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Research article

Escherichia coli and *Listeria innocua* stability in carrot juice preserved by high hydrostatic pressure

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Abstract: The effect of High Hydrostatic Pressure (HHP) on *Escherichia coli* and *Listeria innocua* in carrot juice was evaluated just after pressurization and during refrigerated storage for 14 days. Samples were processed with different variants of pressure (300, 400, 500) and time (1 min, 5 min, and 10 min). The number of bacteria in the populations was analyzed using plating count methods. Required 5 log reduction was achieved only for *L. innocua* strains starting from 400 MPa for 5 min. *E. coli* strains displayed resistance to pressure, and the maximum reduction achieved was 2 log CFU/mL for the harshest process parameters. Sublethal injuries in the bacterial population were observed for all tested strains. According to two standardized ISO methods, selective conventional agars, TBX, and ALOA were used in the storage test. Additionally, the Thin Agar Layer (TAL) method was applied. In both used methods, the possibilities of recovery were provided. The regeneration was observed exclusively for *L. innocua* strains. The recovery of sublethally injured cells on ALOA and TAL did not differ statistically in every strain. In turn, results obtained for *E. coli* suggest that TBX may underestimate the number of HHP-injured bacteria.

Keywords: carrot juice; high hydrostatic pressure; microbial stability; refrigerated storage

1. Introduction

Consumers demand safe and natural products without additives such as preservatives and

humectants. Therefore, unpasteurized juices are becoming more and more popular among users however, their production poses a lot of challenges for both producers and stockists. Most freshly squeezed vegetable juices characterize by numerous bioactive substances, that provide health benefits and support the fight against many civilization diseases. Moreover, vegetable juice consumption prevents the deficiency of many micronutrients in the human body. The most popular and frequently used component in many juices is the carrot. Carrot juice contains a large concentration of carotenoids, vitamins C, E, B, and phenolic acids, such as p-coumarin and caffeic acid [1–3]. Regrettably, fresh vegetable juices are a reservoir of natural microflora reaching from 10^5 to 10^7 CFU/mL [6]. They may be a fomite of pathogenic microorganisms such as some serotypes of *Escherichia coli*, *Salmonella* sp., *Listeria monocytogenes*, and the intestinal protozoan *Cryptosporidium* [4]. Therefore, are not recommended for consumers with reduced immunity systems. While due to the high amount of spoilage microorganisms, raw unpasteurized juices have limited market potential. The shelf life declared by the producers commonly does not exceed 72 hours, depending on the type of juice. Because of the abovementioned circumstances, food manufacturers were looking for new mild and athermal technologies that extend the shelf life without changing its sensory and nutritional properties. HHP technology complies with the above requirements. It has been widespread in the food industry worldwide and has found applications in numerous products [5–7]. Nowadays, it is widely used in the fruit and vegetable industry around the world. Moreover, HHP is recognized as a "clean label" because no preservatives or additives are required. Although the HHP method was designed to eliminate all undesired microbiota, high pressure may generate a heterogeneous population. The pressure acts on various cellular components and triggers changes in cell morphology [8,9]. Cells with different health statuses may subsist equally at the same time in the food matrix after preservation treatment, ranging from intact to dead, including sublethally injured fractions and subpopulations [10]. The reversible cell condition, determined as sublethal injury, may end variously. One possible option is that sublethally injured cells will recover and repair. However, there is also the other possibility when sublethal injuries result in cell death. The presence of injured microorganisms in food is a significant aspect, especially in the case of products preserved by nonthermal technologies. Moreover, the fact that cells possibly recover during storage may have a potential hazard in the food processing industry. The detection of the whole cells in the population, both sublethally injured and healthy ones are important for correct interpretations of achieved results in food quality and safety microbiological laboratories. Microbiological methods, which detect foodborne pathogens, should be appropriate for detecting normal and injured cells in the population. It was reported that sublethally injured bacterial cells have a lower ability to multiply, extend lag phase, and cannot form a colony under selective conditions [11]. Moreover, not all cells can adopt new conditions at the same time or to the same extent. Supposedly, the next subpopulation may be much more resistant to treatment. Therefore, they initiate resistance mechanisms, and the recovery time may be shortened [10]. Many of the approved methods used for the isolation and enumeration of microorganisms in foods (selective media) do not facilitate the resuscitation of injured microorganisms and, in consequence, defeat their detection. Those selective media such as TBX for *E. coli* and ALOA for *Listeria* sp. are recommended by International Organization for Standardization (ISO) and used in the routine analysis in quality control laboratories. The ideal method should provide for the occurrence of sublethally injured cells in a food sample, and admit estimating whole microorganisms with different health statuses [12]. Kang and Fung [13] have proposed a suitable agar method that meets the abovementioned criteria. The Thin Agar Layer method (TAL) consists of two-layer of the solid medium on a Petri dish (Figure 1). The first bottom

