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Behavior of *Listeria innocua* Strains Under Pressure Treatment – Inactivation and Sublethal Injury

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The inactivation and sublethal injury of two strains of *Listeria innocua* (one collection strain and one wild strain isolated from beetroot juice) suspended in beetroot juice and in model solutions, after high hydrostatic pressure (HHP) were investigated. Changes within the population assessed by plating count methods of both *L. innocua* strains suspended in a buffer pH 4.0 were more noticeable than in the natural beetroot juice environment. In beetroot juice the lethal effect was reported after 1 min of pressure treatment at 400 MPa for the collection strain. In the case of the wild type strain, exposure to the maximal parameters of the compression process (400 MPa, 10 min) decreased the population number below 1 log (CFU/mL) but did not cause complete injury. The collection strain of *L. innocua* was easier to inactivate in beetroot juice than the strain isolated from this environment. The maximum level of sublethal injury was observed when the cells were suspended in a buffer pH 7.0. Structural damage in cell membranes after HHP processing was observed using a transmission electron microscope (TEM).

INTRODUCTION

Beetroot is a traditional vegetable distributed in many parts of the world and has been used commercially to produce juice and natural pigments. One of the leading red beet producers is Poland, where fresh beetroot juice has nowadays become increasingly popular because of its multiple health benefits, such as anticancer activity and protection against degenerative diseases [Clifford *et al.*, 2015]. Beetroot contains dietary fiber and carbohydrates of a moderate caloric value. It is a rich source of minerals and important vitamins, and therefore it can play an essential role in the composition of a well-balanced diet [USDA Food Composition Database, 2018; Zielińska-Przyjemska *et al*., 2009]. Due to the fact that edible parts of root vegetables have a direct contact with soil, beetroot juice is one of the most contaminated among the commercially available fresh juices and can be a source of undesirable microbiota including pathogenic microorganisms [Sapers, 2003; Sokołowska *et al.*, 2011]. One of the most virulent foodborne pathogens, widely distributed in the natural environment, is *Listeria monocytogenes*. It has been detected in fruit and vegetables that are contaminated by the soil or by manure used as a fertilizer. Among the investigated samples of unpasteurized commercial root vegetable and fruit juices, 29% (n=17) have been reported to contain *Listeria monocytogenes* [Sokołowska *et al.*, 2011]. This pathogen can survive short pasteurization or freezing, and can be resistant to treatment with food preservatives. Moreover, it can grow in acidic foods traditionally considered as of low risk [Jordan *et al.*, 2001]. The infective dose of *L. monocytogenes* depends on the resistance of the individual host. Fresh fruit and vegetables which are consumed without any further thermal treatment, and are contaminated with *L. monocytogens* at more than 100 CFU/g, are considered to pose a direct risk to human health [Commission Regulation 2073/2005]. Exceeding this number is dangerous, especially for people with compromised immunity, children, the elderly and pregnant women, as it may induce listeriosis and even sepsis [Goulet *et al.*, 2008].

The High Hydrostatic Pressure (HHP) is a technology used worldwide for the preservation of various commercial products, including vegetable juices. However, this technology has not yet been implemented on the industrial scale in Poland. HHP allowed reducing counts of microbes responsible for spoilage and for shortening the shelf-life of beetroot juice [Sokołowska *et al.*, 2013, 2014, 2017], while not markedly changing the sensory and nutritional attributes of the product. Mild, non-thermal technologies used in food preservation, apart from the inactivation, trigger the sublethal injury of bacterial cells. Injury caused by high hydrostatic pressure has been observed in many bacterial cells [Patterson *et al.*, 1995; Yang *et al.*, 2012; Sokołowska *et al.*, 2014; Wang *et al.,* 2016]. The mechanism of microbial inactivation by HHP is re-

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lated to the morphological changes in the cell, modification of the cytoplasmic membrane, damage to the genetic mechanism, and adverse biochemical reaction [Hoover *et al.*, 1989]. Changes in the bacterial cell can be reversible or irreversible depending on the level of environmental stress and physiological condition of the cell. Even though the membrane damage plays a major role in HHP inactivation, the partial loss of its functionality does not always lead to cell death. In a consequence, some of the cells in the population will be sublethally injured [Wesche *et al.*, 2009]. However, the sublethally injured cells may reveal increased sensitivity to inhibitors, which are ingredients of selective agar media [Espina *et al.*, 2016]. The injured survivors are able to recover and resume growth if suitable environmental conditions emerge, and therefore may become dangerous to customers [Mackey *et al.*, 2000]. This is the reason why appropriate identification and quantification of the sublethally injured population play a key role in food safety. An indirect method for evaluating the number of sublethally injured cells is the plating technique which utilizes a selective medium with the addition of NaCl [Yuste *et al.*, 2004; Sokołowska *et al.* 2014], because immediately after HHP processing the damaged cells have no, or a lower, ability to grow on this medium.

