Review

Factors Affecting Microbial Inactivation during High Pressure Processing in Juices and Beverages: A Review

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ABSTRACT

The purpose of this article is to review and discuss the factors affecting high pressure processing (HPP) in juices and beverages. The inactivation of microorganisms by HPP depends on numerous factors, including the magnitude of the pressure and the holding time, process temperature, compression and decompression rates, the microbiota, and the intrinsic properties of juices and beverages. Although extensive HPP research has been performed to characterize many of these factors, a number of issues, such as the rates of compression and decompression, still remain unresolved and need further investigation. In addition, some published results are conflicting and do not provide enough evidence to develop juice HPP "safe-harbor" parameters to achieve a minimum 5-log reduction of the pertinent microorganism and produce safe fruit juices and beverages.

HIGHLIGHTS

- Microorganism inactivation by HPP is proportional to processing pressure and time.
- Bioresistance of microorganisms warrants further research to define critical limits.
- Current HPP science does not support "safe harbor" for HPP-treated juices or beverages.

Minimally processed fruits and vegetables are one of the major growing sectors in the food industry *(91)*. Moreover, beverages, concentrated juices, and purees are vital food products, due to the massive demand of the global market *(130)*. The importance of minimally or nonthermally processed foods with an increased shelf life and, most importantly, better nutritional value is increasing *(77)*. Therefore, there is a growing interest in nonthermal processes, which combine efficient microbial reduction with a maximal retention of the chemical and physicochemical product properties. High pressure processing (HPP) has become a processing technology of choice to fulfill these requirements, since HPP-treated food retains its quality and maintains natural freshness.

HPP is a method of food processing in which food is subjected to elevated pressure in the range from 100 to 1,000 MPa *(111)*. Fruit juices and beverages are normally treated with HPP at 400 to 600 MPa for a few minutes to reduce the numbers of pathogenic and spoilage microorganisms and to prolong the shelf life of food products *(93)*. HPP induces many changes in the bacterial cell, including inhibition of key enzymes, inhibition of protein synthesis, and alterations in cell morphology, as well as affecting the genetic mechanisms of the microorganism, such as disruption of transcription and translation and of cellular functions responsible for survival and reproduction *(49, 82, 92, 107, 129, 134)*.

The sensitivity of microbial cells to HPP in juices and beverages depends on factors like the magnitude of pressure, pressurization time, temperature, compression and decompression rates, microbiota, and intrinsic properties of juices and beverages *(1, 24, 85, 106, 107, 118, 126)*. Considering the limited available information on these factors and the fact that many of them are not fully understood, the purposes of this article are (i) to review and discuss the factors in much more detail and (ii) to address "safe-harbor" parameters that cover juice products with varying compositions, characteristics, and pertinent microorganisms.

PRESSURE AND PRESSURE-HOLDING TIME

The HPP pressure and pressure-holding time affect target microorganisms *(53)*. Generally, as the pressure level and treatment time increased, bacterial cell destruction increased, except in cases where tailing occurred *(2)*. Most research conducted with HPP treatment showed that an increase in inactivation of foodborne pathogens is directly proportional to the pressure level and exposure time *(1, 65)*. For instance, Lavinas et al. *(65)* studied the survival of

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TABLE 1. *Effec of high hydrostatic pressure on* D- *and* z-*values of vegetative cells of* E. coli *and* L. monocytogenes *strains,* Salmonella *serovars, and spoilage microorganisms in selected fruit juices and beverages*

Organism (strain)	Juice	Pressure (MPa)	*D*-value (min)	*z*-value (MPa)	Reference
Escherichia coli (ATCC 25922)	Cashew apple juice	250	16.43	123.5	*65*
		300	11.25		
		350	2.42		
		400	1.21		
E. coli (ATCC 25055)	Apple juice	150	55.0	126	*99*
		200	17.9		
		250	9.22		
		300	2.95		
		350	0.80		
E. coli (KUEN 1504)	Peach juice	300	5.38	450.1	*28*
		400	3.38		
		600	1.22		
	Orange juice	300	2.42	558.4	*28*
		400	1.57		
		600	0.68		
Listeria monocytogenes (4a KUEN, turkey)	Peach juice	300	6.17	506	*27*
		400	3.39		
		600	1.52		
	Orange juice	300	2.87	576	*27*
		400	1.80		
		600	0.87		
Yeast	Mango puree	207	7.2	ND[a]	*40*
Total plate count organisms		207	8.5		*40*
E. coli O157:H7[b]	Whey beverage (pH 3.8)	300	5.24	ND	*116*
		400	2.64	ND	
		500	1.80		
Salmonella serovars[c]	Whey beverage (pH 3.8)	350	1.49		*116*
L. monocytogenes[d]	Whey beverage (pH 3.8)	200	4.86		*116*
		250	1.20		
		300	0.64		
Saccharomyces cerevisiae	Orange juice (pH 3.7)	350	38[e] (76)[f]	106[e]	*90*
		400	7[e] (23)[f]	123[f]	
		450	4[e] (12)[f]		
		500	1[e] (4)[f]		
S. cerevisiae	Orange juice, single strength (pH 3.5, 11.4°Bx)	100	82.2	135	*4*
		200	38.2		
		250	5.4		
	Concentrated orange juice (42°Bx)	200	119	287	
		300	96.8		
		400	23.5		
Leuconostoc mesenteroides	Orange juice, single strength (pH 3.5, 11.4°Bx)	200	26	137	*4*
		300	5.8		
		400	2.0		
	Concentrated orange juice (42°Bx)	200	38.3	251	
		300	15.7		
		400	6.1		

[a] ND, not determined.
[b] Cocktail of five *E. coli* strains: ATCC 43894, ATCC 43895, ATCC 35150, FRIK 125, and 93-062.
[c] Cocktail of five *Salmonella* serovars: Heidelberg, Typhimurium, Gaminara, Enteritidis, and Oranienburg.
[d] Cocktail of five *L. monocytogenes* strains: Scott A, H7969, H7962, H7596, and H7762.
[e] Vegetative cells.
[f] Ascospores.

Escherichia coli (strain ATCC 25922) at a starting cell concentration of approximately 10^6 CFU/mL in apple juice with HPP treatment ranging from 250 to 400 MPa (Table 1). The authors noted that *E. coli* (ATCC 25922) exposed to 250, 300, and 350 MPa declined by 0.12, 0.18, and 1.31 log

CFU/mL, respectively, after 1.5 min. In another study, conducted by Erkmen and Dogan *(27),* peach juice and orange juice were inoculated with *Listeria monocytogenes* at approximately 10^7 CFU/mL and treated with HPP with 200 to 700 MPa at 25°C for specified times. With treatment

at 400 MPa, the *L. monocytogenes* reduction was 2.76 log CFU/mL, compared with 6.47 log CFU/mL at 600 MPa. Increasing the pressure from 150 to 250 MPa at 30°C for 5 min showed significant ($P < 0.05$) reductions in microbial populations, and more than 2.5- and 4-log reductions were observed for red and white grape juices, respectively *(73)*. In the study by Lavinas et al. *(65)*, the reductions in the populations of *E. coli* in cashew apple juice after pressurization for 1.5 min were 0.12, 0.18, 1.31, and 2.19 log CFU/mL at 250, 300, 350, and 400 MPa, respectively.

