices, in the last decade there have been numerous studies demonstrating the interest in using plant-based matrices. In plant-based matrices, the beneficial activity and efficacy of the probiotic could be enhanced by the bioactive components naturally present in the plant matrix, resulting in a final functional product in which there is a synergistic effect on the health benefit between the bioactive components and the probiotic strain [3].

The lulo (*Solanum quitoense* Lam.) stands out among tropical fruits with a pleasant flavor and a distinctive nutritional and phytochemical composition. Although lulo fruit is mainly consumed as juice in the producing countries themselves, it has gained interest in national and international markets due to its organoleptic and functional properties [4]. It has a high potential for the development of functional foods; is rich in vitamin C, fiber, and antioxidant compounds, such as all trans-β-carotene, lutein and zeaxanthin, chlorogenic acids, flavonol glycosides and bioactive amines such as N^1,N^4,N^8-tris(dihydrocaffeoyl) spermidine and N^1,N^8-bis(dihydrocaffeoyl) spermidine. Some in vitro and in vivo studies reveal various mechanisms through which the lulo compounds reduce risk or reverse metabolic- and inflammation-associated diseases [5].

Additionally, some fruit juices have been demonstrated to be a good matrix to proliferate probiotic microorganisms due to its content in carbohydrates, vitamins, and bioactive compounds. Coupled to fruit consumption, the intake of certain microorganisms is capable of decreasing body fat by modifying the intestinal microbiota, stimulating the synthesis of satiety-inducing peptides, reducing the synthesis of proinflammatory cytokines and possibly modifying the lipid profile or suppressing *Helicobacter* populations [6]. Therefore, tropical fruit juices fermented with probiotics may be an effective strategy to meet dietary health requirements.

However, it is known that the survival of lactic bacteria in fruit juices tends to be more complex than in dairy beverages. This is due, mainly, to the natural acidity of fruit, the high level of polyphenols and the absence of lactose, which may interfere in the survival of certain sorts of microorganisms, as well as in their growth and in their resistance to the gastrointestinal digestion process [7]. In this sense, the application of some pretreatments and a suitable selection of strain can improve the probiotic growth, resistance, and, consequently, their release to the specific target site.

Lactobacillus reuteri is a heterofermentative lactic acid bacterium (LAB) found in a variety of ecological niches like fermented food and the gastro-intestinal as well as the urogenital tract of humans and other animals [8]. Its consumption as a supplement has been recognized as safe [9], and some probiotic properties have been demonstrated. It was shown to inhibit *Helicobacter pylori* in the human stomach, thus, providing new opportunities in the treatment of chronic stomach inflammation [10]. Orally administered in combination with *Lactobacillus rhamnosus* enhanced vaginal flora quality and was effective against bacterial vaginosis [11,12]. Additionally, *Lactobacillus reuteri* showed an improve of biomarkers of inflammation and cardiovascular risk in obese adults with metabolic syndrome [13]. *Lactobacillus reuteri* has the capability to ferment a whole range of different carbon sources and some species have been used to ferment fruit juices producing bioactive compounds of great interest such as folates and vitamin B12 [14]. Furthermore, *Lactobacillus reuteri* has the capability to produce reuterin, a compound with potential as a food preservative, especially due to its activity against food spoilage microorganisms such as *Listeria monocytogenes* and *Escherichia coli*. Definitively, its features and its safe status makes it a promising microorganism in the food industry [8]. Some research works show fruit juices as a good carrier for *Lactobacillus reuteri*, but their viability was strongly affected by the kind of juices. Namely, *Lactobacillus reuteri* showed a high viability in pineapple, orange, and apple juices, while the viability was reduced in red-fruit juice [15]. In addition, thermal preservation treatments or exposure to temperatures above refrigeration also have unfavorable effects. In this sense, the viability of probiotic cells can be improved by modifying the properties of the juice after a non-thermal preservation treatment as an alternative to a conventional heat treatment.

High pressures homogenization (HPH) is a non-thermal treatment, which in addition to being used for preservation purposes, also improves the physico-chemical and functional properties of fruit juices, increasing quality, stability, and phytochemical profile [16,17]. It allows the replacement of traditional processes to better preserve sensory and nutritional characteristics, as well as to develop products with differentiated structural characteristics and functional properties [18]. The application of high and/or moderate homogenization pressures to fruit juices has proven to be less destructive for low molecular weight compounds responsible for sensory and nutritional attributes and, at the same time, sufficiently effective to inactivate the microorganisms that are responsible for spoilage [19]. Applied to strains with probiotic effect, homogenization has been shown to increase survival and improve functional properties. For example, in trials with *Lactobacillus paracasei* A13 strains, the application of high homogenization pressures increased their hydrophobicity, which is directly related to their ability to adhere to intestinal cells and their resistance to the digestion process [20]. Moreover, HPH at 50 MPa applied to cells of *Lactobacillus paracasei* A13 were able to modulate the murine immune system inducing a high IgA response, compared to untreated cells. In fact, modifications of the outermost cellular structures by

the hyperbaric treatments play an important role in the final probiotic and immune cells interaction [21].

Finally, to ensure that *Lactobacillus* exerts a beneficial probiotic effect on a host, it needs to survive to gastrointestinal digestion process. In vitro digestion simulation is often used to evaluate probiotic survival in the gastrointestinal tract. This technique is not only faster and less expensive than comparable methods, but also resource-efficient, with significantly lower bio-ethical constraints [22].

Based on all that has been stated, the aim of this study was to evaluate the use of lulo juice as substrate for producing a potentially probiotic beverage with *Lactobacillus reuteri*. Lulo juices at two pH levels and two pressures of HPH treatment have been considered to evaluate the effect of these variables on *Lactobacillus reuteri* growth, physicochemical and antioxidant properties, and the resistance of microbial cells to gastrointestinal digestion in vitro.

2. Materials and Methods

2.1. Raw Materials

Fresh lulo fruits (*Solanum quitoense* Lam.) from Colombia were purchased in the Central Market of Valencia city (Spain). Whole fruits were washed with water, blended (Phillips Advance Collection Standmixer 800W, 2L) and filtered through a 500 µm stainless steel sieve. After sieving, the juices were subjected to a HPH treatment at 100 or 150 MPa, using a laboratory-scale high-pressure homogenizer (Gea Niro Soavi-Panda Plus 2000, Parma, Italy). Then, both the non-homogenized juice and the juices homogenized at 100 or 150 MPa were pasteurized for 3 min at 72 °C. After the thermal treatment, the juices were kept refrigerated at 4 °C for 24 h until further inoculation and after that, frozen until analysis.

Lactobacillus reuteri CECT 925 (animal origin) from the Spanish Type Culture Collection (Parc Científic de la Universitat de València) was used as probiotic strain. The freeze-dried strain was incubated in commercial MRS broth (Scharlau Chemie®, Barcelona, Spain) in 100 mL Erlenmeyer flasks for 24 h at 37 °C in static conditions. *Lactobaciullus reuteri* reached a growth of $1.6 \pm 0.2 \times 10^9$ CFU/mL in MRS broth and it was kept refrigerated for 24 h until its use as starter inoculum.

2.2. Preparation of Lulo Juice with Lactobacillus reuteri CECT 925

To ensure an appropriate growth of *Lactobacillus reuteri* CECT 925 in lulo juice and in accordance with previous studies [19], the pH of the juice was rectified to pH 5.5 and 6 using sodium bicarbonate. In addition, yeast extract (Scharlau Chemie®, Barcelona, Spain) was added in an amount of 5 g/L. The juice was contained in clear borosilicate glass 3.3 bottles, GL45 threaded, 250 mL, with blue stopper and pouring ring, ISO 4796 (Scharlau Chemie®, Barcelona, Spain). The strain was transferred from the MRS broth to the juices (non-homogenized juice, juice homogenized at 100 MPa and homogenized at 150 MPa) in an amount of 4 mL/L. The bottles were completely filled and capped. Subsequently, the juices were incubated at 37 °C for 24 h in static conditions. Fermentation was carried out in triplicate.