This work describes results of the investigation of the survival, sublethal injury, and diversity of the resistance of *Listeria innocua* strains in pasteurized beetroot juice and in model solutions: buffers pH 4.0 and 7.0, after high hydrostatic pressure treatment.

MATERIALS AND METHODS

Microorganisms and growth conditions

Listeria innocua was used in this study. This bacteria is physiologically very close to the previously mentioned *L. monocytogenes*, and is frequently found in the same food products, therefore it is often used for experiments [Escolar *et al.,* 2017].

Two strains of *Listeria innocua* were used in this study: CIP80.11T obtained from the Culture Collection of the Institut Pasteur (Paris, France) and 23/2013 (wild type strain) isolated from unpasteurized Polish beetroot juice obtained from the own collection of the Department of Fruit and Vegetable Product Technology at IAFB (Warsaw, Poland). The strains were stored in Cryobank at a temperature below -27°C±3°C. Broth subcultures were prepared by inoculating a tube containing 10 mL of sterile Brain Heart Infusion (BHI) broth (BioMerieux, I'Etoile, France) with a pure culture immobilized on sterile beads. After inoculation, the tubes were incubated at 37°C for 24 h and then each overnight culture was moved with a 0.1 mL loop on a Petri dish with Tryptic Soy Yeast Extract (TSYE) agar (Biocar Diagnostics, Beauvais, France). Next, the culture from the plate with a 0.1 mL loop was added to 250 mL Erlenmeyer flasks containing 200 mL of Tryptic Soy Broth with Yeast Extract (TSBYE) (Biocar Diagnostics, Beauvais, France) to prepare the second subculture, which was incubated at 37°C for 18 h to obtain the stationary phase culture. Then, 10 mL of the second subculture were added to fresh sterile broth (TSB or TSYEB) and incubated at 37°C for 18 h. The cultures were then harvested by centrifugation (4000×*g*, 10 min, 4°C). The sedimented cells were aseptically re-suspended in phosphate-buffered saline (PBS, pH 7.2) and again centrifuged. The washing procedure was repeated twice. After that, model suspensions of *L. innocua* were prepared in PBS (1:9, v/v). Just before HHP treatment, McIlvain buffers (0.1 M citric acid, 0.2 M disodium phosphate) in pH 4.0 and pH 7.0, and beetroot juice were inoculated with *L. innocua* cells in a concentration of about 10^7 CFU/mL and transferred into sterile polyethylene tubes (Sarstedt, Newton, USA) in 13 mL portions in duplicate.

Model suspensions and beetroot juice

McIlvaine buffers pH 4.0 and pH 7.0, and pasteurized beetroot juice, acidified with citric acid to pH from 3.98 to 4.17 (produced by Victoria Cymes, Poland) were used.

HHP treatment

High pressure treatment was performed using a U 4000/65 device (Unipress, Warsaw, Poland). The apparatus was capable of operating up to 600 MPa, at temperatures ranging from −10°C to +80°C. The maximum volume of the treatment chamber was 0.95 L. The pressure--transmitting fluid was distilled water and polypropylene glycol (1:1, v/v). Each two independent samples were treated in two independent cycles. The treatment was performed at pressures of 200 MPa, 300 MPa, and 400 MPa, at 20°C for 1, 5, and 10 min. Pressure of up to 400 MPa was generated in 70–80 s and the release time was 2–4 s. The total process time did not include the come-up and come-down time of pressurization. After the treatment, the samples were removed from the chamber and placed immediately on ice. The control samples were unpressurized.

Plate count analytical methods

The HHP-treated samples were analyzed immediately after processing. Ten-fold serial dilutions in Tryptone Salt broth (Biokar Dignostics, Beauvais, France) of each sample were prepared. Appropriate dilutions of samples were spread on agars. Counts of total viable cells were determined by spread plate on TSYE agar, while TSYE agar supplemented with 5% NaCl (POCh, Gliwice, Poland) was used to determine uninjured cells in the population [Yuste *et al*., 2004]. This concentration of NaCl was estimated in the laboratory as the maximum concentration that did not change the morphology and number of unstressed *L. innocua* cells. The number of sublethally injured survivors was estimated by the difference between the counts of total viable and uninjured cells in the population [Yuste *et al.*, 2004; Espina *et al*., 2016]. Plates with TSYE agar were incubated for 24 h/37°C, and these with TSYE agar+5% NaCl for 48 h/37°C [Espina *et al*., 2016]. The plates containing less than 300 CFU/mL were selected for counting [Yuste *et al*., 2004].