It can be concluded that higher pressures were found to be more effective in microbial cell destruction. Thus, a progressive increase in HPP pressures causes a decrease in bacterial cell sensitivity to pressure and more rapidly inactivates the pathogens studied. This is also in agreement with the findings reported by Erkmen and Dogan *(27)* for the *L. monocytogenes* inactivation rates in peach and orange juice.

As the time of HPP increased, microbial reduction increased when the same pressure treatment was applied. The log reductions in the populations of *E. coli* in cashew apple juice after 0.5, 1.0, and 1.5 min of HPP treatment at 400 MPa were 2.19, 2.95, and 4.2, respectively *(65)*. *L. monocytogenes* also was found to be more sensitive to increased pressure than to increased pressurization time *(27)*. Because bacterial spores are more resistant to pressure than their vegetative cells, a longer time of HPP treatment normally needs to be applied for their inactivation *(23)*. Raso et al. *(100)* found that HPP treatment at 300 MPa at 25°C reduced the populations of vegetative cells of *Zygosaccharomyces bailii* in grape juice by about 0.5 and 5 log after 5 and 25 min of pressurization, respectively.

Many studies on HPP treatment in juices reported that inactivation of vegetative cells of bacteria such as *E. coli* O157:H7 and *L. monocytogenes* followed a first-order kinetics model *(27, 42, 59, 65, 99)* that assumes that death of microorganisms is log-linear with time. In the context of microbial inactivation as a function of time and pressure, the most common terms are the *D*-value and the "pressure *z*-value," sometimes also called the pressure resistance value (*z*). These values are experimentally determined or calculated and very often used to discuss the results of microbial inactivation data and to determine pressure processing conditions during HPP treatment. The *D*-value is defined as the time in minutes at a constant and specific pressure to reduce the microbial population by 90% (1-log cycle). The *z*-value is derived as the negative inverse slope of a log *D*-value–versus–pressure plot and is the change in pressure required for a 10-fold (1-log) change in the *D*-value. A *D*-value at one pressure, along with a *z*-value, is used to define the pressure resistance of a microorganism and can be used to calculate the *D*-value at any other pressure. The inactivation parameters may be influenced by culture homogeneity, microbial strain, level of pressure, culture medium, and temperature *(89)*. A compilation of pressure resistance characteristics (*D*- and *z*-values) for selected juice products is given in Table 1. Several investigations on *E. coli* O157:H7 (ATCC 25922) indicated that *D*-values ranged from 16.43 to 1.21 min as the processing pressure increased from 250 to 400 MPa *(65)*.

Ramaswamy and others *(99)* showed that increases in pressure levels resulted in decreases in *D*-values. For example, the calculated *D*-values for *E. coli* (strain ATTCC 28055) in apple juice were 9.22, 2.95, and 0.80 min at 250, 300, and 350 MPa, respectively *(99)*. The *D*-values for pear nectar pressurized up to 241 MPa were calculated from first-order kinetics modeling of *Saccharomyces cerevisiae* (strain ATCC 10274), *E. coli* (strain ATCC 11775), and *Listeria innocua* (strain ATCC 51742) survivors and were in the range of 2.0 to 35.3, 0.6 to 20.6, and 9.2 to 588.2 min, respectively *(42)*. Based on the *D*-values from Table 1, it can be concluded that, in comparable experiments, different isolates of *E. coli* vary in their pressure resistance. For instance, *E. coli* ATCC 25922 has been shown to be more pressure resistant than *E. coli* strain ATCC 25055 or *E. coli* strain KUEN 1504 at 300 MPa in apple juice. Also, the same strain of *E. coli* (KUEN 1504) was more pressure resistant in peach juice than in orange juice. The *D*-value at 600 MPa for *E. coli* KUEN 1504 was 1.22 min in peach juice, whereas for orange juice, it was only 0.68 min.

Based on the *D*-values, the *z*-values were calculated to evaluate the pressure sensitivities of the tested microorganisms in juices. Lavinas et al. *(65)* reported a *z*-value of 123.46 MPa for *E. coli* ATCC 25922 in cashew apple juice, which was similar to that reported by Ramaswamy and others *(99)* in apple juice but lower than those reported by Erkmen and Dogan *(28)* for *E. coli* KUEN 1504 in peach juice and orange juice, which were 450.1 and 558.4 MPa, respectively. *z*-values of 120.5, 92.6, and 75.2 MPa were obtained for *S. cerevisiae* (ATCC 10274), *E. coli* (ATCC 10274), and *L. innocua* (ATCC 51742), respectively, in pear nectar treated at up to 241 MPa *(42)*. Evaluation of the *z*-values may can lead to the conclusion that pressure resistance was sustainably different among different juices, tested microorganisms, and processing pressures. By applying appropriate *D*- and *z*-values, it would be possible to modify processing parameters to adjust pressure and time to obtain a desired microbial inactivation. Because pressure resistance can be affected by many factors, including variations in these parameters, it is important, when applying published *D*- and *z*-values to certain juice processes, that the conditions under which the values were obtained should closely match the processor's product or process parameters *(95)*.

Inactivation curves in HPP tend to be exponential, with a rapid initial decrease in bacterial cell populations, and to follow first-order inactivation kinetics *(97)*. However, very often tailing is observed in survival curves, indicating that small fractions of the bacterial populations are resistant to pressure *(94, 132, 138)*. This may cause significant deviations from the linear relationships between the log reductions and treatment times or pressure levels *(20)*. In such deviations, either the Weibull model or other nonlinear models are often applied to adjust microbial inactivation. For instance, the inactivation kinetics of *E. coli* O157:H7 on orange surfaces treated with HPP (400 MPa for 1 min) followed a nonlinear biphasic model *(137)*. The authors also applied the Weibull model, but it was not suitable because of low R^2 values. Zhao et al. *(138)* applied five nonlinear models, including the biphasic, Weibull, modified Gom-

pertz, log-logistic, and Baranyi models, for the inactivation of naturally occurring microbiota in cucumber juice. Those models were fitted to inactivation curves for cucumber juice treated at 300, 350, and 400 MPa for 2 to 15 min and 500 MPa for 2 to 8 min. The biphasic model provided the best fit for the data.

In addition to processing pressure time, there is also the time to achieve a desired processing pressure called come-up time (CUT). For most HPP units, pressure release is achieved in a few seconds, if not almost instantaneously. A long CUT will add significantly to the total process time and contribute to microbial inactivation, but this effect is often ignored, since isobaric-isothermal conditions cannot be assumed in the estimation of kinetic parameters for the design of commercial HPP treatments (114).