2.3. Analytical Determinations

All the analytical determinations were carried out at least in triplicate on the juices before inoculation and after incubation.

The pH values were obtained using a digital pH-meter (Mettler Toledo Inlab, Schwerzenbach, Switzerland), calibrating the equipment prior to analysis with buffer solutions at pHs 7 and 4.

Soluble solids content was determined with a refractometer (Abbe Atago BT, Nar T3, Tokyo, Japan), previously calibrated with distilled water and thermostated at 20 °C, by direct reading of their refractive indexes in Brix [23].

Density was measured with Bingham 25 mL pycnometer, for density and relative density determination in liquids (ASTM D1217, Scharlau Chemie®, Barcelona, Spain) at room temperature (20–22 °C) and distilled water as the reference liquid [24].

Suspended pulp and cloudiness were determined following the methodology described by Betoret et al. [25], according to which the suspended pulp of a juice is equivalent to the volume of the precipitate obtained after centrifugation (Medefriger BL-S P-Select centrifuge) of 10 mL of sample at 365× *g* (for unstable pulp) or 3000× *g* (for stable pulp) at 25 °C for 10 min. The turbidity of the samples corresponds to the transmittance of the supernatant obtained after centrifugation, measured at 650 nm in a UV/Visible spectrophotometer (Thermo Scientific Helios Zeta U/Vis, Loughborough, UK).

Particle size distribution was determined with the Malvern Mastersizer 2000 (Malvern Instruments Limited, Worcestershire, UK), equipped with a 470 nm blue light source and a 0.02–200 micron measuring range. The refractive index of the juices (cloud) and the dispersant (water) were set at 1.5 and 1.33, respectively. Absorption of the cloud particles was taken as 0.1. Particle size distribution of the juices was characterized by both volume (D [4,3]) and area (D[3,2]) equivalent diameters, and by the percentiles d10, d50, and d90, which correspond to the characteristic diameters below which 10%, 50%, and 90% of the particles are included in the distribution [26].

CIE*L*a*b* color coordinates were determined from the surface reflectance spectra obtained between 400 and 700 nm. The equipment was calibrated on white and black backgrounds, considering standard light source D65 and observer 10° (Minolta spectrophotometer CM-3600d, Osaka, Japan). The resulting CIE-L*a*b* color coordinates allowed calculating the psychometric coordinates: tone (h^*_{ab}) and chrome (C^*_{ab}) according to CIE [27].

Antioxidant properties. For antioxidants extraction, the samples were mixed with an 80:20 (*v*/*v*) methanol–water solution at a 1:10 ratio (*w*/*v*), stirred for 1 h using a magnetic device (RTC basic, Staufen, Germany), and centrifuged at 10,000 rpm and 20 °C for 5 min (Medefriger BL-S P-Select centrifuge). Subsequent analyses (total phenols and flavonoids and antiradical capacity) were carried out on the supernatant.

Total phenols were determined following the Folin–Ciocalteu method [28,29], by mixing 0.125 mL of juice, 0.125 mL of Folin–Ciocalteu reagent (Sigma-Aldrich) and 0.5 mL of double-distilled water. This solution was allowed to react for 6 min. Next, 1.25 mL of 7% (*m*/*v*) sodium carbonate solution and 1 mL of double distilled water were added. Absorbance was measured at 765 nm in a spectrophotometer (Thermo Scientific Helios Zeta U/Vis, Loughborough, UK). A reference blank was prepared, wherein the sample was replaced by the same amount of double distilled water and was allowed to react for 90 min. A standard gallic acid curve ranging from 0 to 500 mg/L was obtained to express the results in milligrams of gallic acid equivalent (GAE) per mL.

Flavonoids content was determined following methodology described by Luximon-Rama et al. [30]. This was done by mixing 5 mL of juice and 1.5 mL of a 2% (*w*/*v*) aluminum chloride solution, which was left in the dark for 10 min. Absorbance was measured at 368 nm in a spectrophotometer (Thermo Scientific Helios Zeta U/Vis, Loughborough, UK). The data obtained were compared to a standard quercetin curve ranging from 0 to 350 mg/L. The results were expressed in milligrams of quercetin equivalent (EQ) per mL.

Antiradical capacity was determined following the DPPH (2,2 diphenyl-1-picrylhydrazyl) method, as described by Kuskoski et al. and Stratil et al. [31,32], with some modifications. A solution with 0.1 mL of juice, 0.9 mL of methanol, and 2 mL of a 100 µM methanol–DPPH (39.4 µg/mL) solution was prepared. After 0, 30, and 60 min of reaction, absorbance was measured at 517 nm in a spectrophotometer (Thermo Scientific Helios Zeta U/Vis, Loughborough, UK). The results were expressed as milligrams of Trolox equivalent (TE) per mL, using the Trolox calibration curve within a 0 to 500 mg/L concentration range.

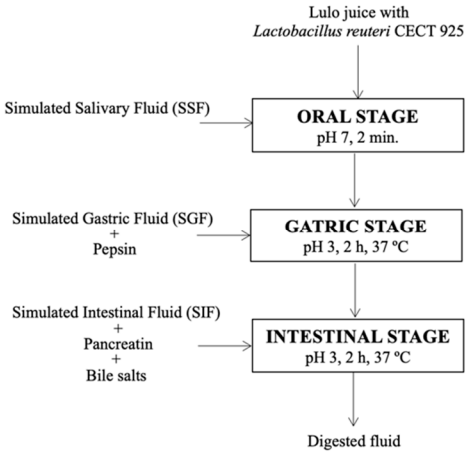
Antiradical capacity was also evaluated following the ABTS + (2,2′-azino-bis-3 ethylbenzothiazoline-6-sulfonic acid) method as described by Re et al. [33]. A solution containing 7 mM of ABTS and 2.45 mM of potassium persulfate was prepared and left in

the dark at room temperature for 16 h. ABTS was mixed with phosphate buffer to reach an absorbance of 0.70 ± 0.02, which was read at 734 nm. A 0.1 mL aliquot of sample was added to 2.9 mL of ABTS solution. Absorbance was measured at 734 nm in a spectrophotometer (Thermo Scientific Helios Zeta U/Vis, Loughborough, UK) after 0, 3, and 7 min of reaction time. The results were expressed as mg of Trolox equivalent (TE) per mL.

2.4. In Vitro Simulation of Gastrointestinal Digestion

To study the resistance of *Lactobacillus reuteri* CECT 925 throughout gastrointestinal digestion process, fermented lulo juices were subjected to an in vitro gastrointestinal digestion, following the standard protocol proposed by Minekus et al. [22].

Figure 1 shows the flow diagram followed in this assay, the salts used, and the volumes needed to prepare the stock solutions of the simulated fluid phases.



Concentration of salts in the stock solutions and their volume (mL/L) used in the preparation of the simulated fluid phases (Minekus *et al.*, 2014)

	Salt content (g/L)	SSF (mL/L)	SGF (mL/L)	SIF (mL/L)
KCl	37.3	30.2	27.6	27.2
KH_2PO_4	68	7.4	3.6	3.2
$NaHCO_3$	84	13.6	50	170
NaCl	117	-	47.2	38.4
$MgCl_2(H_2O)_6$	30.5	1	1.6	4.4
$(NH_4)_2CO_3$	48	0.12	2	-
$CaCl_2(H_2O)_2$	44	0.05	0.01	0.08

Figure 1. Stages, conditions, salts used, and the volumes needed to prepare the stock solutions of the simulated fluid phases in the simulated gastrointestinal digestion.

In each stage, the previously digested phase (lulo juice in the oral stage) and the associated simulated fluid were mixed in a ratio 1:1 (*v*/*v*) and kept in stirring (Intell-Mixter RM-2 Elmi ltd, Riga, LV-1006, Letino, Italy) at 55 rpm until the end of stage. The quantity of enzymes used (pepsin in the gastric stage and pancreatin in the intestinal stage) was calculated according to the enzymatic activity to ensure 20,000 U/mL of pepsin and 100 U/mL of pancreatin. NaOH (1 M) and HCl (6 M) were used for pH adjustment.