Cell morphology assessment by transmission electron microscopy (TEM)

After exposure to 400 MPa for 5 min, the bacteria cells in the PBS buffer (pH 7.2) were fixed with 2.5% glutaraldehyde cacodylic buffer and incubated for one hour, then washed with 0.1 M cacodylic buffer. Next, they were postfixed in 1% OsO_4 in ddH_2O for 1 h and washed three times in ddH_2O. Af-

ter postfixation, the samples were dehydrated through a graded series of EtOH (30% – 10 min, 50% – 10 min, 70% – 24 h, 80% – 10 min, 90% – 10 min, 96% – 10 min, anhydrous EtOH – 10 min, acetone – 10 min) and infiltrated with epon resin in acetone (1:3 – 30 min, 1:1 – 30 min, 3:1 – 2h), infused twice for 24 h in pure epon resin and polymerized at 60°C for 24 h. Next, 60 nm sections were prepared using RMC ultramicrotome MT-X (RMC Boeckeler Instruments, Tucson, USA), contrasted with uranyl acetate and lead citrate according to Reynolds [1983], and examined on LIBRA 120 electron microscope produced by Zeiss (Oberkochen, Germany). Images were captured with the Slow-Scan CCD camera (Proscan) using EsiVision Pro 3.2 software (Soft Imaging Systems GmbH). Measurements were performed using the analySIS® 3.0 image-analytical software (Soft Imaging Systems GmbH, Münster, Germany).

Statistical analysis

The results of survival and sublethal injuries of bacteria were analyzed by two-way ANOVA statistical model with Scheffe's test using Statistica version 13 (TIBCO Software Inc., Palo Alto, CA, USA). Statistical comparison was made for results obtained at different times of the process. The differences were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

Effect of HHP on bacterial cells

The results of the experiment showed that the inactivation and injury of *L. innocua* cells subjected to HHP depended on the origin of the strain, as well as the medium and parameters of the process. Survival rates of the population under the studied conditions and in all media tested are presented in Figures 1–3.

For both strains suspended in beetroot juice, increasing the pressurization time from 1 to 10 min under the pressure of 200 MPa had no significant effect on their survival ($p\geq0.05$) (Figure 1). The maximum reduction was less than 1.1 log (CFU/mL). A higher reduction was achieved when the pressure was increased up to 300 MPa. After 5 min of treatment, the population of the collection strain suspended in beetroot

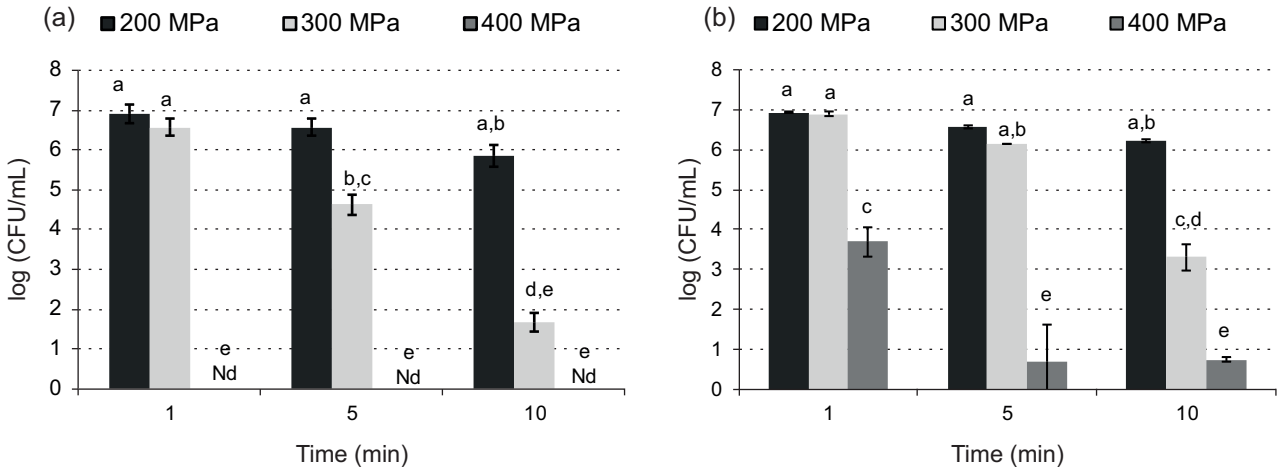

FIGURE 1. Effect of high hydrostatic pressure on the survival of *L. innocua* CIP80.11T (a) and wild type strain 23/2013 (b) in beetroot juice. The bars with different letters are significantly different at $p<0.05$; Nd – not detected.