PROCESS TEMPERATURE

The processing temperature during HPP treatment plays a critical role in microbial inactivation and should be considered when pressure treating fruit juices to ensure microbiological safety (39, 66). As a rule, HPP treatment conducted above or below room temperature of the product and pressure fluid (20°C) increases the inactivation rate of microorganisms (30). For instance, Tonello et al. (128) observed that HPP treatment was more effective at 4°C than at room temperature and resulted in a 6-log reduction of *S. cerevisiae* in grape juice subjected to pressurization at 300 MPa for 5 min. The decrease in resistance to pressure at low temperatures may be cause by changes in bacterial membrane structure and fluidity due to weakening of hydrophobic interactions and crystallization of phospholipids (19). In another study, Linton et al. (66) found that pressure treatment of 550 MPa for 5 min at 20°C produced a 6-log inactivation of *E. coli* O157:H7 in orange juice over the pH range of 3.4 to 4.5. However, increasing the processing temperature to 30°C also provided a 6-log reduction, but at pH 5. In a similar study done by Bayindirli et al. (5), a significant reduction of more than 8 log of *E. coli* O157:H7 in orange juice was obtained at a much lower pressure (350 MPa for 5 min) but at the higher temperature of 40°C. Another study, done by Cheng et al. (21), showed a significant effect of temperature ($P < 0.05$) on pathogen inactivation. At temperatures significantly below room temperature, for example 5°C, apple juice inoculated with *E. coli* O157:H7, *Salmonella enterica,* and *Listeria monocytogenes* at initial concentrations of 10^6 to 10^7 CFU/mL achieved only 1.58-, 4.02-, and 6.38-log reductions, respectively, whereas at 20°C, greater reduction values of 3.48, 7.61, and 6.65, respectively, were obtained. On the other hand, Buzrul et al. (14) observed no significant impact of the processing temperature (−10, 0, or 20°C) on *E. coli* ATCC 11775 and *L. innocua* ATCC 33090 in pineapple juices treated at 300 MPa for 5 min. Studies have shown that bacterial spores are highly resistant to pressure, showing a remarkable tolerance to pressures above 1,000 MPa near room temperature. Nevertheless, sterilization of low-acid foods, such as some fruit derivatives, is possible through combined high-pressure (500 to 900 MPa) and increased-temperature (90 to 120°C) processing for about 5 min (25).

Pressure treatments ranging from 300 to 800 MPa at ambient temperature lead to the unfolding and partial or complete denaturation of bacterial cell enzymes and proteins (102, 119). Bacterial cells are relatively less sensitive to hydrostatic pressure at 20 to 35°C but become more sensitive to pressurization above 35°C owing to phase transition of membrane lipids and changes in their fluidity (57, 61, 78). Temperatures between 45 and 50°C appear to increase the rates of inactivation of pathogens and spoilage microorganisms and, thus, warrant the development of processes which incorporate a uniform initial food temperature in this range.

Because bacterial cell death increased with increases in temperature, the temperature distribution of the tested food should be an integral part of the HPP microbial inactivation process (39). Very often a relationship between process pressure and temperature was observed (11). Therefore, it would be possible to lower the temperature and increase the process pressure to achieve the same microbial inactivation. Mert et al. (73) showed that applying a mild heat treatment (≥30°C) could be useful to allow the pressure value to be lowered to ≤250 MPa but that longer HPP holding times, at least 5 and 10 min under the same conditions, were required for microbial inactivation in white and red grape juices, respectively.

COMPRESSION AND DECOMPRESSION RATES

There are only a few studies that discuss the effects of compression and decompression rates of HPP treatments. Generally, these studies usually compared slow to fast rates, and rarely to medium rates, of compressions and decompression. During compression, the pressure increases, which causes increases in the temperature in the food, pressure medium, and vessel due to adiabatic heating. The typical change in the temperature of juice and beverages is 3°C per 100 MPa increase from an initial temperature of 25°C (3). For homogeneous foods, such as juices and beverages, the adiabatic temperature increase is uniform throughout the food product. Contrariwise, during decompression, the temperature of the food decreases to a temperature which may be below the precompression temperature if heat has dissipated from the food during the holding time.

One of the first studies on this topic was done by Smelt (120), who assumed that a slow compression might have induced a stress response of bacterial cells that would result in a lower microbial inactivation and make HPP less effective. Rapid decompression applied after continuous pressurization induced the fast adiabatic expansion of water and generated an impulsive force. According to Hayakawa et al. (45), the combination of this impulsive force and pressurization causes much more bacterial inactivation than pressurization alone. Hayakawa et al. (45) reported that rapid decompression of 1.3 to 1.64 ms increased the lethality to *Bacillus stearothermophilus* (strain IFO 12550) spores treated at 500 MPa for 1 min. The researchers also noticed that the inactivation rate was directly proportional to the decompression rate and pressure. Noma et al. (85) reported similar findings for the inactivation of vegetative cells of *E. coli* K-12, *Salmonella enterica* serovar Typhimurium, and *Pseudomonas aeruginosa* when rapid

decompression (1 ms) was used rather than slow decompression (>30 s) during HPP treatment at pressure levels ranging from 70 to 400 MPa.

However, there were also studies that showed results contradictory to the research discussed above. Chapleau et al. *(18)* observed the highest microbial inactivation of *Salmonella* Typhimurium and *L. monocytogenes* when the slowest rates of compression (1 MPa/s) and decompression (5 MPa/s) were applied. Rademacher et al. *(98)* found no significant differences between treatments for inactivation of *L. innocua* suspended in Tris buffer when fast compression (8.3 MPa/s) was followed by slow decompression (1.7 MPa/s) or slow compression was followed by fast decompression with the same pressurization ramps. The researchers concluded that the rates of pressurization and depressurization (slow versus fast) did not have a direct effect on the inactivation kinetics of *L. innocua* if the temperature gradients at the fast and slow ramps were approximately the same.

Studies on the effects of compression and decompression rates during HPP treatment of juices and beverages are very limited. There is one study conducted by Syed et al. *(124)* on the effects of compression and decompression rates on the lethality of *E. coli* O157:H7 in orange juice. HPP treatments were carried out using slow (1.3 MPa/s), medium (3.6 MPa/s), and fast (11.4 MPa/s) compression rates along with slow (2.6 MPa/s), medium (6.0 MPa/s), and fast (12.9 MPa/s) decompression rates at 600 MPa pressure for 3 min at 4°C. The lowest inactivation count of *E. coli* O157:H7 (1.49 log CFU/mL) was obtained for the medium compression followed by the medium decompression. The authors concluded that during HPP of orange juice, fast compression and slow decompression rates are more effective than slow and medium compression and fast and medium decompression rates.

The controversial results of different research groups on compression and decompression rates have not yet been fully investigated, and the role of depressurization remains an area for further research. Since vegetative bacterial cells, yeasts, and bacterial spores exhibit various degrees of sensitivity to pressure, there is a need to investigate the behavior of each microorganism under different compression and decompression conditions.

STRAINS (ISOLATES) OF BACTERIA

It is well established that different species of pathogenic foodborne bacteria and different strains of the same species can exhibit considerable variation in hydrostatic pressure resistance *(2, 6, 55, 96, 108, 132)*. This may explain the variation in levels of microbial inactivation obtained by researchers using different strains of the same species. For example, *L. monocytogenes* strain Scott A showed greater sensitivity than *L. monocytogenes* strain CA in phosphate buffer at 275.6, 310, and 344.5 MPa *(123)*.