Viable microbial counts were performed on four samples obtained throughout the simulated digestion: at the beginning and at the end of the gastric stage, and at the beginning and at the end of the intestinal phase. In addition, antioxidant properties were determined at the end of the whole process.

2.5. Microbiological Analysis

Colony counts were measured by serial decimal dilution in PBS, 10X solution (Scharlau Chemie®, Barcelona, Spain) and aliquots of 1 mL of proper dilutions were plated on MRS Agar (Scharlau Chemie®, Barcelona, Spain) [34]. The plates were incubated in anaerobiosis at 37 °C for 24 h. Survival rate (Rs) of the microbial strain to each stage of the in vitro gastrointestinal digestion was then calculated as the ratio between the microbial concentration at the end of the stage and the microbial concentration at the end of the previous one, both referred to the same basis.

2.6. Statistical Analysis

All determinations were made in triplicate. The statistical analysis of the data was performed in a Statgraphics Centurion XVII software package, making use of a simple or

multifactorial analysis of variance (ANOVA) at a 95% confidence level ($p \leq 0.05$). Significant differences ($p \leq 0.05$) among groups were determined using the Fisher LSD test.

3. Results and Discussion

3.1. Lactobacillus reuteri CECT 925 Growth in Lulo Juices. Effect on Physicochemical and Antioxidant Properties

A multifactorial analysis of variance revealed a significant effect of pH ($p \leq 0.05$). Viable cell counts in the juices showed values above 8 (log CFU/mL) in all samples and above 9.5 in the juices at pH 5.5. HPH treatment had no significant effect ($p \leq 0.05$) on the viable cell counts when compared to that achieved in the non-homogenized samples. Results are shown in Figure 2.

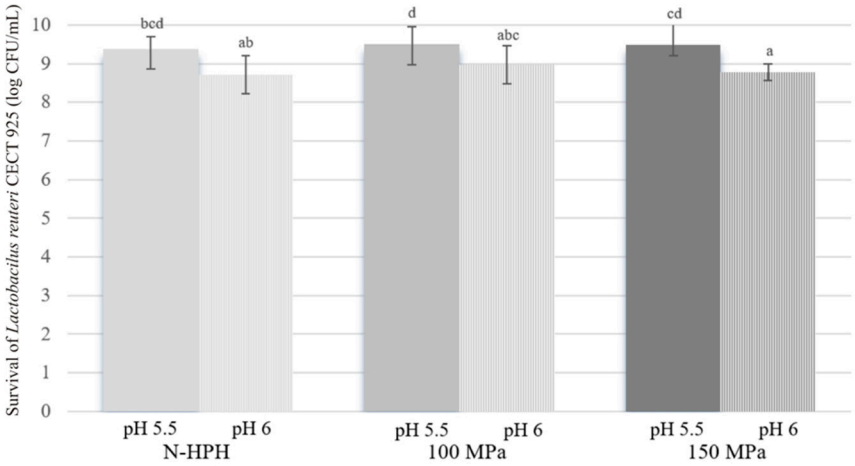


Figure 2. Impact of pH value and high homogenization pressures on survival of *Lactobacillus reuteri* CECT 925 in lulo juices after fermentation. Different letters denote significant differences ($p \leq 0.05$).

In relation to physicochemical properties such as pH and soluble solids content (Brix), the influence of HPH depends on the food product. Zhou et al. [35] found no significant changes in pH and soluble solids content of mango juice after HPH at pressures up to 190 MPa. Patrignani et al. [20] found no effect of HPH at 100 MPa on pH of carrot juice, while a decrease of pH of apricot juice after the same HPH treatment was observed. In this research, the application of the HPH treatment significantly decreased the Brix of the non-fermented juices (Table 1). As expected, fermentation was the most important variable decreasing both pH and soluble solids content. This decrease was more pronounced in the juices at pH 6 where pH rectification provided a higher initial value for Brix.

The growth of *Lactobacillus reuteri* was the only variable that significantly influenced the density of the juices, slightly raising its value.

Pulp sedimented and transmittance of supernatant at two centrifugation speeds were measured to evaluate physical stability. According to Stokes Law, the sedimentation velocity of spherical and rigid particles is proportional to the particle size (diameter) and inversely proportional to the dispersed medium viscosity [36]. Therefore, particle size reduction due to HPH contributes to the delay of juice cloud loss by decreasing the particle's Stokes diameter and, thus, slowing down the pulp sedimentation and improving physical stability [37]. It is important to note that the possibility of increasing physical stability by employing HPH is very interesting for fruit juices since there will be no need for hydrocolloids or other stabilizer addition.

Table 1. Physicochemical properties, before and after fermentation, of lulo juices at pH 5.5 and 6, non-homogenized and homogenized at 100 MPa and 150 MPa. Mean values ± standard deviation.

			pH	Brix	ρ (g/cm^3)	% Pulp 365× *g*	Transmit. 365× *g*	% Pulp 3000× *g*	Transmit. 3000× *g*
Before fermen.	N-HPH	pH 5.5	5.53 ± 0.01 ^{g}	8.33 ± 0.12 ^{fg}	1.048 ± 0.011 ^{a}	2.79 ± 0.17 ^{cd}	13.7 ± 2.2 ^{a}	1.13 ± 0.03 ^{c}	51.3 ± 0.6 ^{b}
		pH 6	6.04 ± 0.01 ^{h}	8.97 ± 0.06 ^{i}	1.055 ± 0.007 ^{ab}	3.3 ± 0.3 ^{e}	13 ± 3 ^{a}	1.35 ± 0.11 ^{ef}	49.2 ± 0.5 ^{b}
	100 MPa	pH 5.5	5.52 ± 0.01 ^{f}	8.43 ± 0.12 ^{g}	1.071 ± 0.015 ^{ab}	2.8 ± 0.3 ^{cd}	16.7 ± 2 ^{ab}	1.41 ± 0.07 ^{f}	64 ± 2 ^{d}
		pH 6	6.12 ± 0.01 ^{i}	8.67 ± 0.15 ^{h}	1.062 ± 0.011 ^{ab}	2.28 ± 0.14 ^{b}	12.64 ± 0.12 ^{a}	1.34 ± 0.10 ^{f}	54.7 ± 0.5 ^{c}
	150 MPa	pH 5.5	5.51 ± 0.01 ^{f}	8.07 ± 0.06 ^{de}	1.061 ± 0.017 ^{ab}	2.34 ± 0.08 ^{b}	13 ± 2 ^{a}	1.07 ± 0.06 ^{c}	54.6 ± 0.6 ^{c}
		pH 6	6.21 ± 0.01 ^{j}	8.17 ± 0.06 ^{ef}	1.05 ± 0.03 ^{a}	2.24 ± 0.03 ^{b}	22 ± 2 ^{c}	0.82 ± 0.06 ^{b}	74 ± 2 ^{f}
After fermen.	N-HPH	pH 5.5	4.81 ± 0.01 ^{a}	7.93 ± 0.12 ^{cd}	1.074 ± 0.001 ^{ab}	3.0 ± 0.3 ^{de}	19 ± 5 ^{bc}	1.11 ± 0.07 ^{c}	72.5 ± 1.1 ^{f}
		pH 6	5.19 ± 0.01 ^{d}	8.33 ± 0.15 ^{fg}	1.08 ± 0.04 ^{b}	2.42 ± 0.13 ^{b}	14.1 ± 0.6 ^{a}	1.16 ± 0.05 ^{cd}	65 ± 0.95 ^{d}
	100 MPa	pH 5.5	4.86 ± 0.01 ^{b}	7.80 ± 0.02 ^{bc}	1.069 ± 0.012 ^{ab}	3.16 ± 0.01 ^{e}	20 ± 2 ^{bc}	1.56 ± 0.02 ^{g}	80.8 ± 0.97 ^{g}
		pH 6	5.23 ± 0.01 ^{e}	8.03 ± 0.06 ^{de}	1.073 ± 0.009 ^{ab}	2.54 ± 0.18 ^{bc}	14.2 ± 0.2 ^{a}	1.39 ± 0.07 ^{f}	38 ± 2 ^{a}
	150 MPa	pH 5.5	4.96 ± 0.01 ^{c}	7.6 ± 0.12 ^{a}	1.072 ± 0.016 ^{ab}	2.75 ± 0.01 ^{cd}	22.7 ± 0.4 ^{c}	1.27 ± 0.07 ^{de}	69.2 ± 1.4 ^{e}
		pH 6	5.24 ± 0.01 ^{e}	7.7 ± 0.13 ^{ab}	1.063 ± 0.004 ^{ab}	1.28 ± 0.11 ^{a}	32 ± 2 ^{d}	0.67 ± 0.02 ^{a}	83.8 ± 1.1 ^{h}

Values with different superscript letters in a column are significantly different ($p \leq 0.05$).