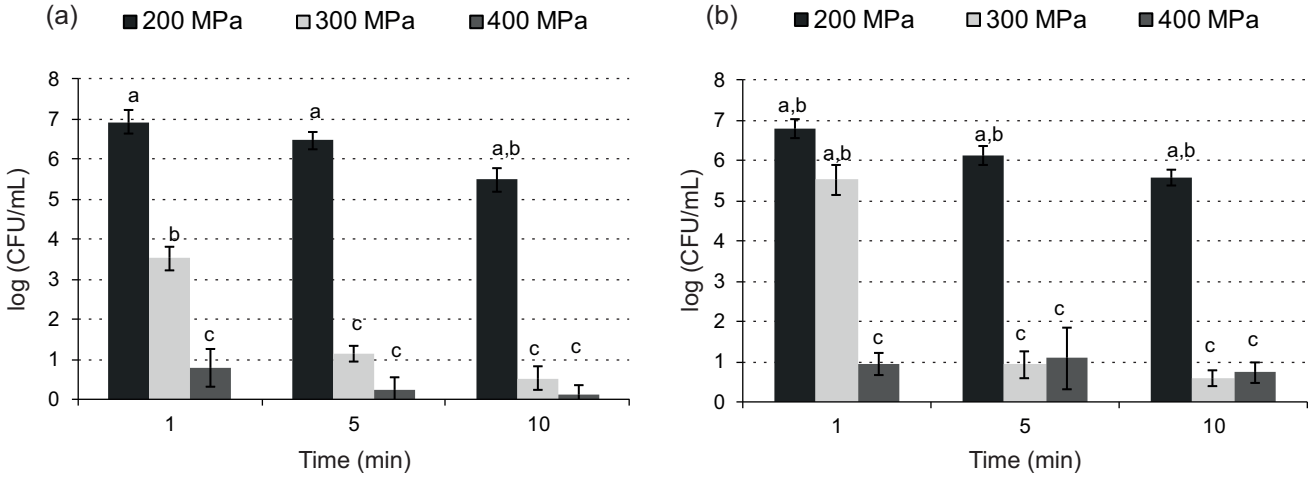

FIGURE 2. Effect of high hydrostatic pressure on the survival of *L. innocua* CIP80.11T (a) and wild type strain 23/2013 (b) in buffer pH 4. The bars with different letters are significantly different at $p<0.05$.

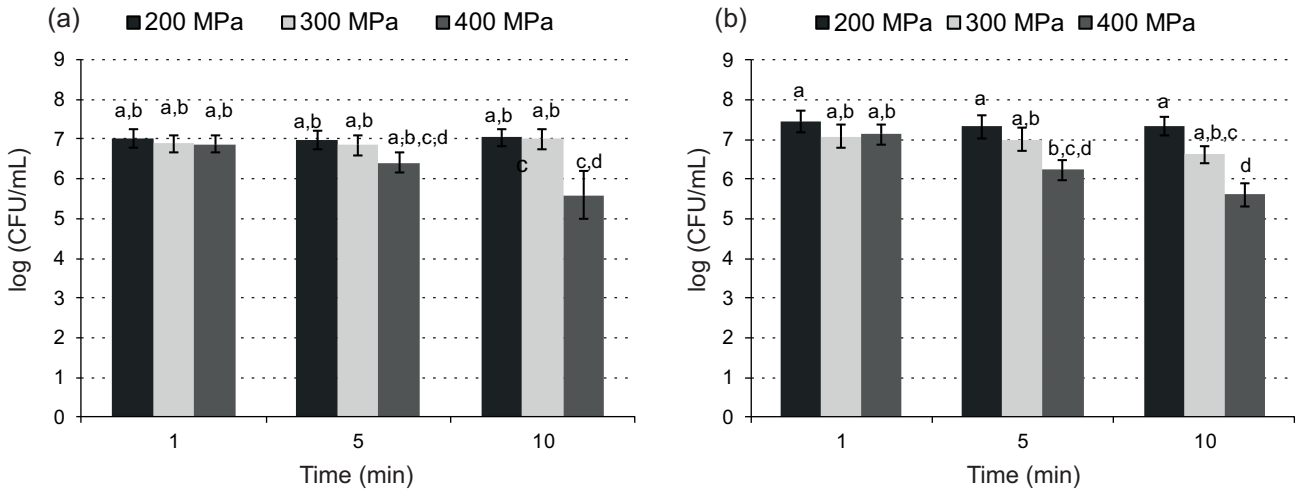

FIGURE 3. Effect of high hydrostatic pressure on the survival of *L. innocua* CIP80.11T (a) and wild type strain 23/2013 (b) in buffer pH 7. The bars with different letters are significantly different at $p<0.05$.