Pathogenic microorganisms with marked characteristics of tolerance to pressure have been identified and described *(43, 58, 108)*. A strain of *E. coli* K-12 (MG1655) that was found to be highly resistant to pressure of at least 800 MPa was isolated by applying cycles of exposure in a range from 280 to 450 MPa *(43)*. *L. monocytogenes* Scott A

strain AK01, resistant to pressure up to 400 MPa for 20 min, was isolated and characterized in terms of growth characteristics, resistance to different pressures, and the effects of growth phase and temperature *(58)*. Robey et al. *(108)* demonstrated that strain C9490 was one of the most pressure-resistant strains of *E. coli* O157:H7 and that it was able to withstand 500 MPa for 5 min with little loss of viability. A significant variation in pressure sensitivity in phosphate-buffered saline (PBS) at up to 700 MPa at 20°C was also observed among three strains of *L. monocytogenes* (NCTC 11994, Scott A, and 2433), three strains of *E. coli* O157:H7 (NCTC 12079, H631, and H1071), and two strains of *Salmonella* (*Salmonella* Typhimurium NCTC 74 and *Salmonella* Enteritidis phage type 4) *(94)*. Among the tested strains, *L. monocytogenes* NCTC 11994 and *E. coli* O157:H7 NCTC 12079 were the most pressure resistant. In addition, *Salmonella* Typhimurium was less pressure resistant than *Salmonella* Enteritidis when tested under the same conditions. Alpas et al. *(2)* also noticed that variation in pressure resistance among strains existed mainly at a lower temperature (25°C) and not at a higher temperature (50°C).

Among the bacterial species, pathogenic foodborne bacteria like *L. monocytogenes, Salmonella* spp., *E. coli* O157:H7, and *Staphylococcus aureus* are among the most extensively studied in juices and beverages in terms of HPP *(10, 21, 55, 66)*. Tables 2 and 3 show the reductions (CFU per milliliter) in pathogenic and spoilage microorganisms in selected fruit juices and beverages, respectively, processed by HPP. However, limited studies have been conducted on the variations in hydrostatic pressure resistance among different pathogenic species or strains in juices and beverages. Research has mostly used bacterial strains from other research studies that were particularly resistant to high pressure in various food matrices or media, such as phosphate buffer *(2)*. For example, *L. monocytogenes* strain NCTC 11994, which was the most pressure resistant of three strains tested by Simpson and Gilmour *(117)*, was tested in commercially prepared fruit juices *(55)*. In another study, *E. coli* O157:H7 strain NCTC 12079, which was highly resistant to pressure *(94)*, was chosen for a study on the effect of HPP in orange juice *(66)*. Buzrul et al. *(14)* used *E. coli* ATCC 11775 and *L. innocua* ATCC 33090 in kiwifruit and pineapple juices treated with HPP, based on previous research showing that these strains were particularly resistant to high pressure *(14)*. Because fruit juices and beverages are mostly acidic, some strains of *E. coli,* including the pathogenic O157:H7 strain, are acid resistant and can survive for long periods in acidic foods, especially at low temperatures *(55, 75)*. Thus, the most appropriate pathogenic strains to be used for HPP research should not only be pressure resistant but also acid resistant. For this reason, *E. coli* O157:H7 strain C9490, which was highly resistant not only to pressure but also to acid *(6, 55),* was examined in commercially prepared fruit juices *(55)*. It was also noted that the bacteria from animal sources may be more pressure resistant due to the temperatures at which the cells grow, unlike those of environmental origin *(113)*. Ogawa et al. *(86)* reported that yeasts resistant to heat, such as *S. cerevisiae* or *Rhodotorula glutinis* and *Hansenula*

TABLE 2. *Pathogenic and spoilage microorganism reduction in selected fruit juices subjected to HPP*

| Juice type | HPP condition | | | Pathogen or spoilage microorganism | Log reduction (CFU/mL) | Shelf life (days)/storage temp (°C) | Reference |
	Pressure (MPa)	Time (min)	Temp (°C)				
Apple (pH 4.5)	550	1	5	*Escherichia coli* O157:H7	2.53	NA	*21*
				Salmonella enterica	4.90		
				Listeria monocytogenes	7.07		
			20	*E. coli* O157:H7	6.75		
				S. enterica	5.73		
				L. monocytogenes	7.02		
Apple (pH 3.5)	500	5	Room temp	*E. coli* O157:H7	>5	NA	*55*
Apple (pH 4.12, 11.19°Bx)	300	7.5	25	*E. coli* O157:H7	1.62	NA	*65*
					2.55		
	400	2		*E. coli* O157:H7	4.2	NA	
					5.57		
		3		Aerobic mesophilic bacteria	4.32	56/4	
				Yeast and fungi	3.31		
Apple (pH 3.8, 12.09°Bx)	500	1	25	*L. monocytogenes*	4.85	NA	*115*
				Staphylococcus aureus	2.40		
				E. coli	5.01		
				Salmonella Typhimurium	7.0		
				Saccharomyces cerevisiae	4.9		
Apricot (pH 3.8)	350	5	30	*S. aureus*	ca. 6.74	NA	*5*
				E. coli O157:H7	ca. 7.0		
				Salmonella Enteritidis	ca. 7.47		
Cherry (pH 3.30)	350	5	30	*S. aureus*	ca. 7.24		*5*
				E. coli O157:H7	ca. 7.39		
Cactus (pH 3.7)	600	10	15	Yeast and molds	4.2	NA	*79*
				Acid-tolerant microorganisms	4.2		
Cucumber (pH 6.6, 1.9°Bx)	400	4	25	Yeast and molds	3–4	50/4	*138*
	500	2			3–4		
Keiskei (pH 6.0)	550	1.5	Room temp	Coliform	6.05	8/4	*17*
				Yeast and molds	4.7		
				Pseudomonas	5.33		
				Bacillus cereus	1.3		
Kiwifruit (pH 3.32)	350	5	20	*E. coli* ATCC 11775	>5	NA	*14*
				Listeria innocua ATCC 33090	>5		
Mandarin (pH 7.8)	120	0.1	30	*Lactobacillus plantarum*	2.4		*15*
Navel orange (pH 3.75)	600	1	20	Yeast and molds	3.1	84/4 or 10	*13*
Valencia orange (pH 3.8, 11.6°Bx)	250	1–30	25–40	*Lactobacillus brevis*	ca. 4.0	NA	*63*
	200–350	1–10	35	*L. plantarum*	ca. 4.3		
Orange (pH 3.4–5.0)	400–550	1	20–30	*E. coli* O157:H7	5–6	NA	*66*
Orange (pH 3.4)	700	5	4	*S. aureus*	6.2–6.6	15/4	*125*
Orange (pH 3.58, 14.72°Bx)	400	1	25	*E. coli* O157:H7	2.4	NA	*137*
Peach (pH 5.21)	400	20	25	*L. monocytogenes*	ca. 7.5	NA	*27*
	600	10	25	*L. monocytogenes*	ca. 7.5	NA	
Pineapple (pH 3.77)	300	5	20	*E. coli* ATCC 11775	2.5	NA	*14*
				L. innocua ATCC 33090	3.5		
Tomato (pH 4.5)	300–500	10	25	Yeast and molds	3.6	28/4	*50*
				Enterobacteria	2.1		
				Lactic acid bacteria	4.2		
Tomato (pH 4.1)	350	5	20	*E. coli* O157:H7 C9490	>5	NA	*55*
				L. monocytogenes NCTC11994	>5		

[a] NA, not applicable.

anomala, tend to also be highly resistant to pressure in mandarin juice.