layer is an appropriate selective agar for the enumeration of the target microorganism. While the overhead layer is nonselective agar. The injured microorganisms are inoculated directly on nonselective agar. In the first few hours of incubation, injured cells are possible to repair on the nonselective medium. After that, selective agents diffuse through a nonselective agar and create a selective environment. Those previously regenerated cells interact with selective agents and produce a typical reaction while other microorganisms are inhibited. It has been confirmed that the TAL method enables resuscitation of heat, cold, acid injured microorganisms, such as *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* Typhimurium, *Staphylococcus aureus*, *Yersinia enterocolitica*, [13–17] *Campylobacter coli* [18] or *Vibrio parahaemolyticus* [19].

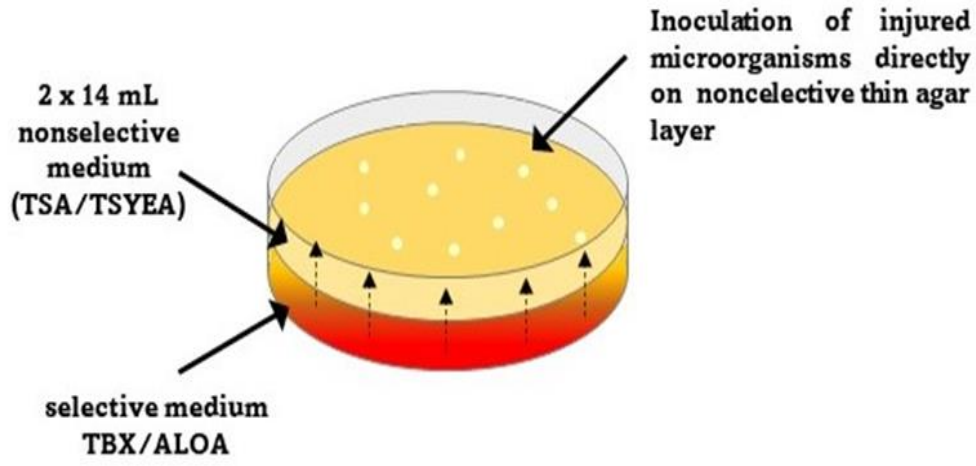

Figure 1. Thin Agar Layer (TAL) scheme.

Many quantitative studies on the high-pressure inactivation of microorganisms have been conducted only with the usage of a nonselective medium. Nonselective agars allow the growth of both non-injured and sublethally injured cells. However, they cannot differentiate target pathogens from a mixed population [12]. Additionally, there is still insufficient knowledge about the behavior of sublethally injured cells during storage in food matrices.

This study aimed to evaluate *E. coli* and *L. innocua* stability of pressurized carrot juice treated at 300, 400, and 500 MPa for three different pressure durations (1 min, 5 min, and 10 min) at ambient temperature. The second purpose of this research was to evaluate the possibilities of regeneration of HHP-sublethally injured cells on conventional selective mediums obligated for certificated laboratories and the usage of the TAL method.

2. Materials and methods

2.1. Tested microorganisms and inoculum culture

E. coli ATCC 7839 (obtained from American Type Culture Collection, Manassas, USA) and *L. innocua* CIP80.11T (obtained from the Culture Collection of the Institute Pasteur, Paris, France) were used in this investigation. Additionally, isolates from unpasteurized, commercial beetroot juice: *L. innocua* 23/13 and *E. coli* 61/14 (obtained from the Department's collection of Fruit and Vegetable Product Technology at IAFB, Warsaw, Poland), were chosen as wild-type strains. Firstly, strains were activated from Cryobanks stored at −27 ± 3 ℃ and grown overnight at 37 ℃ in 10 mL of sterile Brain Heart Infusion (BHI) broths (BioMerieux, I'Etoile, France). Next, cultures were moved with a 10 μL loop on a Petri dish, through streak plate technique with Tryptic Soy (TSA) agar (Biocar Diagnostics, Beauvais, France) for *E. coli* or Tryptic Soy Yeast Extract (TSYE) agar (Biocar Diagnostics, Beauvais, France) for *L. innocua*. Then the culture was added to 250 mL Erlenmeyer flasks containing 200 mL of Tryptic Soy Broth (TSB) (Biocar Diagnostics, Beauvais, France), or Tryptic Soy Broth with Yeast Extract (TSBYE) (Biocar Diagnostics, Beauvais, France) using 10 μL loops to prepare the second subculture. The next steps were as follows: incubation of cultures at 37 ℃ for 18 h; adding 10 mL of the second subculture to the fresh, sterile broth (TSB or TSYEB); incubation of them under the abovementioned conditions. The next process included: harvesting each strain by centrifugation at 4000 × g for 10 min at 4 ℃; washing them three times with phosphate-buffered saline (PBS, pH 7.4), and resuspending them in 10 mL of PBS. Shortly before HHP treatment, carrot juice (Vital Fresh, pH 6.0–6.7) was inoculated with bacterial suspensions in PBS (final concentration was approximately 7 log CFU/mL) and transferred into sterile polyethylene tubes (Sarstedt, Newton, USA) in 13 mL portions in duplicate.