juice decreased by 2.3 log (CFU/mL), while under the same conditions the reduction for the wild type strain was 0.9 log (CFU/mL). When the treatment time was extended up to 10 min, the inactivation of both strains increased by 2.9 log (CFU/mL). In the beetroot juice samples, after the application of 400 MPa for 1 min the collection strain of *L. innocua* was not detected, while the inactivation of the wild type strain was 3.3 log (CFU/mL). Increasing the time of exposure up to 10 min resulted in a significant decrease ($p<0.05$) in the population number of the wild type strain but did not provide its complete inactivation. Our previous studies have shown that the HPP treatment at 400 MPa and 20°C for 10 min of the same beetroot juice (pH 4.18, °Bx 12.35), resulted in 6.2 log (CFU/mL) reduction of *E. coli* ATCC 7839, whereas HPP treatment at 300 MPa and 20°C for 10 min caused about 3.5 log (CFU/mL) reduction of *Saccharomyces cerevisiae* NCFB 3191 [Sokołowska *et al.*, 2013, 2014].

Changes within the population of *L. innocua* suspended in buffer pH 4.0 were more noticeable (Figure 2) than in a natural beetroot juice environment, however there were no significant differences between both strains ($p\geq0.05$). This was most probably due to the presence of molecules, such as lipids and carbohydrates, in product. This modified the effect of HHP on microorganisms, which was confirmed in our previous study [Sokołowska *et al.*, 2013]. After 10 min of the treatment under 200 MPa, the population numbers of the collection and wild type strains decreased by 1.4 and 1.6 log (CFU/mL), respectively. When the samples of the collection strain in an acid model solution were treated under 300 MPa for 1 min, their inactivation reached 3.3 log (CFU/mL). Under the same conditions, the level of reduction of the wild type strain was only 1.6 log (CFU/mL). A decline in the population numbers of both strains at the level of about 1 log (CFU/mL) was observed after 5 min of the treatment. Further enhancement of the process parameters had no significant effect on the studied bacterial populations ($p\geq0.05$). Jofré *et al*. [2010] studied the inactivation of five strains of *L. monocytogenes* of different origins suspended in a complex medium (pH: 5.1; 6.3, 7.4). The results have demonstrated that treatment at 400 MPa for 10 min greatly affected the viability of each strain. We have shown similar findings in an acid model solution. However, the results of bacterial inactivation, in a near-neutral pH environment, were totally different. In our study, the survival rates of *L. innocua* suspended in buffer 7.0 under pressure reaching up to 400 MPa for 5 min have shown no significant differences ($p\geq0.05$) (Figure 3). Maximum inactivation of both strains was observed after the treatment at 400 MPa for 10 min and was below 1.5 log (CFU/mL). On the contrary to our results. Patterson *et al.* [1995] showed 5 log (CFU/mL) reduction of *L. monocytogenes* in a phosphate buffer (pH 7) after the treatment at 375 MPa for 15 min. In another study, it has been reported that the treatment at 207 MPa for 10 min at 25°C, caused a 0.7 log (CFU/mL) reduction in population numbers of two strains of *L. monocytogenes* suspended in a peptone solution (pH 7.2) [Alpas *et al.*, 2000]. Stewart *et al.* [1997] described the effect of HHP on the injury and destruction of two strains of *L. monocytogenes* (Scott A and CA) in buffer suspensions. They observed complete sterility in buffer pH 4.0 in the case of the samples pressurized at 404 MPa for 10 min. However, under the same HHP conditions in buffer 6.0, both strains were reduced by 4.0 log (CFU/mL) and 6.0 log (CFU/mL), respectively. On the other hand, the number of cells of both strains decreased by 5.0 log (CFU/mL) in pH 4.0 and by 3.0 log (CFU/mL) in pH 6.0 upon pressurization at 300 MPa, 25°C for 10 min [Stewart *et al.*, 1997].

Sublethal injury to bacterial cells

Microorganisms are said to be sublethally injured if they survive an inactivation treatment. Some of the damages might be repaired, especially while microorganisms are stored under favorable conditions [Jofré *et al.*, 2010]. Pressure treatment at 300–600 MPa, at ambient temperature for a few minutes destroys pathogenic bacteria, such as *Listeria*, *Escherichia*, *Salmonella*, as well as causes sublethal injuries [Patterson *et al.*, 1995]. However, under these conditions some bacteria are sublethally injured. This phenomenon has been confirmed in our study (Table 1). As aforementioned, survivors may re-

TABLE 1. The level of sublethal injuries of *L. innocua* strains after HHP treatment.