For conducting bacterial inactivation challenge studies, National Advisory Committee on Microbiological Criteria for Foods guidelines *(84)* do not specify any bacterial strains to be used for HPP studies. However, the same document recommends that the selection of the organisms should be based on the likelihood of pathogen association with the specific food and pathogen pressure resistance, as well as the public health objective of the process and the intended use of the product *(84)*. Since there is considerable variation in results obtained with different species or strains

TABLE 3. *Pathogenic and spoilage microorganism reduction in selected beverages subjected to HPP*

| Beverage, puree, or nectar | HPP condition | | | Pathogen or spoilage microorganism | log reduction (CFU/mL) | Shelf life (storage time [days]/ temp [°C]) | Reference |
	Pressure (MPa)	Time (min)	Temp (°C)				
Acidified apple puree (pH 3.41, 11.2°Bx)	400	5	20	Total aerobic mesophilic/ psychrotrophic bacteria	3.31	14–21/4	*64*
				Yeast and molds	3.22		
Cantaloupe puree (pH 6.9, 11.2°Bx)	300	5	8	Total aerobic bacteria	4.3	10/4	*81*
				Yeast and mold	1.4		
Kiwifruit puree (pH 3.2)	500	3	Room temp	Total mesophilic bacteria	3.44	Up to 70/4	*32*
Mango nectar (pH 3.95, 8.2°Bx)	600	1	20	Total aerobic bacteria	>2	NS[a]	*67*
				Yeast and molds	>1		
Mango pulp (pH 3.9)	400	1	NP[b]	Total aerobic bacteria	1.64	NS	*68*
				Yeast and molds	1.96		
	500	1		Total aerobic bacteria	3.28		
				Yeast and molds	ND[c]		
Strawberry puree (pH 3.2)	500	1.5 or 15	0 or 50	Total aerobic bacteria	3.6	NS	*71*
				Yeast and molds	2.8		
Tomato puree (pH 4.35)	700	0.5–2.0	20–90	*Bacillus stearothermophilus*	4.6–6.1	56/4	*63*
Smoothie (pH 3.8, 12.2°Bx)[d]	350	7	<25	Aerobic mesophilic bacteria	1.8	28/4	*51*
				Psychrotrophic bacteria	2.5		
				Yeast and molds	1.8		
				Enterobacteria	2.4		
Smoothie (pH 3.44, 13.7°Bx)[e]	300	5	−5	*Listeria monocytogenes*	5.6	NS	*113*
			45		6.3		

[a] NS, not studied.
[b] NP, not provided.
[c] ND, not determined.
[d] Strawberries, oranges, black and white grapes, blackberries, gooseberries, bananas, and lime.
[e] Wild berries, bilberries, blackberries, raspberries, red currants, strawberries, apples, and oranges.

of the same species in response to hydrostatic pressure, the strains and species most resistant to pressure should be selected carefully. Also, their selection needs to be justified to ensure that these organisms would not survive in pressure-treated juices.

GRAM-POSITIVE VERSUS GRAM-NEGATIVE BACTERIA

The published work on the pressure resistance of Gram-positive and Gram-negative bacteria provides strong evidence that the structure of the bacterial cell wall determines resistance to HPP treatment *(19, 69, 136)*. Specifically, the peptidoglycan polymer (murein), which serves as the skeletal structure and gives cells their structural strength, plays an important role in that determination *(74)*. Generally, Gram-positive bacteria are more resistant than Gram-negative bacteria to high pressure, probably due to their thicker cell walls, which are composed predominantly of peptidoglycan (90%), with layer after layer forming around the cell membrane. In contrast, the cell walls of Gram-negative bacteria contain only 5 to 10% peptidoglycan layers *(10)*.

Wuytack et al. *(136)* studied the high hydrostatic pressure (200 to 400 MPa for 15 min at 25°C) resistance of five Gram-positive bacteria (*Enterococcus faecalis, S. aureus, Lactobacillus plantarum, L. innocua,* and *Leuconostoc dextranicum*) and six Gram-negative bacteria (*Salmonella* Typhimurium*, Shigella flexneri, Yersinia enter-*

ocolitica, Pseudomonas fluorescens, and two strains of *E. coli*). Generally, the Gram-negative bacteria were inactivated at a lower pressure than Gram-positive microorganisms. *S. aureus* and *E. faecalis* were the most resistant Gram-positive bacteria, while *L. innocua* and *L. plantarum* had intermediate resistance and *Y. enterocolitica* and *Salmonella* Typhimurium were the most sensitive Gram-negative bacteria *(136)*. However, there is considerable overlap between the Gram-positive and Gram-negative bacteria studied. For instance, *L. dextranicum* was less resistant to high hydrostatic pressure than several Gram-negative bacteria, such as *S. flexneri, E. coli,* and *Salmonella* Typhimurium. A similar pattern of findings was reported by Cheng et al. *(21)*. The authors showed that Gram-positive bacteria like *L. monocytogenes* were also less resistant to HPP than Gram-negative bacteria like *E. coli* O157:H7 or *Salmonella enterica* in apple juice pressurized at 550 MPa for 1 min at 5 and 20°C. Simpson *(116)* also reported that *E. coli* O157:H7 strains were the most barotolerant compared with *L. monocytogenes* and *Salmonella* strains in whey beverages subjected to HPP (300 to 500 MPa) for 2 to 8 min at 25°C.

GROWTH STAGE OF MICROORGANISMS

Researchers who have studied HPP in juices and beverages mostly have used bacteria in their early, mid-, or late stationary stages (Table 4). They showed that bacterial cells in stationary phases were less susceptible than

TABLE 4. *Growth phase of tested pathogens and their incubation conditions subjected to HPP in fruit juices*

Organism	Growth phase	Incubation conditions (temp [°C]/time [h], medium)[a]	Juice type(s)	Reference
Escherichia coli O157:H7	Stationary	37/6, TSBYE	Orange, apple, tomato	55
E. coli	Early stationary	37/15–21, TSBYE	Kiwifruit, pineapple	14
E. coli	Early stationary	30/12, TSB	Pear nectar	42
Listeria monocytogenes	Stationary	35/5 days, BHI	Peach and orange	27
	Mid-/end exponential	35/24, BHI		
L. monocytogenes	Stationary	37/overnight, TSB	Orange, apple tomato	55
Listeria innocua	Early stationary	37/15–21, TSBYE	Kiwifruit, pineapple	14
L. innocua	Early stationary	30/11, TSB	Pear nectar	42
Salmonella	Stationary	37/20, BHI	Orange	13
Lactobacillus plantarum	Stationary	37/16–20, MRS	Mandarin	15
Saccharomyces cerevisiae	Early stationary	30/27, TSB	Pear nectar	42
S. cerevisiae	Late stationary	37/ND, TSBYE	Wild berry smoothies	113

[a] TSBYE, tryptone soy broth with yeast extract; TSB, tryptone soy broth; BHI, brain heart infusion broth; MRS, de Man Rogosa Sharpe broth; ND, not determined.

exponential-phase cells to environmental stresses induced by high pressure, starvation, or acidification *(19, 62, 88, 121)*. In addition, Manas and Mackey *(70)* reported that after the pressure treatment at 200 MPA for 8 min, exponential-phase cells of *E. coli* strain J1 showed physical perturbations of the cell envelope structure, loss of osmotic responsiveness, and loss of protein and RNA to the extracellular medium. These changes were not observed in the cytoplasmic membrane of stationary-phase cells, which were able to withstand pressure up to 600 MPa for 8 min.