2.2. HHP equipment and process parameters

Samples were pressurized using U 4000/65 device (Unipress, Warsaw, Poland). The detailed description of the HHP device was as follows: maximum volume of treatment chamber—0.95 L; maximum working pressure—600 MPa; working temperature from −10 ℃ to +80 ℃; transmitting fluid—a mixture of distilled water and polypropylene glycol (1:1, v/v); the time needed to obtain the pressure up to 400 MPa—75 s; release time 2–4 s. Due to the adiabatic heating, the temperature increased approximately 3 ℃ per 400 MPa. Nine different combinations including time (1 min, 5 min, 10 min) and pressure (300 MPa, 400 MPa, 500 MPa) were used for any of the tested strains. The pressurization was carried out at temperatures in a range of 18 ℃ to 20 ℃ and reported times do not include the come-up and come-down times. Afterward, two sets of the HHP parameters were selected for further research concerning refrigerated storage as the sublethal high-pressure value: 400 MPa/5 min for *L. innocua* strains and 500 MPa/5 min for *E. coli* strains. Different HHP parameters for species were dictated by their different sensitivity to pressure. Samples were pressurized in two independent cycles.

2.3. Plate count methods

The number of bacterial survivals after HHP treatment was estimated using pure plate methods. The number of all viable cells of *E. coli* and *L. innocua* populations was estimated using nonselective agars, TSA, and TYEA, respectively. Simultaneously, the number of non-injured cells was analyzed

on the same agars, however, they were supplemented with 5% NaCl (w/v). It was a critical concentration of NaCl, that did not affect the number of tested strains [20]. Plates were incubated at 37 ℃ for 24 h or 48 h, and the results were expressed as a colony-forming unit (CFU)/mL. The number of sublethally injured cells was calculated according to the following equation:

$$sublethal\ injuy\ \log number = LOG\ \frac{CFU\ nonselective}{CFU\ selective(5\%NaCl)} \quad (1)$$

Equation 1. The number of sublethally injured cells log CFU/mL.

The number of bacterial cells suspended in carrot juice was enumerated at regular intervals during refrigerated storage. The surface-plated method on conventional selective media was used. The number of *E. coli* strains was estimated using TBX (BioRad, California, USA), according to the procedure included in ISO 16649-2 [21]. The number of *L. innocua* strains was estimated using ALOA (BioMerieux, I'Etoile, France) according to the procedure included in ISO 11290-2 [22]. Additionally, TAL methods were used, and plates were incubated at 37 ℃ up to 48 h.

2.4. Statistical analysis

Statistical analysis of the results was performed by two-way ANOVA statistical model with Tukey's test, using Statistica version 13 (TIBCO Software Inc., Palo Alto, CA, USA). The differences were considered significant at $p < 0.05$. Statistical comparison was made for results, obtained for strains of the same species.

3. Results

3.1. HHP impact on the vitality and sublethal injuries of L. innocua and E. coli suspended in carrot juice

Changes in the *E. coli* and *L. innocua* strains population suspended in carrot juice after HHP MPa up to 10 min. Significant changes ($p < 0.05$) were observed after pressure at 500 MPa for 10 min. The inactivation of the wild-type strain was about 2.0 log CFU/mL, but in the case of collection, the strain did not exceed 1.0 log CFU/mL. Results have shown that *L. innocua* strains were more sensitive to HHP than *E. coli*. After pressurization at 300 MPa, up to 10 min, the reduction for both *L. innocua* strains did not exceed 1.0 log CFU/mL. Above this pressure value changes in the cell number of *L. innocua* populations were observed. Pressurization at 400 MPa for 5 min decreased the number of *L. innocua* collection strains by 4.0 log CFU/mL. In turn, under these conditions inactivation of the wild-type *L. innocua* strain was about 2.8 log CFU/mL. Extending treatment time up to 10 min resulted in no growth of *L. innocua* collection strain. In turn, a further 3 log CFU/mL reduction was observed for the wild-type strain. Exposure to 500 MPa for 1 min reduced the number of collection and wild-type strains by 3.5 CFU/mL and 2.3 CFU/mL, respectively. Treatment time increase to 5 min resulted in an additional c.a. 2.5 log reduction for both *L. innocua* strains. The most rigorous parameters (500 MPa, 10 min) affected no *L. innocua* growth on the plate. The pressure and time both correlated positively with bacterial inactivation in carrot juice. The number of HHP-sublethal injured cells in the bacterial population is shown in Table 1. There were no significant changes in the number of sublethally injured cells within all populations after pressurization at 300 MPa up to 10 min. Pressurization at 400 MPa for 1 min prompted major injuries, exclusively in *L. innocua* collection strains ($p < 0.05$) in the amount