Strains/HHP parameters	200 MPa			300 MPa			400 MPa		
	1 min	5 min	10 min	1 min	5 min	10 min	1 min	5 min	10 min
Sublethal injuries in beetroot juice (log CFU/mL)									
Listeria innocua CIP 80.11T	0.11±0.26^{a}	0.08±0.23^{a}	0.37±0.26^{a}	-0.03±0.27^{a}	0.72±0.26^{a}	0.58±0.69^{a}	Nd^{a}	Nd^{a}	Nd^{a}
Listeria innocua – wild type strain 23/13	-0.02±0.00^{a}	0.01±0.05^{a}	-0.09±0.09^{a}	0.13±0.04^{a}	0.10±0.00^{a}	2.34±0.26^{b}	0.83±0.13^{a,b}	-0.05±1.02^{a}	0.01±0.17^{a}
Sublethal injuries in McIlvain buffer pH 4.0 (log CFU/mL)									
Listeria innocua CIP 80.11T	-0.06±0.27^{a}	0.05±0.25^{a}	0.12±0.22^{a}	0.54±0.29^{a}	0.45±0.67^{a}	0.54±0.00^{a}	0.81±0.00^{a}	0.24±0.00^{a}	0.15±0.00^{a}
Listeria innocua – wild type strain 23/13	-0.19±0.22^{a}	-0.09±0.24^{a}	0.07±0.26^{a}	0.42±0.39^{a}	0.28±0.92^{a}	0.45±0.21^{a}	0.30±0.16^{a}	0.30±1.01^{a}	0.42±0.34^{a}
Sublethal injuries in McIlvain buffer pH 7.0 (log CFU/mL)									
Listeria innocua CIP 80.11T	0.10±0.21^{a}	0.09±0.28^{a}	0.17±0.25^{a}	0.14±0.24^{a}	0.17±0.26^{a}	0.31±0.27^{a}	0.67±0.25^{a,b}	2.64±0.34^{c}	3.83±0.32^{d}
Listeria innocua – wild type strain 23/13	0.04±0.28^{a}	0.08±0.20^{a}	-0.01±0.25^{a}	0.03±0.23^{a}	0.05±0.22^{a}	0.24±0.27^{a}	0.28±0.24^{a}	1.21±0.23^{b}	2.39±0.25^{c}

All data were the mean ± SD, n=2. Values in rows (a-d) denoted with different letter are significantly different at $p<0.05$. Nd: not detected.

veal increased sensitivity to inhibitors, such as sodium chloride [Mackey, 2000]. In our study, it has been observed that the maximum level of sublethal injury occurred when the cells were suspended in buffer pH 7.0 (Table 1) and exposed to the pressure of 400 MPa for 10 min. The level of sublethal injury was 3.83 log (CFU/mL) and 2.39 log (CFU/mL) for the collection strain and wild type strain, respectively. Pressure treatment at 200 MPa and 300 MPa in buffer pH 7.0 caused no significant changes ($p\geq0.05$) in the levels of sublethal injury of bacterial cells. The same observation was made in buffer pH 4.0 ($p\geq0.05$) (Table 1). In turn, beetroot juice samples exposure to 300 MPa for 10 min significantly ($p<0.05$) affected the level of sublethal injury of the wild type strain compared with the collection strain (Table 1). Sokolowska *et al.* [2014] confirmed that the pressure of 400 MPa triggered sublethal injury of *E. coli* cells in PBS. After 5 and 10 min of HHP treatment, 2.4 log (CFU/mL) and 2.7 log (CFU/mL) of sublethally injured cells were observed, while in beetroot juice the counts of injured cells reached 1.5 log (CFU/mL) and 0.8 log (CFU/mL), respectively. On the other hand, the application of 400 MPa for 10 min on five strains of *L. monocytogenes* suspended in a complex medium caused that the number of sublethally injured cells in population was less than 1 log (CFU/mL) [Jofré *et al.,* 2010]. The number of sublethally injured survivors in the population depends on high pressure treatment parameters, as well as on the type of microbiota and medium. In some cases, the level of sublethally injured cells might be almost 100%. The adequate identification and quantification of the sublethally injured population plays an important role in food safety.