The results of pulp content and transmittance at the two centrifugation speeds show a higher percentage of pulp at 365× *g* and a higher transmittance at 3000× *g*. The lower centrifugation speed allows the less stable and larger particles to settle while keeping the smaller particles in suspension. The smaller particles will sediment at 3000× *g*, considerably increasing the transmittance of the supernatant.

Fermentation did not affect the percentage of pulp but did increase the transmittance especially in the samples treated at 150 MPa (e.g., 22.7 ± 0.4 > 13 ± 2 or 83.8 ± 1.1 > 74 ± 2). In the samples homogenized at 100 MPa, the effect was small at low centrifugation speeds and non-existent at higher speeds.

Both pH and homogenization pressure had a significant effect on suspended pulp and turbidity at the two centrifugation speeds. However, the way in which the HPH affected both variables was conditioned by the pH of the juice. At pH 5.5, the centrifugation speed of 365× *g* did not allow to detect the changes that the application of 100 MPa caused in the suspended pulp and turbidity (e.g., 2.79 ± 0.17 ≈ 2.8 ± 0.3). However, increasing the centrifugation speed did detect an increase in the percentage of settled pulp (1.13 ± 0.03 < 1.41 ± 0.07) and in the transmittance of the supernatant (51.3 ± 0.6 < 64 ± 2). The application of a HPH of 150 MPa did induce detectable changes at both centrifugation speeds. It appears that the changes induced by the 100 MPa pressure occurred on the more stable pulp and, therefore, require a higher centrifugation speed to be detected. However, when the 150 MPa pressure is applied, the percentage of pulp decreases and the supernatant transmittance decreases, reflecting an increase in the stability of the juice cloud.

At pH 6, the effect of HPH treatment is more pronounced following the same trend as at pH 5.5 when the pressure is 100 MPa but decreasing the percentage of pulp and increasing the transmittance when the pressure is 150 MPa. Moreover, in this case, the changes can be associated with an increase in the stability of the juice cloud. The particles remaining in suspension may be small enough to allow more light to pass through, thus confirming the increase in transmittance.

One of the main applications of high homogenization pressures in fruit juices is to achieve pulp stabilization so that the pulp remains in suspension instead of settling [38]. In a study by Betoret et al. [25] analyzing transmittance in mandarin orange juices, it was observed that the turbidity of the juices increased when higher homogenization pressures were applied. Welti-Chanes et al. [39] observed the same effect in orange juice. In other cases, because of chemical changes and the increased contact surface between smaller particles generated by the application of high homogenization pressures, they may aggregate and precipitate more easily, decreasing the stability of the juice cloud [37,40]. The decrease in the percentage of pulp observed at a pressure of 150 MPa in the lulo juice makes it possible to rule out this phenomenon.

It is widely referenced in scientific literature that during HPH treatment in fruit juices, shear stress causes the disruption of suspended particles and a reduction of particle size. Moreover, different works have described an asymptotic behavior; higher pressures cause less and less changes in particle diameter [37,41]. Thus, it indicates that disruption occurs preferentially in larger particles and cell clusters, with the small particles and cell fragments less susceptible to subsequent disruptions. This effect has been verified in lulo juice after evaluating the particle size distribution by using light scattering technique (Table 2).

Table 2. Main parameters of particle size, before and after fermentation, of lulo juices at pH 5.5 and 6, non-homogenized and homogenized at 100 MPa and 150 MPa. D[4,3]: volume-weighed mean diameter; D[3,2]: surface based mean diameter; d10, d50, and d90: characteristic diameters below which 10%, 50%, and 90% of the particles are included in the distribution. Mean values ± standard deviation.

			D[4,3]	D[3,2]	d10	d50	d90
Before fermen.	N-HPH	pH 5.5	234 ± 10^{h}	76.5 ± 1.3^{fg}	62.71 ± 0.95^{a}	176 ± 3^{g}	465 ± 27^{g}
		pH 6	220 ± 8^{g}	76.3 ± 1.2^{cd}	64.1 ± 0.9^{a}	169 ± 2^{f}	420 ± 28^{h}
	100 MPa	pH 5.5	83 ± 2^{de}	31.4 ± 1.2^{cd}	14.4 ± 0.8^{b}	69 ± 2^{d}	172 ± 3^{e}
		pH 6	80.1 ± 0.9^{cd}	29.58 ± 0.12^{ef}	13.8 ± 0.2^{c}	65.1 ± 1.1^{c}	170 ± 2^{de}
	150 Mpa	pH 5.5	69.7 ± 1.2^{b}	27.6 ± 0.5^{bc}	12.0 ± 0.3^{d}	59.0 ± 0.8^{b}	144 ± 2^{c}
		pH 6	56.8 ± 1.2^{a}	20.8 ± 0.2^{h}	8.86 ± 0.14^{g}	41.4 ± 0.5^{a}	128 ± 3^{a}
After fermen.	N-HPH	pH 5.5	220 ± 6^{h}	73.4 ± 0.9^{de}	61.7 ± 0.6^{a}	177 ± 2^{g}	460 ± 13^{f}
		pH 6	214 ± 7^{f}	72.7 ± 0.8^{b}	61.2 ± 0.7^{a}	170 ± 2^{f}	412 ± 15^{f}
	100 Mpa	pH 5.5	88 ± 2^{e}	30.3 ± 1.2^{cd}	13.2 ± 0.7^{c}	73 ± 2^{de}	184 ± 3^{d}
		pH 6	74.8 ± 1.3^{bc}	25.5 ± 0.3^{g}	11.9 ± 0.2^{f}	59.7 ± 0.8^{b}	160 ± 3^{c}
	150 Mpa	pH 5.5	75.4 ± 1.3^{bc}	26.4 ± 0.5^{fg}	10.96 ± 0.20^{e}	62.0 ± 1.2^{b}	160 ± 3^{b}
		pH 6	72 ± 2^{b}	25.5 ± 0.9^{a}	13.2 ± 0.5^{f}	63 ± 2^{b}	144 ± 4^{d}

Values with different superscript letters in a column are significantly different ($p \leq 0.05$).

pH, HPH treatment, and fermentation had a significant effect on all parameters related to particle size. However, the most important changes were due to the HPH treatment; all parameters decreased as HPH pressure increased. pH and fermentation had a much weaker effect, slightly decreasing particle size.

The data shown in Table 2 correspond in all cases to monomodal distribution curves with a single peak and a similar narrow distribution. The maximum corresponds to a diameter of about 210 µm, 105 µm, and 93 µm for the samples non-homogenized and those homogenized at 100 and 150 Mpa, respectively. These values decreased to about 200 µm, 100 µm, and 90 µm after fermentation with *Lactobacillus reuteri*. Although other authors [37,41] observed narrower distributions with increasing homogenization pressure, this effect was not observed for lulo juice. It is probable that the filtration step in the processing provided a more homogeneous juice as compared to non-filtered fruit juices.

Table 3 shows the results of the CIE-L*a*b* coordinates and the psychrometric h^*_{ab} and C^*_{ab} coordinates of the lulo juices before and after fermentation and as a function of homogenization pressure applied and pH. To better explain the effect of pH, HPH treatment, and fermentation with *Lactobacillus reuteri*, color coordinates for fresh lulo juice published by Hinestroza-Córdoba et al. [17] have been added to the Table 3.