of 1.7 log CFU/mL. In the case of other strains, the number of sublethal injuries did not exceed 1.0 log CFU/mL. A similar effect was observed after 400 MPa for 5 min, however, the number of sublethally injured cells slightly increased for most strains. Extending treatment time to 10 min resulted in 1.0 log CFU/mL and 2.2 number of sublethal injuries in *E. coli* collection and wild-type strain population, respectively. The number of sublethally injured cells of *the L. innocua* wild-type strain was 0.4, whereas the as collection strain was not detected. Pressure at 500 MPa resulted in a significant increase in the level of *E. coli* cell damage which positively correlated with the extension of treatment time. After 5 and 10 min treatment, it was approximately 3.1–3.5 log CFU/mL and 5.5–4.3 log CFU/mL for collection and wild-type strain, respectively ($p < 0.05$) (Table 1). The application of 500 MPa for 1 min induced a similar level of sublethal injures for *L. innocua* collection strain than after 400 MPa for 5 min. Appropriately 1.3 log CFU/mL and 1,2 log CFU/mL was obtained. The same HHP parameters did not significantly affect sublethal damages within the *L. innocua* wild-type strain population. The extension of treatment time did not increase the number of sublethally injured cells, neither collection nor wild-type strain of *L. innocua*. After 10 min treatment at 500 MPa, both *L. innocua* strains were below the detection level (1.0 log CFU/mL).

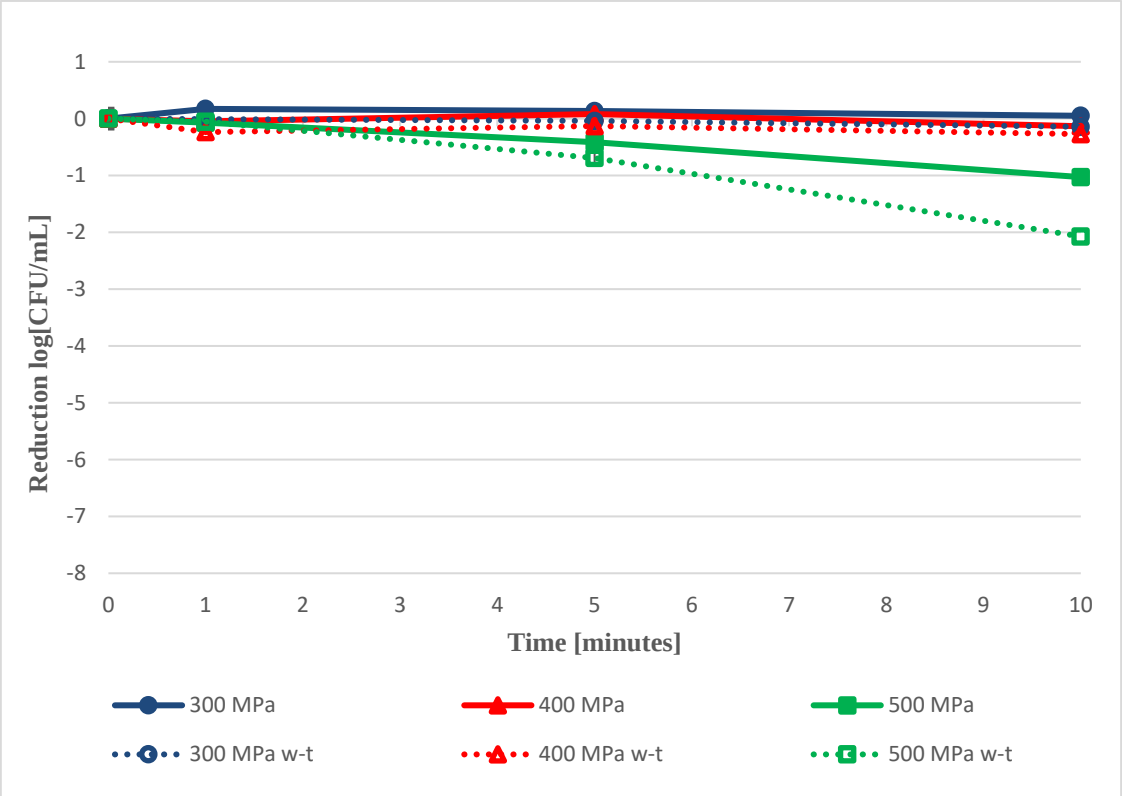

Figure 2. Reduction curves of HHP-treated *E. coli* strains suspended in carrot juice. Closed symbols and continued lines indicate *E. coli* ATCC 7839. Open symbols and dotted lines indicate the results obtained for *E. coli* 61/14 (wild-type).