Erkmen and Dogan *(27)* compared the effects of HPP at 300 MPa on old (stationary-phase) and young (mid- to end-exponential-phase) cells of *L. monocytogenes* in peach and orange juices. About 3.36 log CFU of old cells per mL and 3.97 log CFU of young cells per mL were inactivated under the operational pressure conditions at 300 MPa after 20 min. HPP was much more effective on cells that were in mid- to end exponential phase than on old cells in a stationary phase of growth. In general, old cells were about 0.40 times more resistant to HPP than young cells (Erkmen and Dogan *(27)*). In another study *(15)*, mandarin juice was inoculated with *L. plantarum* in early stationary phase (37°C for 16 to 20 h in de Man Rogosa Sharpe [MRS] broth). Several authors also have observed that as the growth temperature increased, there was a corresponding increase in the pressure resistance of stationary-phase cells and a corresponding decrease in the pressure resistance of exponential-phase cells *(16)*.

RECOVERY CONDITIONS FOR SUBLETHALLY INJURED CELLS

Determining the pressure resistance of microorganisms requires that the surviving viable populations be accurately enumerated after pressure treatment. Recovery conditions after pressure treatment influence microbial survival *(29)*. A high-pressure treatment may not always completely inactivate microorganisms, but it may injure a proportion of the bacterial population. Microbial cells surviving pressurization may become sublethally injured, but they are not fully dead. This condition is called "viable but nonculturable" (VBNC). VBNC cells develop sensitivity to physical and

chemical environments to which the normal cells are resistant *(56, 57)*. The injured bacterial cells cannot be detected on selective media, but they can repair themselves in a nonselective medium containing the necessary nutrients for growth. The repair process involves the synthesis of ATP, RNA, DNA, and micropeptides *(22, 88, 135)*. Under subsequent storage and conditions of optimum pH and temperature, the cells could start growing in juices and affect the microbiological safety, especially in low-acid juices and beverages. Thus, it is important to enumerate sublethally injured bacterial cells. Nonselective media will allow the growth of both viable (noninjured cells) and sublethally injured cells. Thus, the injured survivors are usually counted as the differences between log CFU of bacteria per milliliter in pressurized samples on nonselective and selective media *(28, 103–105, 126)*. The variation in procedures for recovery on different nonselective media may also have an effect on different estimates of bacterial counts. This may be due to the use of plating media containing different selective agents (e.g., NaCl, bile salts, deoxycholate) and different metabolic inhibitors to repair *(103)*.

Bacterial cells subjected to pressurization processes may form two types of bacterial injuries, called I1 and I2 *(9)*. I1 indicates an injury that bacterial cells can repair by themselves, and the I2 type is an injury that the cells cannot repair due to structural damage to the bacterial cell wall. Cells with an I1-type injury can form colonies on both selective and nonselective media, while cells with an I2-type injury can only form colonies on nonselective media. The authors observed that after pressure treatments at 450 and 550 MPa for 10 min and during storage at 4°C for up to 4 weeks, a complete recovery was observed only for *L. monocytogenes* and not for *S. aureus* and *Salmonella* Enteritidis. Based on the results of the study, it is very important to conduct a shelf-life study after HPP treatment and enumerate bacterial cells over a period that is necessary for potential repair of I2-type injury.

There are several factors that influence the number of injured bacterial cells during pressure treatment. As the rate of pressure and time of pressurization increase, the number

of injured bacterial cells increases *(27, 55)*. Erkmen and Dogan *(27)* reported rates of injury of *L. monocytogenes* cells in peach and orange juices from 4.5 to 100% when subjected to HPP treatment at 300 MPa after 1 min and 60 min, respectively. Approximately a 1-log reduction in *E. coli* O157:H7 was observed in orange juice (2.37- to 3.23-log cycles) and apple juice (0.62- to 1.28-log cycles) when pressurization time was extended from 1 to 2 min *(126)*. The rate of compression and decompression during HPP may also affect cell injury. Noma et al. *(85)* investigated the injurious effects of HPP treatments at pressure levels from 70 to 400 MPa combined with slow and fast decompression (30 s and 1 ms, respectively). The authors concluded that a rapid decompression procedure could enhance the injury, which causes the higher bactericidal effect of HPP treatments. Low-temperature storage may result in further injury of bacterial cells after HPP in fruit juices *(55)*. The numbers of injured cells of *E. coli* O157 in tomato juice after pressure treatment at 350 MPa for 5 min increased from 1.06 to 4.9 log CFU/mL after 24 h of storage of tomato juice at 4°C *(55)*. This additional injury during refrigerated storage was mostly attributed to changes in the bacterial membrane after HPP that made the cells more susceptible to subsequent acid injury.

a_W AND BRIX

Water activity (a_w) is considered a critical factor for the inactivation of bacteria using pressure *(7, 36, 47, 89, 93)*. The protective effect of lowering water activity during HPP has been observed not only with bacteria but also with yeasts and molds *(38, 87, 89)*. It is expected that microorganisms may be sublethally injured by pressure *(44, 57)* and, thus, become more sensitive to low a_w and consequently fail to recover *(119)*. The influence of a_w on the survival of pressure-treated microorganisms is illustrated by the work of Palou et al. *(89)*. The authors studied the effect of different a_w values, ranging from 0.998 to 0.900, on inhibition of *Z. bailii* in a model system subjected to HPP treatment of 345 MPa at 21°C for 5 min. When the a_w was higher than 0.98, the yeast was inhibited below the detection level of 10 CFU/mL after the HPP treatment. As the a_w decreased, the number of surviving cells of *Z. bailii* increased. For a_w values of 0.92, 0.91, and 0.90, the HPP treatment reduced the initial population by less than 1 log.

The a_w of the finished juice or beverage can affect HPP treatment efficacy based on a number of factors. These factors include strains and serotypes, growth and storage conditions tested, and other intrinsic and extrinsic properties of a food, such as the physical and chemical food composition, test medium, and medium used to recover surviving and pressure-damaged cells. Increasing the concentration of solutes in juices reduces a_w and results in enhanced pressure resistance of microorganisms *(19)*. The addition of chemical components like soluble carbohydrates (i.e., sucrose, fructose, and glucose) to juices or beverages could protect bacterial cells against pressure treatment by lowering a_w *(86, 89)*. A higher Brix level corresponds to lowering a_w and juice compressibility, which is defined as the free volume between molecules *(76)*. It correlates with an increase in pressure resistance of microorganisms *(54, 76)*. This correlation might be due to (i) cell shrinkage, which causes thickening of the cell membrane, reducing the membrane permeability and protecting cells from destruction by HPP and temperature *(89)*, and/or (ii) a decrease in the mechanical energy transferred during pressure treatment as the result of lower compressibility of a cell's cytoplasm *(31)*. An increase in Brix level has been reported to correlate with an increase in pressure resistance of *E. coli, S. cerevisiae, Rhodotorula rubra,* and *Z. bailli (31, 89, 112)*. The nature of the solute (i.e., sugar or salt) can have a significant effect on cell survival after pressure treatment. For instance, ionic solutes like NaCl or $CaCl_2$ offer more protection to *Bacillus coagulans* than do nonionic solutes like sucrose and glycerol *(92)*, due to their effect on osmotic pressure. The media used for recovery after pressure treatment also influence the survival of organisms. For instance, the recovery of pressure-treated *E. coli* cells was much lower when 2% salt was added to the tryptic soy agar medium *(94)*.