Table 3. CIE-L*a*b*, h^*_{ab} and C^*_{ab} coordinates of the lulo juices before and after fermentation and as a function of homogenization pressure applied and pH. Mean values ± standard deviation.

			L*	a*	b*	h^*_{ab}	C^*_{ab}
Before fermen.	N-HPH	pH 5.5	5.91 ± 0.06^{f}	25.8 ± 0.3^{d}	26.5 ± 0.3^{d}	44.27 ± 0.02^{de}	77.08 ± 0.5^{d}
		pH 6	5.37 ± 0.02^{c}	23.12 ± 0.14^{b}	23.73 ± 0.13^{b}	44.25 ± 0.01^{d}	76.92 ± 0.19^{cd}
	100 MPa	pH 5.5	5.23 ± 0.02^{b}	24.7 ± 0.3^{c}	25.3 ± 0.3^{c}	44.37 ± 0.02^{g}	78.0 ± 0.4^{f}
		pH 6	6.15 ± 0.03^{g}	24.8 ± 0.3^{c}	25.3 ± 0.3^{c}	44.15 ± 0.01^{a}	76.1 ± 0.4^{a}
	150 MPa	pH 5.5	5.70 ± 0.10^{e}	25.8 ± 0.8^{d}	26.4 ± 0.8^{d}	44.32 ± 0.02^{f}	77.5 ± 1.2^{e}
		pH 6	3.89 ± 0.02^{a}	19.46 ± 0.05^{a}	19.85 ± 0.06^{a}	44.44 ± 0.01^{i}	78.70 ± 0.07^{h}
After fermen.	N-HPH	pH 5.5	7.16 ± 0.04^{k}	31.23 ± 0.11^{h}	32.08 ± 0.10^{h}	44.233 ± 0.013^{c}	76.80 ± 0.15^{c}
		pH 6	7.12 ± 0.09^{j}	30.4 ± 0.2^{g}	31.2 ± 0.3^{g}	44.236 ± 0.008^{c}	76.8 ± 0.4^{c}
	100 MPa	pH 5.5	6.827 ± 0.006^{i}	30.76 ± 0.03^{gh}	31.51 ± 0.03^{g}	44.311 ± 0.002^{e}	77.48 ± 0.04^{e}
		pH 6	7.63 ± 0.03^{l}	31.75 ± 0.09^{i}	32.65 ± 0.09^{i}	44.196 ± 0.007^{b}	76.49 ± 0.12^{b}
	150 MPa	pH 5.5	6.45 ± 0.06^{h}	29.36 ± 0.02^{f}	30.065 ± 0.006^{f}	44.325 ± 0.014^{f}	77.61 ± 0.02^{e}
		pH 6	5.51 ± 0.02^{d}	26.7 ± 0.17^{e}	27.26 ± 0.17^{e}	44.402 ± 0.006^{h}	78.3 ± 0.2^{g}
Fresh lulo juice (Hinestroza et al., 2021)			40.4 ± 0.4	9.5 ± 0.2	34.9 ± 1.4	36.2 ± 1.3	74.7 ± 0.2

Values with different superscript letters in a column are significantly different ($p \leq 0.05$).

A multifactorial analysis of variance shows a significant effect of pH, HPH, and fermentation on color coordinates, with fermentation being the variable with the largest significant effect.

To understand these effects, the structural characteristics of the fruit juices and the effect of the treatments on them should be considered. Fruit juices are colloidal suspensions integrated by the insoluble pulp dispersed in a viscous solution. Pulp is constituted of cell fragments including insoluble polymers and molecules. The viscous solution is an aqueous solution of soluble polysaccharides, sugars, salts, and acids. The interactions within each phase and between them will determine physical properties such as the color. Since pH, HPH, and fermentation affect both aqueous and pulp phases, they also influence the color.

It can be observed that the brightness of the juices drops when sodium bicarbonate is added to adjust the pH and HPH treatment is applied (5.91 ± 0.06 in N-HPH juice vs. 3.89 ± 0.02 in 150 MPa treated juice). This effect is associated with an increase in suspended particles and with an increased cloud stability. In addition, after fermentation and regardless of HPH treatment, the color coordinate a* undergoes a noticeable increase in color intensity towards red ($31.23 \pm 0.11 > 25.8 \pm 0.3$ in N-HPH juice), and the color coordinate b* also shows a slight increase reflecting a gain of yellow color intensity ($32.08 \pm 0.10 > 26.5 \pm 0.3$). In relation to the value of h^*_{ab}, it can be observed that it is higher than that of the fresh lulo juice, which is consistent with the redder shades reflected by the coordinate a*. Finally, the psychrometric coordinate that refers to the chromaticity (C^*_{ab}), which measures the purity and saturation of color, underwent slight changes with the pH adjustment, the HPH treatment, or the fermentation step.

Figure 3 shows total phenols content, flavonoids content, and antiradical capacity measured by DPPH and ABTS methods of the lulo juices before and after fermentation and as a function of the homogenization pressure applied and the pH.

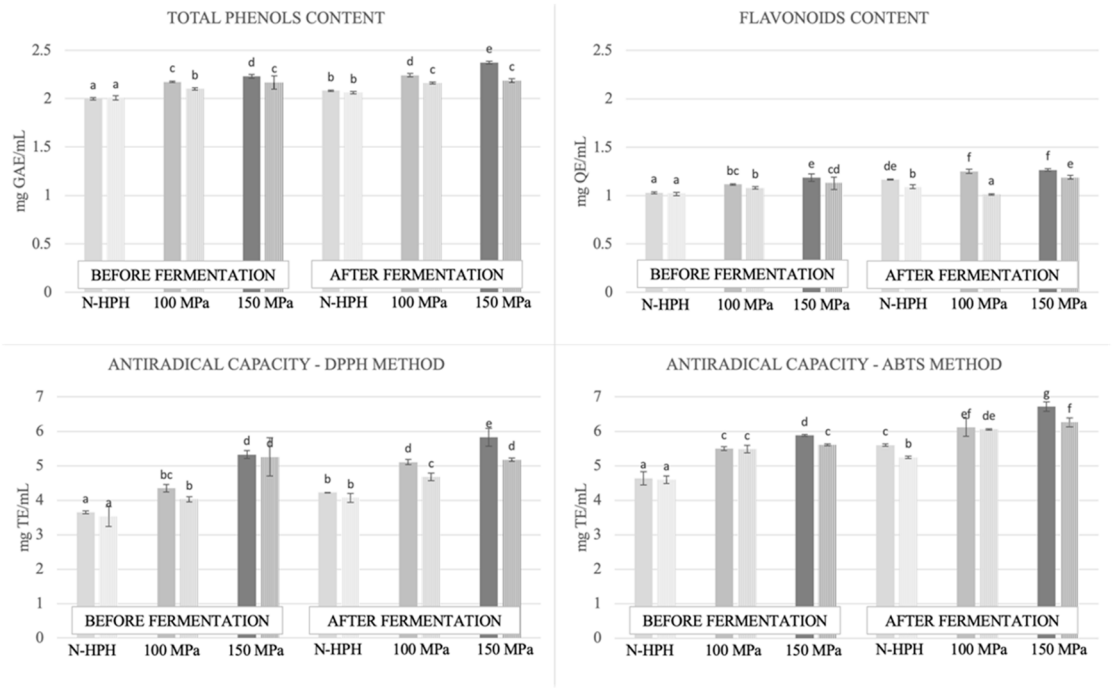


Figure 3. Total phenols content, flavonoids content, and antiradical capacity by DPPH and ABTS methods of the lulo juices before and after fermentation and as a function of homogenization pressure applied and pH. Solid bars represent juices at pH 5.5 and the striped bars represent juices at pH 6. Different letters denote statistically significant differences ($p \leq 0.05$).

The content of total phenols and flavonoids in both fermented and non-fermented lulo juice increased as it did the homogenization pressure. In addition, juices at pH 5.5 (solid bars) showed slightly higher contents of these two types of compounds compared to those at pH 6 (striped bars). The effect of the two factors was statistically significant ($p \leq 0.05$).