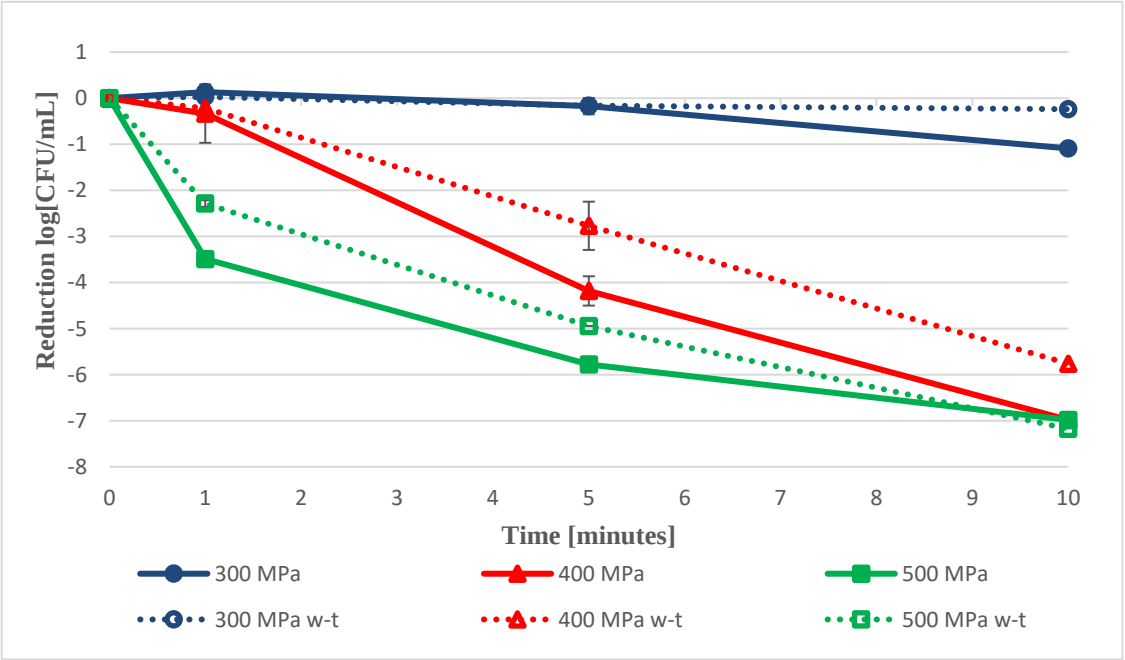

Figure 3. Reduction curves of HHP-treated *L. innocua* strains suspended in carrot juice. Closed symbols and continued lines indicate *L. innocua* CIP80.11T. Open symbols and dotted lines indicate the results obtained for *L. innocua* 23/13 (wild-type).

Table 1. The number of sublethally injured cells of bacterial population suspended in carrot juice.

Strains/parameters	HHP 300 MPa			HHP 400 MPa			HHP 500 MPa		
	1 min	5 min	10 min	1 min	5 min	10 min	1 min	5 min	10 min
E. coli ATCC 7839	0.15 ± 0.00^{a}	0.13 ± 0.00^{a}	0.17 ± 0.01^{a}	0.19 ± 0.04^{a}	0.47 ± 0.00^{ab}	1.01 ± 0.00^{c}	0.35 ± 0.02^{ab}	3.11 ± 0.18^{e}	5.50 ± 0.00^{g}
E. coli 61/14	0.17 ± 0.06^{a}	0.13 ± 0.01^{a}	0.05 ± 0.00^{a}	0.12 ± 0.03^{a}	0.70 ± 0.04^{bc}	2.15 ± 0.00d	0.22 ± 0.11^{a}	3.46 ± 0.38^{e}	4.34 ± 0.00^{f}
L. innocua CIP80.11T	0.11 ± 0.00^{ab}	0.25 ± 0.16^{abcd}	0.14 ± 0.00^{abc}	1.68 ± 0.00^{f}	1.34 ± 0.30^{ef}	Nd^{a}	1.24 ± 0.00^{e}	0.55 ± 0.00^{d}	Nd^{a}
L. innocua 23/13	−0.01 ± 0.02^{a}	0.06 ± 0.04^{a}	0.05 ± 0.00^{ab}	0.23 ± 0.21^{abcd}	0.53 ± 0.00^{cd}	0.37 ± 0.30^{abcd}	0.36 ± 0.04^{abcd}	0.45 ± 0.00^{bcd}	Nd^{a}

All data were the mean ± SD, n = 2. Values in rows (a–g) denoted with a different letter are significantly different at $p < 0.05$, obtained for strains of the same species. Nd: not detected.