Cheng et al. *(21)* reported that apple juice with an a_w of 0.99 inoculated with *E. coli* O157:H7, *Salmonella,* and *L. monocytogenes* and subjected to HPP treatment (550 MPa for 1 min at 20°C) showed 6.75-, 5.73-, and 7.02-log reductions, respectively. The effect of HPP treatment on inactivation of *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* in coconut water that has a water activity of 0.995 was studied *(26, 33)*. The research confirmed that treatments of 500 and 600 MPa for 120 s reduced all tested pathogens by more than 5 log. Buerman et al. *(12)* determined the effects of different a_w values of 0.94 (41.0 degrees Brix [°Bx]), 0.96 (32.0°Bx), 0.98 (19.8°Bx), or 1.00 (6.5°Bx) on HPP apple juice pH 4.6 to prevent fungal spoilage. Fungi subjected to HPP at 450 MPa for 1.5 min or 600 MPa for 15 min to resemble industrial application were more resistant at lower a_w. Fungi such as *Aspergillus pseudoglaucus, Byssochlamys nivea,* and *Aspergillus fischeri* that are known to be heat-resistant species were also more resistant to HPP than the other organisms tested. Typical spoilage fungi *Byssochlamys spectabilis, Aspergillus niger,* and *Penicillium* were reduced by 4.8 log or more at a_w values of 0.98 and above. Reductions ranged from 2.5 to 4.9 log at 0.94 a_w and from 3.3 to 5.5 log at 0.96 a_w. These authors concluded that the HPP-treated products should be at or above 0.98 a_w to minimize the risk of fungal spoilage *(12)*.

ACIDITY AND PRIMARY ACIDULANT

The acidity of juices and beverages can significantly affect the inactivation of microorganisms by pressure *(7, 41, 42, 47, 93)*. Yeasts and molds are more susceptible to pressure at a pH below 4.0 *(120)*. Vegetative cells of bacteria are also more susceptible to pressure at lower pH values *(1, 60, 66, 107, 122, 134)*. Bacterial spores are most resistant to pressure at neutral pH, but at pressures over 1,000 MPa, they are killed more rapidly at low pH *(8, 101, 127)*. Pressure treatment between 50 and 300 MPa would activate the nutrient-stimulated germination pathways *(43)* and initiate spore germination, which would proceed faster at neutral pH *(120)*. The germinated spores then become more sensitive to HPP treatment.

More acidic conditions in combination with high pressure greatly increase microbial lethality. For example, Stewart et al. *(122)* reported an additional 3-log reduction in *L. monocytogenes* CA when pressurized in buffer at pH 4.0 compared with the reduction at pH 6.0 at 353 MPa and 45°C for 10 min. Linton et al. *(66)* found that pressure treatment of 550 MPa for 5 min at 20°C produced a 6-log inactivation of *E. coli* O157:H7 in orange juice over the pH range of 3.4 to 4.5 but that lower reduction was obtained at pH 5. Erkmen and Dogan *(28)* reported higher inactivation rates for aerobic bacteria and *E. coli* in orange juice than in peach juice treated with HPP from 200 to 600 MPa at 25°C because of the difference in pH values of those juices, with a pH of 5.21 for peach juice and a pH of 3.55 for orange juice. At 400 MPa, the calculated D-values for *E. coli* were about 3.38 and 1.57 min for peach juice and orange juice, respectively. For aerobic bacteria at 400 MPa, the D-values were 4.9 min for peach juice and 2.20 min for orange juice. The lower D-values obtained for orange juice indicated shorter times required to inactivate 90% of the microbial populations than for peach juice. Similarly, a synergistic effect of low pH with pressure treatment in the inactivation of bacteria was also observed by Jordan et al. *(55),* who reported a 5-log and a 1-log reduction in viable cell numbers of *E. coli* O157:H7 in apple (pH 3.5) and orange juice (pH 3.8), respectively, after treatment at 500 MPa at 20°C for 5 min. The additional microbial reductions in acidic juices after HPP treatment may be caused by sublethal injury of a large portion of the bacterial cells that fail to recover. In addition, after HPP treatment, the organic acids present in fruit juices may exist in the form of undissociated molecules that would enhance antimicrobial properties *(105, 119)*. Thus, lower pH values not only enhance microbial inactivation during treatment but also inhibit outgrowth of cells sublethally injured due to pressure. This will result in a reduced resistance of the bacteria injured by HPP treatment to low pH during storage *(35)*. For example, in high-acid juices, pathogens like *E. coli* O157:H7 may survive the initial pressure treatment but will die off within a relatively short period of time during cold storage under acidic conditions *(14, 15, 83)*. As a result of storage of beetroot juice (pH 4.0 to 4.2) treated with HPP (300 MPa for 10 min) at 5°C for up to 28 days, additional reductions of 5.15 and 6.53 log CFU/mL after pressure treatment were obtained for *L. innocua* and *E. coli,* respectively *(83)*. That was in agreement with the results obtained by Buzrul et al. *(14),* who studied the effects of storage temperature (4, 20, and 37°C for up to 3 weeks) on the additional reduction of *E. coli* and *L. innocua* after HPP treatment (5 pulses of 350 MPa at 20°C for 60 s) in kiwifruit and pineapple juices. Both kinds of bacteria were reduced by more than 7 log at all storage temperatures during the first 24 h, except at 4°C in pineapple juice. The subsequent storage enhanced further inactivation even at 4°C, and no recovery of the bacteria was detected during 3 weeks of storage at 4, 20, and 37°C *(14)*.

During pressurization, a decrease in the pKa of the acids and a pH reduction were observed *(89)*. Heremans *(48)* reported a lowering of pH in apple juice by 0.2/100 MPa increase in pressure. Thus, the pH shift and its magnitude must be determined for each food treatment process to assure product safety. Methods for the routine measurement of pH at pressures up to 250 MPa in acidic solutions were proposed by Hayert et al. *(46)* and Salerno et al. *(110)*.

PROPERTIES OF THE JUICES AND BEVERAGES

The pressure resistance of microorganisms may depend on the food matrix, which can be a very complex structural and chemical system containing components like protein, fats, sugars, and minerals. All these components have been shown to have a protective effect on microorganisms *(34, 37, 78, 89, 94, 133)*. This could be due to the fact that HPP only affects noncovalent bonds (i.e., ionic, hydrophobic, and hydrogen bonds) but does not denature covalent bonds, which leaves primary protein structure largely unaffected *(82, 102, 109)*.