TEM observations

The character of sublethal injuries of *L. innocua* triggered by high hydrostatic pressure, was illustrated by TEM microscopy. Changes in *L. innocua* cells morphology are shown in Figure 4. TEM images of untreated samples demonstrated intact, characteristic rod-shaped *L. innocua* cells, single or dividing. Cell membrane and walls were clearly defined with centrally located genome surrounded by the integrated cytoplasmic area and tickly packed ribosomes (Figure 4 a,b). The mechanisms of microbial inactivation by HHP have been mostly associated with the damage of cell membrane, as the major target of pressure treatment. Loss of membrane integrity and swelling leads to the leakage of cellular materials and nucleoid condensation [Hauben *et al.*, 1996; Mañas & Mackey, 2004]. According to plate count results, the reduction of both strains suspended in buffer pH 7.0 after HHP treatment at 400 MPa for 5 min was less than 1 log (CFU/mL). The level of sublethal injury was 2.64 log (CFU/mL) and 1.21 log (CFU/mL) for collection and wild type *L. innocua* strain, respectively (Figure 3). It was coherent with the results which we achieved using the transmission electron microscopy technique. Most of the cells in the population observed by TEM had an intact cell membrane. Only a few cells of *L. innocua* wild type strain have presented surface damage (Figure 4 f). TEM observations confirmed aggregation of cytoplasm. Disorganization of the genome area containing fibrillar regions was observed in all populations of both strains (Figure 4 c,d,e,f). Alterations in the appearance of the interior of the *Escherichia coli* cells were reported after HHP treatment at 300 and 600 MPa for 5 min [Prieto-Calvo *et al.*, 2014] and of *Listeria monocytogenes* cells interior after the treatment at 450 MPa for 5 min [Huang *et al*., 2015]. Monitoring of the cellular ultrastructure by TEM showed the cellular enlargement, disruption of cellular membranes, condensation of the cytoplasmic material and disorganization of the genome area [Huang *et al*., 2015; Prieto-Calvo *et al.*, 2014]. Mackey *et al.* [1994] observed that cells of *L. monocytogenes* treated under 250 MPa were characterized with unusual symmetrical areas in the cytoplasm. These changes were related to the deprivation of ribosomes, resembling gas bubbles, which could have been due to the osmotic effects or phase changes in the membrane. Under the same treatment conditions, amorphous compacted regions were