3.2. The comparison of conventional selective media and TAL for regeneration of HHP-sublethally injured cells during refrigerated storage

The next aim of this study was to estimate the number of healthy and recovered cells obtained with two different culturing procedures: ISO recommendations and the TAL method. The second purpose was to compare which procedure enables better regeneration of sublethally injured cells. The growth of tested bacteria on conventional selective media, TBX and ALOA, in comparison to TAL, is shown in Figures 4 and 5. The cell number of the HHP-treated *E. coli* collection strain gradually decreased during cold storage. Finally, after 14 days of storage, the decrease was 2.5 log CFU/mL in reference to initial microbial counts, just after HHP treatment. In turn, the level of *E. coli* wild-type strain did not decrease below 6.0 log CFU/mL (Figure 4). Significant differences were observed between the growth of TBX and TAL. In most cases, the number of viable cells on TAL plates was higher than on TBX. The differences between the log CFU/mL of *E. coli* collection strain on TAL and TBX ranged from 0.05 to 0.88. Exclusively on the 14th day of storage, there was an opposite situation in which the recovery was better on TBX. The distinction between colony numbers of *E. coli* wild-type strain on both media ranged from 0.22 to 0.58 log CFU/mL. The number of *L. innocua* population in carrot juice increased during 2-week storage in reference to initial microbial counts (Figure 5). However, there were no significant changes between the results obtained on TAL and ALOA ($p \geq 0.05$). The growth of bacteria on both agars was comparable. The differences between the log CFU/mL on TAL and ALOA did not exceed 0.16 log CFU/mL. Exclusively on day 14th the distinction between colony numbers of *L. innocua* collection strain on TAL and ALOA was 0.71 log CFU/mL.

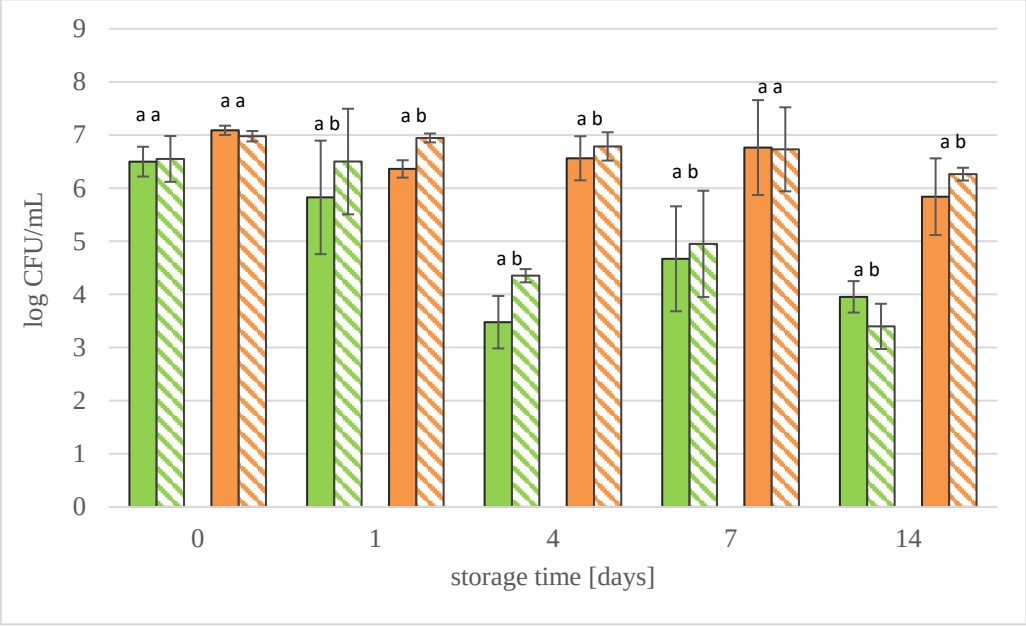

Figure 4. The growth of *E. coli* ATCC 7839, (■) 61/14 (■) in HHP treated (400 MPa/5 min) carrot juice stored at 5 °C for 14 days on different selective media: TBX (plain bars) and TAL (texture bars).