The effect of sugar content on inactivation of bacteria in beverages treated with HPP has already been discussed in the section on water activity. However, the published information on the effects of juice and beverage composition, especially different proteins and lipids, on microorganisms subjected to HPP is almost nonexistent. One study using a model food system researched the effects of HPP at 300, 350, 375, 400, and 450 MPa for up to 30 min on *L. monocytogenes* at ambient temperature in a matrix containing different levels of protein (1, 2, 5, and 8% [w/v] bovine serum albumin [BSA] in PBS) and lipid (30% [v/v] olive oil) *(117)*. This study showed that increasing the concentration of BSA in the suspending medium of PBS resulted in decreasing levels of inactivation of *L. monocytogenes*. However, the minimum concentration of BSA required to increase survival to a level greater than that observed with PBS alone varied depending on the strain and on the duration of treatment. Also, the survival of *L. monocytogenes* was greater in the olive oil–PBS emulsion than in PBS alone at all treatment times. One explanation of the protective effect on microorganisms in a medium containing proteins and lipids and subjected to HPP may be related to the a_w of the medium used during pressurization. It is well documented that the presence of proteins in the suspending medium will decrease a_w and, thus, the level of free water *(95)*. Decreasing a_w has also been found to increase the resistance of bacteria to high pressure *(87)*. The denaturation of proteins that occurs during high pressure can cause breakage of hydrophobic and electrostatic interactions but not covalent bonds, such as peptide bonds *(80)*. The increase of free water during pressurization will cause an increase in the hydration of protein due to the electrostriction of water molecules around newly exposed charged groups *(72)*. Once the pressure is removed after the HPP cycle and if the level of free water is too low, the proteins may return to their native state, since they would not have enough support to remain in the denatured conformation *(117)*. Thus, reducing the a_w of the medium again would have a protective effect on microorganisms. A similar explanation may be applied to media containing lipids when subjected to HPP treatment. A change in the level of free water, especially for those bacteria exposed to the interface of lipid and water with a high concentration of hydrophilic

TABLE 5. *Factors affecting microbial inactivation during HPP in juices and beverages*[a]

Factor	Factor's role in microbial inactivation	Reference(s)
Pressure and pressure holding time	Bacterial inactivation is directly proportional to the pressure level and pressure holding time	*1*
Temp	Temp above or below room temp increases the inactivation rate of microorganisms during HPP treatment; temp in the range from 10 to 40°C correspond to the greatest resistance of bacterial cells to pressure	*30*
Compression and decompression rates	A very rapid decompression process (1 ms) during HPP treatment ranging from 70 to 400 MPa could enhance the injury and cause the higher microbial inactivation during HPP treatments; contradictory results have been also reported	*3, 4, 18, 85*
Species and strain (isolate) of pathogenic microorganism	Different species and different strains of the same species could exhibit a considerable variation in hydrostatic pressure resistance; highly pressure-resistant bacterial strains have been identified and characterized	*2, 6, 55, 95, 108*
Gram-positive and Gram-negative bacteria	Generally, Gram-positive bacteria are more resistant than Gram-negative bacteria to high pressure	*19, 69, 74, 136*
Growth stage	Bacterial cells in stationary phases were more resistant than exponential-phase cells to high pressure	*19, 69, 121*
Sublethally injured cells	Microbial cells surviving pressurization become stressed and sublethally injured, but they are not fully dead: this condition is called "viable but nonculturable" (VBNC); VBNC cells can recover under certain conditions and regrow and, thus, could pose a health hazard risk if not controlled for	*44, 56, 57*
Water activity	Lowering water activity (a_w) protects microorganisms subjected to HPP	*26, 33*
pH (acidity)	Microorganisms are more susceptible to pressure at lower pH values	*7, 47, 93, 107*
Composition and properties of the juices and beverages	Proteins, carbohydrates, lipids, salts, and minerals have a protective effect on bacteria during HPP treatment and, thus, increase microbial resistance to pressure	*78, 106*
Post-HPP storage time and temp	Conflicting results have been published on recovery of pathogenic bacteria during storage after HPP	*14, 55, 83, 126, 131*

[a] There may be specific products that may have additional critical factors which should be controlled for.

groups of emulsifier, would have a protective effect on microorganisms.

POST-HPP STORAGE TIME AND TEMPERATURE

The storage period and temperature after HPP may influence the safety of HPP-treated fruit juices and beverages *(14, 55, 83, 126, 131)*. Conflicting results have been published on recovery of pathogenic bacteria during storage after HPP. For example, no recovery of *E. coli* or *L. innocua* was observed during 3 weeks of storage at 4°C for kiwifruit and pineapple juices after HPP treatment at 350 MPa at 20°C for 60 s in 5 pulses *(14)*. The counts of a pressure-resistant strain (strain C9490) of *E. coli* O157:H7 in orange juice were reported to be reduced after the juice was treated with HPP at 500 MPa for 5 min and then held at 4°C for 0, 3, 7, or 24 h *(55)*. Nasilowska et al. *(83)* also observed no recovery of *E. coli* (ATCC 7839) in HPP-treated (300 MPa for 5 min) beetroot juice (pH 4.0 to 4.2) during storage for up to 28 days at 5°C.

Other studies have shown that pathogens may recover and grow during shelf life *(83, 126, 131)*. *L. innocua* survived and grew in HPP-treated (400 and 500 MPa for 5 min) carrot juice (pH 6.0 to 6.7) during the entire storage at 5°C up to 28 days *(83)*. Teo et al. *(126)* stated that additional studies conducted in their laboratory showed that pressure-injured cells of *E. coli* O157 and *Salmonella* were capable of surviving in acidic fruit juices for 7 days at 4°C.

Similarly, Usaga and Worobo *(131)* showed that at a pH of >4.4, *L. monocytogenes* could recover and grow in apple juice (pH 4.5) during shelf life, even when a 5-log reduction had been achieved.

CONCLUSION AND SAFE HARBOR DISCUSSION

Observations made by researchers have pointed out important considerations when evaluating HPP. The inactivation of bacteria by HPP depends on numerous factors, including the magnitude of the pressure and the holding time *(1, 65)*, the process temperature *(21)*, compression and decompression rates *(3, 85)*, the microbiota *(118)*, and intrinsic properties of juices and beverages *(106, 126)*. In addition to the above-mentioned factors, studies have shown that pathogens may recover and grow during shelf life *(126, 131)*.

Although extensive HPP research has already been carried out to characterize many factors influencing HPP, a number of issues still remain unresolved and not fully understood. To date, much of the information has been obtained using pathogenic or nonpathogenic microorganisms treated in buffer systems, where the levels of inactivation are not always in agreement when compared with those obtained in food products *(89, 123)*. Microbial baroresistance is usually higher in low-acid food products than in buffer systems *(117)*, but the mechanism(s) by which food products protect bacterial cells are not fully understood.

It also appears there is no common protocol for preparing bacterial strains for validation studies, no consensus on the HPP parameters required for treatment of juices, and no common approach on how shelf-life studies are conducted. In addition, conflicting results presented in published articles and government documents do not provide enough evidence to develop a generic processing industry guideline showing that HPP can adequately control the potential hazards and produce a safe product. These could be some of the reasons for a limited application of HPP technology in the beverage industry and for the lack of a broad safe-harbor process for HPP that covers juice products with varying compositions, characteristics, and pertinent microorganisms. While specific safe-harbor guidelines have not been developed, many of the factors influencing microbial inactivation during HPP have been identified here and summarized in Table 5. Even though the common processing conditions of 75,000 to 85,000 lb/in^2 for up to 180 s are commonly used in the juice industry, a manufacturer wanting to use one validation study for a group of juice and/or beverage products must provide valid scientific justification to show that the relevant foodborne hazards are controlled in each of its products. Conducting a robust validation study is critical to ensure that the process will consistently deliver a minimum of a 5-log reduction of the pertinent microorganism(s) for as long as the shelf life of the product during normal and moderate-abuse conditions.

The complexity of the effects of HPP technology, especially with regard to bioresistance of pathogenic and spoilage microorganisms as a function of the food matrix, pH, and water activity, warrants further scientific research (52).

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