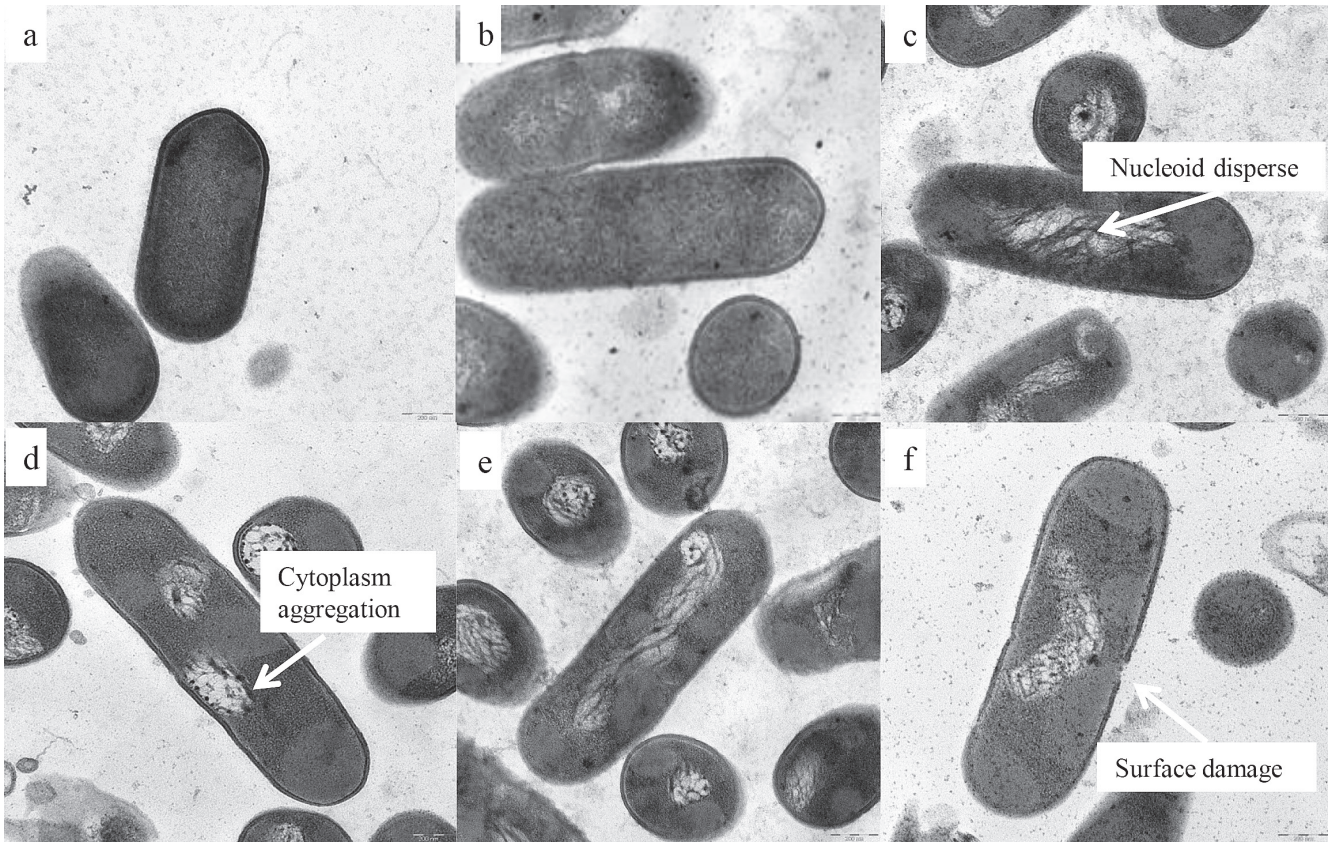

FIGURE 4. TEM images of untreated *L. innocua* strains (a) CIP80.11T and (b) wild type strain 23/2013 and after exposure to 400 MPa for 5 min (c-d) and (e-f) respectively. Scale bar, 200 nm. Representative images of the samples are shown.

noticed in *Salmonella* Thompson. It was probably induced by denaturation of cytoplasmic protein [Mackey *et al.*, 1994]. Increased pressure up to 500 MPa resulted in extreme condensation of the cytoplasm, whilst the outline of the cells was intact [Mackey *et al.*, 1994].

Variation in resistance to HHP

Numerous studies have demonstrated that variations in the resistance of microorganisms to high pressure occurred not only among the different species of bacteria, but also among the strains belonging to the same species [Alpas *et al.*, 1999; Jordan *et al.,* 2001; Boeijen *et al.*, 2010; Huang *et al.*, 2015]. It has been reported that some bacterial strains with very high pressure resistance were isolated from the natural environment. Because of biodiversity of microorganisms, the results that were obtained in different studies varied significantly [Alpas *et al.*, 2000]. The studies that we have conducted on *Listeria*, which was suspended in beetroot juice, showed a certain phenomenon. The collection strain was easier to inactivate than the strain isolated from the natural environment (Figure 1). Moreover, the wild type strain was not completely inactivated in an acidic medium, even being treated in a very harsh way (Figure 2). Alpas *et al.* [1999] studied the variation in pressure resistance among nine strains of *L. monocytogenes*. They observed that after pressure treatment at 345 MPa for 5 min at 25°C, some strains were more resistant to pressure than others. The viability loss of cells ranged from 0.9 to 3.5 log (CFU/mL). In another work, all the survivors of two strains of *L. monocytogenes* suspended in a peptone solution were completely injured after being exposed to the aforementioned factors. The diversity between 24 piezotolerant variants of *L. monocytogenes*, which were resistant to pressure treatment at 350 MPa was examined by Boeijen *et al.* [2010]. Those 24 strains were compared with the wild type strain. In most cases the wild type strain revealed greater sensitivity than the used variants. Most of them were also resistant to other stresses besides HHP, such as high temperature and low pH. Differences among the variants were observed in *e.g.* acid resistance, growth rate or motility. The authors suggested that this population diversity may be essential to the persistence of pathogens such as *L. monocytogenes* in a range of environments [Boeijen *et al.*, 2010].

CONCLUSIONS

It has been confirmed in our study that high pressure can result in the loss of viability of *L. innocua* cells. It was found that the level of reduction by HHP treatment at 20°C in beetroot juice and buffer solutions was strictly dependent on a couple of factors, including: the pressure applied, the duration of the process, as well as the origin of the strain. In spite of the fact that the pH of both media was similar, the survivability of both strains was greater in beetroot juice than in the buffer. It can be explained by the content of organic compounds which are known to be able to produce a protective layer for bacterial cells which could inhibit the effect of pressure treatment.

On an industrial scale, juices are exposed to pressures of 300–600 MPa for a few minutes at 20°C or below. This environment is sufficient to reduce the number of spoilage microorganisms such as: yeast, moulds, and lactic acid bacteria. However, the results of this study have proved that the mentioned above factors are not always sufficient enough to inactivate pathogens and ensure consumer safety.

To attain safe standards of high pressure processed foods, particular attention should be paid to the potential presence of sublethal injured cells. Moreover, baroresistance among microbial species and strains should also be taken into consideration. The conditions of pressure processing should be properly selected for the type of product as well as the expected conditions and duration of storage. It should be particularly taken into account that the possibility of recovery of sublethally injured cells may occur. Therefore, it is worth considering the coupling of HHP and other treatments to ensure microbiological stability and health safety of juices and beverages from beetroots or other root vegetables.

RESEARCH FUNDING

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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