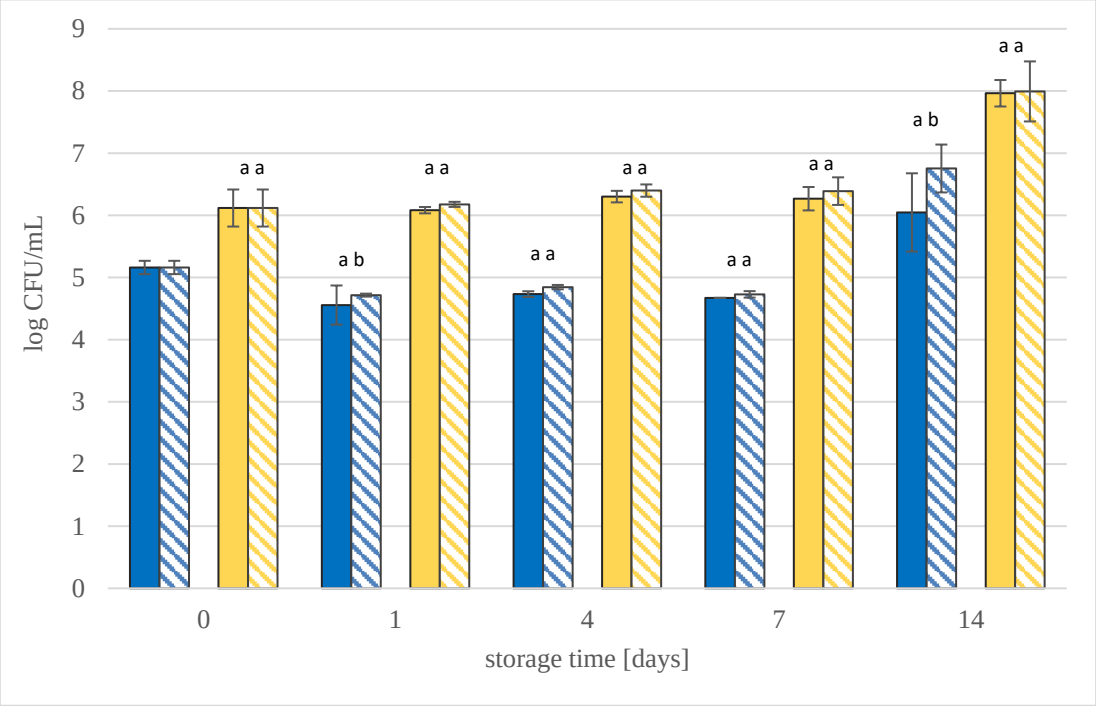

Figure 5. The growth of *L. innocua* CIP80.11T, (■) 23/13 (■) in HHP treated (400 MPa/5 min) carrot juice stored at 5 ℃ for 14 days on different selective media: ALOA (plain bars) and TAL (texture bars).

4. Discussion

4.1. HHP impact on the vitality and sublethal injuries of bacteria

Pokhrel et al. [23] described the effectiveness of HHP (200-500 MPa) on the inactivation of *L. innocua* ATCC 51742 and *E. coli* ATCC 11755 in thermally pasteurized carrot juice (pH 6.4). Similar to our results, pressurization up to 300 MPa was not effective in the inactivation of both microorganisms. Application of pressure at 500 MPa for 2 min resulted in a 4 log CFU/mL reduction of *L. innocua*, corresponding to our findings. However, opposite to our experiment, the 5 log CFU/mL reduction for *E. coli* was observed in these HHP conditions. In another study, Pokhrel et al. [24] showed that 400 MPa for 3 min decreased the number of *L. innocua* ATCC 51742 suspended in carrot-orange juice (pH 6.0) by over 6 log CFU/mL. Similar results were presented by Patterson et al. [25], in which numbers of *L. monocytogenes* decreased more than 6 log CFU/mL in carrot juice after pressurization at 500 MPa for 1 min. Opstal et al. [26] studied the inactivation kinetics of *E. coli* MG1655 in fresh carrot juice treated by HHP. They applied pressure in a range of 150–600 MPa at different temperatures (5–45 ℃). They observed that inactivation kinetics was described by the first-order relationship, which means that all cells in the population have equivalent resistance to the lethal treatment. They reported that 8.6 min HHP treatment is required to achieve a decimal reduction of *E. coli* in carrot juice at 500 MPa at 20 ℃ and 15 min at 400 MPa. Resembling studies had been conducted by Pilavtepe-Çelik et al. [27]. Based on the 5 log reduction simulations, they suggested that carrot juice (pH 6.22) had a protective effect on *E. coli* O157:H7 933, treated by HHP at 300 MPa. This conclusion

corresponds to our achievements in which, it has been demonstrated that there is no reduction of *E. coli* in carrot juice just after HHP treatment at 300 MPa up to 10 min. Additionally, *L. innocua* turned out more sensitive to pressure than *E. coli*. However, our findings do not correspond with the general opinion that Gram-negative bacteria are more sensitive to physical stress than Gram-positive bacteria. Numerous studies confirmed that HHP triggers sublethal injuries of vegetative cells [28,29]. However, there is sparing data concerning this phenomenon in alkaline vegetable juices. The problem of sublethal injuries is minimalized in acidic food matrices, while it occurs when the food matrices have a neutral pH. According to the food legislation in the USA, a 5 log CFU/mL reduction of "pertinent microorganisms" is sufficient to claim preservation treatment [30]. Full commercialization of HHP technology to low acid foods has been realized much later than to acidic products due to a lack of available scientific information concerning the process. Nowadays, according to Juice HACCP Hazards and Controls Guidance, low acid juices, such as carrot juice, need to be distributed under refrigeration [27]. Nevertheless, the sublethal injury phenomenon should be taken into account with the choice of HHP conditions, notably for products that carry a high risk of dangerous pathogens. Scientists suggest that survivability depends not only on the strain and the device's parameters. Food matrices have an impact on the result and exhibit either a protective or sensitizing effect on microorganisms [27].