The mechanical effect of homogenization pressure on suspended particles results, in many cases, in the breakdown of cell structure and the subsequent release of chemical compounds, including phenols and flavonoids, into the aqueous phase making them more accessible to react with radicals [35]. Furthermore, a restriction of enzymatic degradation of phenolic compounds as a consequence of the HPH effect on enzymatic activity may have occurred. Velázquez-Estrada et al. [42] found the same effect on phenols and flavonoids in orange juice.

The results for the antiradical capacity (DPPH and ABTS methods) followed a similar trend as for phenols and flavonoids. The ABTS and DPPH methods determine the antiradical capacity of compounds with different hydrophilic nature: the ABTS radical reacts with compounds of a more hydrophilic nature than the DPPH radical. However, the great diversity of components of different nature with antiradical capacity existing in the juice of lulo resulted in high values in both cases. Moreover, Hinestroza-Córdoba et al. [17] determined the profile of phenolic compounds by liquid chromatography coupled with mass spectroscopy and showed a considerable increase in the diversity of phenolic compounds when applying a homogenization treatment to lulo juice. Considering that the antioxidant capacity of a mixture is not only given by the sum of the antioxidant capacities of each of its components, since the compounds interact with each other and can produce synergistic or inhibitory effects, it would be possible that the increase in the diversity of phenolic compounds is associated, in lulo juice, with an increase in the antiradical capacity of the juices when applying the HPH treatment.

After juice samples fermentation, a significant improvement in the antioxidant properties of the juices was observed. Both total phenol and flavonoid contents increased after the fermentative action of *Lactobacillus reuteri*. Similar results were obtained by Balli et al. [43], who found that *Lactobacillus* increased the content of total phenols in cereals by 30%. The

results were attributed to the release of phenols that were physically or chemically trapped in the soluble and fermentable fiber of the feed.

The antiradical capacity determined by both DPPH and ABTS methods also experienced a significant increase after fermentation, being more evident for the results obtained by ABTS. This increase was positive with the homogenization pressure applied and more pronounced at pH 5.5 than at pH 6. In addition to the release of components with antiradical activity and the generation of secondary metabolites in the fermentation process, the microbial cells themselves may have contributed to the increased antiradical capacity. Although there are no studies demonstrating this activity for *Lactobacillus reuteri*, there is strong evidence of the antioxidant activity of *Lactobacillus plantarum*, *Lactobacillus helveticus*, *Lactobacillus acidophilus*, *Lactobacillus fermentum*, *Lactobacillus casei*, and some bifidobacteria strains [44].

3.2. Effect of Gastrointestinal Digestion on the Viable Cell Content in Lulo Juices at Different pH and Homogenization Pressures

Figure 4 includes viable cell counts and survival rate (Rs) (calculated as explained in Section 2.5) of *Lactobacillus reuteri* along gastric and intestinal stages of the in vitro simulated gastrointestinal digestion process for all the fermented lulo juices. Analysis of variance ($p \leq 0.05$) showed that both the initial pH and the homogenization pressure had a significant effect on the survival of *Lactobacillus reuteri* to the in vitro simulated gastrointestinal digestion process. Furthermore, there was a significant interaction between the two factors.

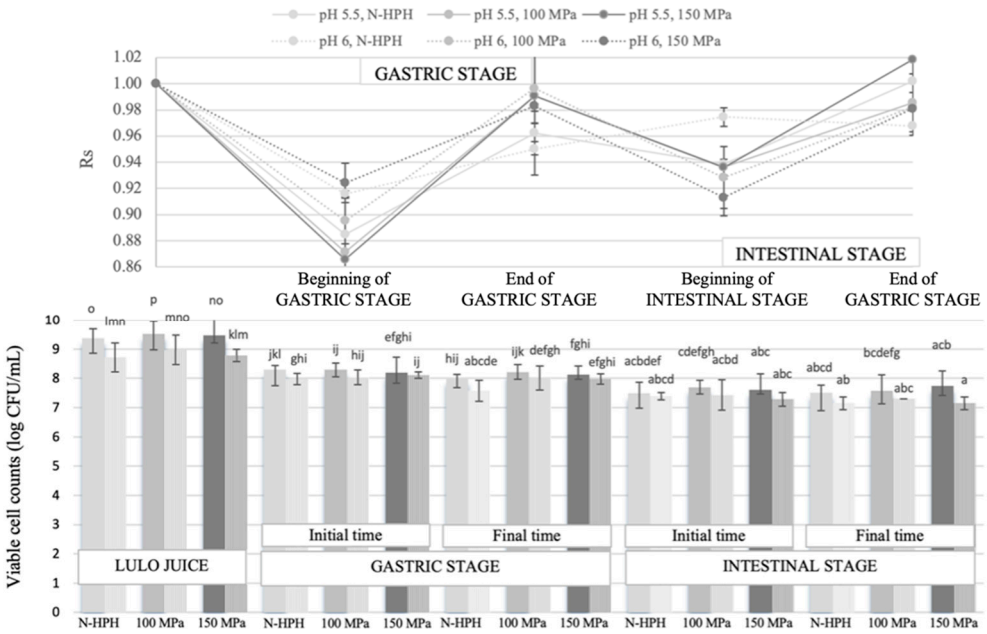


Figure 4. Viable cell counts and survival rate (Rs) of *Lactobacillus reuteri* along gastric and intestinal stages of the simulated gastrointestinal digestion process in vitro. Fermented lulo juices at pH 5.5 and 6, non-homogenized (N-HPH) and homogenized at 100 MPa (100 MPa) and 150 MPa (150 MPa) has been included. The solid bars refer to juices with pH 5.5 and the striped bars refer to juices with pH 6. Different letters denote significant differences ($p \leq 0.05$).

In all cases, the increase in acidity and the presence of pepsin associated with the beginning of the gastric stage caused a decrease in the number of viable cells of up to 1.5 log units. However, at the end of this stage, the viable cell content remained almost constant in the juices subjected to the HPH treatment, and slightly decreased in the case of non-homogenized ones. Similarly, the onset of the intestinal phase caused a pronounced decrease in viable cell content, with less changes 2 h later. This evolution in the viable count during the simulation of the gastrointestinal process is similar to that reported by García-

Hernández et al. [45] for *Lactobacillus reuteri* ATCC 55730 in raw and fried tomato puree. As for the enhancement in the microbial strain survival to simulated gastric conditions in HPH juices, reference should be made to their higher content in both total phenols and flavonoids content and to the polyphenols ability to enhance microorganisms' survivability to gastric conditions by protecting them from oxygen toxicity [45,46].

Microbial cells growth in juices at pH 5.5 showed lower tolerance to the drastic conditions of gastric stage than that growth in juices adjusted to pH 6. However, the microbial cell in juices at pH 5.5 showed better survival rate after 2 h in this stage specially when the HPH treatment was applied. The survival rate in intestinal stage was different being higher for microbial cell in juices at pH 5.5 both at the beginning and at the end of the stage. Only, the non-homogenized juices at pH 6 show a different behavior between the initial and final time of intestinal stage. In relation to the HPH treatment, the highest survival was achieved by cells incubated in juices treated at 150 MPa.

Overall, the best resistance to simulated gastrointestinal digestion was observed in the juice at pH 5.5 and homogenized at 150 MPa with 82% of survival (calculated as the ratio between the microbial concentration at the end of the intestinal stage and the microbial concentration before in vitro digestion, both referred to the same basis), although in the other juices it was only 5% lower, reaching values of 80–81%. In all cases, levels above 7 $\log_{10}$ units per mL of juice were reached at the end of the intestinal stage; these are levels above the minimum necessary for a food to exert a probiotic effect [47]. Islam et al. [48] found a survivability of 84–85% of *Lactobacillus acidophilus* LA-5 in a whey-pineapple beverage (25% whey) at pH 4.3 after simulated gastrointestinal digestion. Moreover, this survival only decreased by 5% after more than 50 days of refrigerated storage. However, in this work, the survival was much higher than that achieved by Calabuig-Jiménez et al. [49] in mandarin juice at pH 3.7 with *Lactobacillus salivarius subsp.salivarius*, which was only 60%.