4.2. The influence of culture media and long-term storage on the regeneration of HHP-sublethally injured bacterial cells

The study of pathogens injury induced by HHP and the effect of storage on their recovery has been reported by several groups of researchers [25,31–33], however, most of them are concerned with fruit juices. Carrot juice preserved by HHP has been well examined just after processing as well as during storage, but in the most of research total, natural microbiota has been investigated, not strictly pathogens. Patterson et al. [25] reported that the number of pressurized (500 MPa/1 min) *E. coli* cells in carrot juice decreased during refrigerated storage, whereas after 10 days, it was under detectable level. A similar observation has been made for a cocktail of *L. monocytogenes* under the same HHP conditions, while bacteria reached an undetectable level in 14 days of subsequent refrigerated storage. Contrary to the abovementioned studies, we observed propagation of *L. innocua* in pressurized (400 MPa, 5 min) carrot juice, stored at 5 ℃ for 2 weeks. This phenomenon was not observed for *E. coli*. The number of *E. coli* strains slowly decreased or was stable during refrigerated storage of carrot juice, referring to initial cell counts. Pokhrel et al. [23] carried out the shelf-life studies of freshly squeezed carrot juice. The total plate counts were about 1 log CFU/mL in carrot juice after HHP (500 MPa, 20 ℃). The number of microorganisms increased up to 3 logs CFU/mL after 28 days of refrigerated storage. Szwajgier et al. [34] reported that pressurization at 500 MPa for 15 min at room temperature was insufficient to eliminate psychrotrophic bacteria in beet/carrot (80/20 v/v) juice. Despite juice pH being 4.0, the number of microorganisms increased by over 5.5 log CFU/mL after 3 weeks of refrigerated storage. There are numerous studies in which pressure treatment of low pH products and subsequent cold storage prevented the outgrowth and caused further inactivation of the sublethally injured vegetative cells [32,35,36]. However, in our investigation pH of carrot juice did not decrease below 6.0 during 14-days of refrigerated storage. It was measured at the same intervals as plate count analysis was made. There are numerous accessible research data relating to TAL application for the enumeration of pathogens preserved by physical and chemical treatments [13–18,37,38]. Those studies

confirmed that TAL allowed for improved isolation of single colonies for pure and mixed cultures. However, there are only a few data for the survivors' evaluation after HHP using TAL. Lavieri et al. [39] studied the recovery of pressured and heat-inured cultures of mixture *L. monocytogenes* in TSYEB. The obtained results demonstrated that the recovery of thermally injured cells was significantly better on TAL than on selective medium (MOX). This phenomenon was not observed for pressure-injured cells. It corresponds to our achievements for pressure-treated *L. innocua* strains. In turn, there was a significant difference between colony numbers of *E. coli* strains on TAL and TBX in our study. The abovementioned results demonstrated that selective media, used for microorganisms enumeration and detection, might not be suitable for the recovery of HHP-injured cells for all types of microorganisms. Selective media can underestimate its presence and be especially dangerous in the case of foodborne pathogens. The essential point of previously mentioned studies is that the TAL method has been used to recover injured cells after preservation treatment. Moreover, it provided typical colony morphologies of target pathogens and color reactions on the respective agars. However, there is no report of the TAL application in the storage investigation.

5. Conclusion

The results indicate the lethality of *L. innocua* bacteria in carrot juice after HHP treatment at ambient temperature. In turn, the application of 500 MPa for 10 min on *E. coli* strains did not meet the FDA's required inactivation criteria (>5-log CFU/mL reduction). Carrot juice is a complex matrix because of its non-acid environment. Hence, numerous studies confirmed that unsupported HHP treatment is unsuitable for carrot juice preservation and subsequent long-term storage. Only hurdle technology or combining procedures with other treatments or others additives may bring the expected effect. Second, a remarkable observation is that sublethally injured cells of *L. innocua* may resuscitate in carrot juice during cold storage. TAL effectively recovered bacteria injured by HHP. However, the effectiveness of the TAL method may be specific to the type of applied injury and species of microorganism. In conclusion, this study will contribute to collecting the data on the regeneration of HHP-sublethally injured cells on a conventional selective medium recommended by ISO versus TAL.

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Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of the article.

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