Among the factors that affect the probiotic bacteria viability in a fruit juice after the gastrointestinal digestion process are the microbial strain and its resistance to acidic conditions and gastric fluids, the fruit juice composition (acidity, carbohydrate content, nitrogen sources, mineral content), and the possible interactions of the probiotic strains with the food matrix component [45]. In this case, it would be both, adaptation of strain to juice acidity and the good nutrient composition of the lulo juice, the main factors responsible for the favorable survival levels obtained after in vitro gastrointestinal digestion. Moreover, the homogenization treatment at 150 MPa would have contributed to the availability of nutrients due to the smaller size of the suspended particles and the greater stability of the cloud.

4. Conclusions

HPH treatment in lulo juice affected, mainly, suspended particles size, with a smaller effect when increasing the pressure from 100 to 150 MPa. This effect on particle size resulted in a juice with greater pulp stability and a different color characterized by a loss of brightness and an increase in color intensity towards red. The HPH treatment also favored the release of components of an antiradical nature, affecting them in a similar way regardless of their hydrophilic or hydrophobic nature.

Regarding the growth of *Lactobacillus reuteri*, it was mainly affected by the pH of the medium, being the rectified juice at pH 5.5 the most appropriated one.

The growth of *Lactobacillus reuteri* slightly modified the physicochemical properties of both non-homogenized and homogenized juices, following the same trend, but mainly increased the antiradical capacity of the juices, possibly due to the release of components with this capacity that were trapped in the soluble and fermentable fiber fraction.

In general, *Lactobacillus reuteri* showed good resistance to in vitro gastrointestinal digestion conditions, reaching levels above 10^7 CFU/mL in all cases. The highest resistance was observed in the juice treated at 150 MPa followed by the juice homogenized at 100 MPa.

Overall, fermentation of lulo juice with *Lactobacillus reuteri* at pH 5.5 and after an HPH treatment of 150 MPa improves the antioxidant properties of the juice and is favorable for providing the bacterial cells in adequate quantities to exert their potential probiotic effect after gastrointestinal digestion. The results could be used to develop beverages with tropical flavors and potential antioxidant and probiotic properties. In vivo studies would be necessary to assess organoleptic acceptability and health effects.

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References

1. Butnariu, M.; Sarac, I. Functional food. *Int. J. Nutr.* **2019**, *3*, 7–16. [CrossRef]
2. Hill, C.; Guarner, F.; Reid, G.; Gibson, G.R.; Merenstein, D.J.; Pot, B.; Morelli, L.; Canani, R.B.; Flint, H.J.; Salminen, S.; et al. The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat. Rev. Gastroenterol. Hepatol.* **2014**, *11*, 506–514. [CrossRef]
3. De Bellis, P.; Sisto, A.; Lavermicocca, P. Probiotic bacteria and plant-based matrices: An association with improved health-promoting features. *J. Funct. Foods* **2021**, *87*, 104821. [CrossRef]
4. Messinger, J.; Lauerer, M. Solanum quitoense, a new greenhouse crop for Central Europe: Flowering and fruiting respond to photoperiod. *Sci. Hortic.* **2015**, *183*, 23–30. [CrossRef]
5. Forero, D.P.; Masatani, C.; Fujimoto, Y.; Coy-Barrera, E.; Peterson, D.G.; Osorio, C. Spermidine derivatives in lulo (*Solanum quitoense Lam.*) fruit: Sensory (Taste) versus biofunctional (ACE-inhibition) properties. *J. Agric. Food Chem.* **2016**, *64*, 5375–5383. [CrossRef]
6. Dahiya, D.K.; Renuka; Puniya, M.; Shandilya, U.K.; Dhewa, T.; Kumar, N.; Kumar, S.; Puniya, A.K.; Shukla, P. Gut microbiota modulation and its relationship with obesity using prebiotic fibers and probiotics: A review. *Front. Microbiol.* **2017**, *8*, 563. [CrossRef] [PubMed]
7. Chakkaravarthi, S.; Aravind, S.M. Fruit juice added with prebiotics and probiotics. *Probiotics Prebiotics Foods* **2021**, *62*, 219–232.
8. Stevens, M.; Vollenweider, S.; Lacroix, C.; Zurich, E.T.H. The potential of reuterin produced by Lactobacillus reuteri as a broad spectrum preservative in food. *Prot. Cult. Antimicrob. Metab. Bacteriophages Food Beverage Biopreserv.* **2011**, 129–160. [CrossRef]
9. Casas, I.A.; Dobrogosz, W.J. Validation of the probiotic concept: Lactobacillus reuteri confers broad-spectrum protection against disease in humans and animals. *Microb. Ecol. Health Dis.* **2009**, *12*, 247–285.
10. Francavilla, R.; Lionetti, E.; Castellaneta, S.P.; Magistà, A.M.; Maurogiovanni, G.; Bucci, N.; De Canio, A.; Indrio, F.; Cavallo, L.; Ierardi, E.; et al. Inhibition of *Helicobacter pylori* infection in humans by *Lactobacillus reuteri* ATCC 55730 and effect on eradication therapy: A pilot study. *Helicobacter* **2008**, *13*, 127–134. [CrossRef]
11. Petricevic, L.; Unger, F.M.; Viernstein, H.; Kiss, H. Randomized, double-blind, placebo-controlled study of oral lactobacilli to improve the vaginal flora of postmenopausal women. *Eur. J. Obstet. Gynecol. Reprod. Biol.* **2008**, *141*, 54–57. [CrossRef]
12. Anukam, K.C.; Osazuwa, E.; Osemene, G.I.; Ehigiagbe, F.; Bruce, A.W.; Reid, G. Clinical study comparing probiotic Lactobacillus GR-1 and RC-14 with metronidazole vaginal gel to treat symptomatic bacterial vaginosis. *Microbes Infect.* **2006**, *8*, 2772–2776. [CrossRef]
13. Tenorio-Jiménez, C.; Martínez-Ramírez, M.J.; Tercero-Lozano, M.; Arraiza-Irigoyen, C.; Del Castillo-Codes, I.; Olza, J.; Plaza-Díaz, J.; Fontana, L.; Migueles, J.H.; Olivares, M.; et al. Evaluation of the effect of Lactobacillus reuteri V3401 on biomarkers of inflammation, cardiovascular risk and liver steatosis in obese adults with metabolic syndrome: A randomized clinical trial (PROSIR). *BMC Complement. Altern. Med.* **2018**, *18*, 306. [CrossRef]
14. Santos, F.; Wegkamp, A.; De Vos, W.M.; Smid, E.J.; Hugenholtz, J. High-level folate production in fermented foods by the B12 producer Lactobacillus reuteri JCM1112. *Appl. Environ. Microbiol.* **2008**, *74*, 3291–3294. [CrossRef]
15. Perricone, M.; Corbo, M.R.; Sinigaglia, M.; Speranza, B.; Bevilacqua, A. Viability of Lactobacillus reuteri in fruit juices. *J. Funct. Foods* **2014**, *10*, 421–426. [CrossRef]

16. Kubo, M.T.K.; Tribst, A.A.L.; Augusto, P.E.D. *High Pressure Homogenization in Fruit and Vegetable Juice and Puree Processing: Effects on Quality, Stability and Phytochemical Profile*; Elsevier: Amsterdam, The Netherlands, 2021; Volume 3, ISBN 9780081005965.
17. Hinestroza-Córdoba, L.I.; Barrera, C.; Seguí, L.; Betoret, N. Potential use of vacuum impregnation and high-pressure homogenization to obtain functional products from lulo fruit (*Solanum quitoense* Lam.). *Foods* **2021**, *10*, 817. [CrossRef]
18. Patrignani, F.; Lanciotti, R. Applications of high and ultra high pressure homogenization for food safety. *Front. Microbiol.* **2016**, *7*, 1132. [CrossRef]
19. Betoret, E.; Calabuig-Jiménez, L.; Patrignani, F.; Lanciotti, R.; Dalla Rosa, M. Effect of high pressure processing and trehalose addition on functional properties of mandarin juice enriched with probiotic microorganisms. *LWT* **2017**, *85*, 418–422. [CrossRef]
20. Muramalla, T.; Aryana, K.J. Some low homogenization pressures improve certain probiotic characteristics of yogurt culture bacteria and Lactobacillus acidophilus LA-K1. *J. Dairy Sci.* **2011**, *94*, 3725–3738. [CrossRef] [PubMed]
21. Tabanelli, G.; Patrignani, F.; Vinderola, G.; Reinheimer, J.A.; Gardini, F.; Lanciotti, R. Effect of sub-lethal high pressure homogenization treatments on the in vitro functional and biological properties of lactic acid bacteria. *LWT* **2013**, *53*, 580–586. [CrossRef]
22. Minekus, M.; Alminger, M.; Alvito, P.; Ballance, S.; Bohn, T.; Bourlieu, C.; Carrière, F.; Boutrou, R.; Corredig, M.; Dupont, D.; et al. A standardised static in vitro digestion method suitable for food-an international consensus. *Food Funct.* **2014**, *5*, 1113–1124. [CrossRef] [PubMed]
23. ISO 2173:2003. *Fruit and Vegetable Products—Determination of Soluble Solids—Refractometric Method*; International Organisation for Standardization: Geneva, Switzerland, 2003.
24. ISO 758:1976. *Liquid Chemical Products for Industrial Use—Determination of Density at 20 °C*; International Organisation for Standardization: Geneva, Switzerland, 1976.
25. Betoret, E.; Betoret, N.; Carbonell, J.V.; Fito, P. Effects of pressure homogenization on particle size and the functional properties of citrus juices. *J. Food Eng.* **2009**, *92*, 18–23. [CrossRef]
26. Malvern Instruments. *Manual: Sample Dispersion & Refractive Index Guide (Mastersizer 2000) Reference Manual*; Malvern Instruments Ltd.: Malvern, UK, 2000.
27. CIE. *Colorimetry*, 2nd ed.; Technical Report CIE 15.2; Commission Internationale de L'Eclairage: Paris, France, 1986.
28. Singleton, V.L.; Orthofer, R.; Lamuela-Raventós, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods Enzymol.* **1999**, *299*, 152–178.
29. Wolfe, K.L.; Liu, R.H. Apple peels as a value-added food ingredient. *J. Agric. Food Chem.* **2003**, *51*, 1676–1683. [CrossRef]
30. Luximon-Ramma, A.; Bahorun, T.; Soobrattee, M.A.; Aruoma, O.I. Antioxidant activities of phenolic, proanthocyanidin, and flavonoid components in extracts of Cassia fistula. *J. Agric. Food Chem.* **2002**, *50*, 5042–5047. [CrossRef]
31. Kuskoski, E.M.; Asuero, A.G.; Troncoso, A.M.; Mancini-Filho, J.; Fett, R. Aplicación de diversos métodos químicos para determinar actividad antioxidante en pulpa de frutos. *Ciência Tecnol. Aliment.* **2005**, *25*, 726–732. [CrossRef]
32. Stratil, P.; Klejdus, B.; Kubáň, V. Determination of total content of phenolic compounds and their antioxidant activity in vegetables—Evaluation of spectrophotometric methods. *J. Agric. Food Chem.* **2006**, *54*, 607–616. [CrossRef]
33. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef]
34. Messer, J.W.; Johnson, C.H. Total viable counts. Pour plate technique. *Encycl. Food Microbiol.* **2000**, *3*, 2154–2158.
35. Zhou, L.; Guan, Y.; Bi, J.; Liu, X.; Yi, J.; Chen, Q.; Wu, X.; Zhou, M. Change of the rheological properties of mango juice by high pressure homogenization. *LWT* **2017**, *82*, 121–130. [CrossRef]
36. Kubo, M.T.K.; Augusto, P.E.D.; Cristianini, M. Effect of high pressure homogenization (HPH) on the physical stability of tomato juice. *Food Res. Int.* **2013**, *51*, 170–179. [CrossRef]
37. Lacroix, N.; Fliss, I.; Makhlouf, J. Inactivation of pectin methylesterase and stabilization of opalescence in orange juice by dynamic high pressure. *Food Res. Int.* **2005**, *38*, 569–576. [CrossRef]
38. Quan, W.; Tao, Y.; Qie, X.; Zeng, M.; Qin, F.; Chen, J.; He, Z. Effects of high-pressure homogenization, thermal processing, and milk matrix on the in vitro bioaccessibility of phenolic compounds in pomelo and kiwi juices. *J. Funct. Foods* **2020**, *64*, 103633. [CrossRef]
39. Welti-Chanes, J.; Ochoa-Velasco, C.E.; Guerrero-Beltrán, J.Á. High-pressure homogenization of orange juice to inactivate pectinmethylesterase. *Innov. Food Sci. Emerg. Technol.* **2009**, *10*, 457–462. [CrossRef]
40. Augusto, P.E.D.; Ibarz, A.; Cristianini, M. Effect of high pressure homogenization (HPH) on the rheological properties of tomato juice: Time-dependent and steady-state shear. *J. Food Eng.* **2012**, *111*, 570–579. [CrossRef]
41. Silva, V.M.; Sato, A.C.K.; Barbosa, G.; Dacanal, G.; Ciro-Velásquez, H.J.; Cunha, R.L. The effect of homogenisation on the stability of pineapple pulp. *Int. J. Food Sci. Technol.* **2010**, *45*, 2127–2133. [CrossRef]
42. Velázquez-Estrada, R.M.; Hernández-Herrero, M.M.; Rüfer, C.E.; Guamis-López, B.; Roig-Sagués, A.X. Influence of ultra high pressure homogenization processing on bioactive compounds and antioxidant activity of orange juice. *Innov. Food Sci. Emerg. Technol.* **2013**, *18*, 89–94. [CrossRef]
43. Balli, D.; Bellumori, M.; Pucci, L.; Gabriele, M.; Longo, V.; Paoli, P.; Melani, F.; Mulinacci, N.; Innocenti, M. Does fermentation really increase the phenolic content in cereals? A study on millet. *Foods* **2020**, *9*, 303. [CrossRef] [PubMed]
44. Fernandes Pereira, A.L.; Rodrigues, S. Turning fruit juice into probiotic beverages. *Fruit Juices* **2018**, 279–287. [CrossRef]

45. García-Hernández, J.; Hernández-Pérez, M.; Peinado, I.; Andrés, A.; Heredia, A. Tomato-antioxidants enhance viability of *L. reuteri* under gastrointestinal conditions while the probiotic negatively affects bioaccessibility of lycopene and phenols. *J. Funct. Foods* **2018**, *43*, 1–7. [CrossRef]
46. Kemsawasd, V.; Chaikham, P.; Rattanasena, P. Survival of immobilized probiotics in chocolate during storage and with an in vitro gastrointestinal model. *Food Biosci.* **2016**, *16*, 37–43. [CrossRef]
47. Koirala, S.; Anal, A.K. Probiotics-based foods and beverages as future foods and their overall safety and regulatory claims. *Futur. Foods* **2021**, *3*, 100013. [CrossRef]
48. Islam, M.Z.; Tabassum, S.; Harun-ur-Rashid, M.; Vegarud, G.E.; Alam, M.S.; Islam, M.A. In vitro bioaccessibility of isoflavones from a soymilk-based beverage as affected by thermal and non-thermal processing MaríaDevelopment of probiotic beverage using whey and pineapple (*Ananas comosus*) juice: Sensory and physico-chemical properties and pr. *J. Agric. Food Res.* **2021**, *4*, 100144. [CrossRef]
49. Calabuig-Jiménez, L.; Betoret, E.; Betoret, N.; Patrignani, F.; Barrera, C.; Seguí, L.; Lanciotti, R.; Dalla Rosa, M. High pressures homogenization (HPH) to microencapsulate *L. salivarius* spp. salivarius in mandarin juice. Probiotic survival and in vitro digestion. *J. Food Eng.* **2019**, *240*, 43–48. [CrossRef]