

Supplementary Information (SI)

Title: Enhanced control of *Listeria monocytogenes* by combining High Hydrostatic Pressure (HHP) and nisin: Impact of strain tolerance, pressure level, nisin concentration and medium

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Supplementary Table ST1. *L. monocytogenes* strains used in this study.

Strain	Origin/Serotype	Reference
<i>Listeria monocytogenes</i>		
LO28	Healthy pregnant carrier, 1/2c	(Aryani et al., 2015)
L6	Milk, 1/2b	(Aryani et al., 2015)
FBR13	Frozen endive a la crème, 1/2a	(Aryani et al., 2015)
10403S	Human skin lesion, 1/2a	(Karatzas et al., 2010)
F2365	Jalisco cheese, 4b	(Aryani et al., 2015)

Supplementary Table ST2. Inoculum concentrations for five *L. monocytogenes* strains (LO28, FBR13, 10403S, L6, and F2365) used in the MIC assay.

<i>L. monocytogenes</i> strains	(Log ₁₀ CFU/mL)
LO28	5.49 (0.03)
FBR13	5.52 (0.06)
10403S	5.54 (0.08)
L6	5.42 (0.06)
F2365	5.50 (0.05)

Values represent mean viable cell counts (log₁₀ CFU/mL) with standard deviation (SD) of three independent biological replicates.

Supplementary Table ST3. Initial concentration in viable numbers of *L. monocytogenes* (LO28, FBR13, 10403S, L6, and F2365) stationary phase cells in BHI and resuspended in ACES buffer.

<i>L. monocytogenes</i> strains (Log ₁₀ CFU/mL)					
	LO28	FBR13	10403S	L6	F2365
BHI	9.43 (0.03)	9.53 (0.06)	9.49 (0.01)	9.49 (0.06)	9.57 (0.04)
ACES buffer	9.41 (0.06)	9.46 (0.05)	9.46 (0.06)	9.58 (0.06)	9.55 (0.06)

Values represent the average of three different biological replicates with the standard deviations.

Supplementary Table ST4. Initial pH values of BHI and ACES buffer cultures for each *L. monocytogenes* strain prior to HHP treatment.

<i>L. monocytogenes</i> strains					
pH	LO28	FBR13	10403S	L6	F2365
BHI	5.69 (0.22)	5.43 (0.10)	5.56 (0.10)	5.71 (0.12)	5.65 (0.09)
ACES buffer	7.05 (0.02)	7.11 (0.1)	7.02 (0.05)	7.08 (0.04)	7.10 (0.04)

Values represent the average of three biological replicates with the standard deviations (SD).

Supplementary Table ST5. Log₁₀ reductions in viable numbers of five *L. monocytogenes* strains in BHI exposed to different nisin concentrations for 45-60 min at 20 ± 2 °C.

Nisin (IU/mL)	<i>L. monocytogenes</i> strains – Log ₁₀ N/No				
	LO28	FBR13	10403S	L6	F2365
100	0.33 (0.11) ^c	0.24 (0.10) ^c	0.09 (0.06) ^b	0.26 (0.07) ^c	0.23 (0.13) ^c
250	0.64 (0.08) ^b	1.10 (0.26) ^b	0.30 (0.09) ^b	0.75 (0.11) ^b	1.11 (0.18) ^b
500	0.86 (0.11) ^a	1.59 (0.25) ^a	1.22 (0.49) ^a	1.71 (0.13) ^a	1.67 (0.15) ^a

Values are expressed as log₁₀ reductions (log₁₀ N/No) and represent the average of three biological replicates with the standard deviations.

^{a-c}: indicate the statistically significant differences between the different nisin concentrations within the same strain (Tukey HSD test, *p* < 0.05). Means that do not share a letter are significantly different.

Supplementary Table ST6. Log₁₀ reductions in viable numbers of five *L. monocytogenes* strains in ACES buffer exposed to different nisin concentrations for 45-60 min at 20 ± 2 °C.

Nisin (IU/mL)	<i>L. monocytogenes</i> strains – Log ₁₀ N/No				
	LO28	FBR13	10403S	L6	F2365
100	1.16 (0.24) ^a	1.70 (0.24) ^b	0.98 (0.20) ^b	1.48 (0.15) ^b	1.26 (0.13) ^a
250	1.24 (0.33) ^a	1.87 (0.22) ^{ab}	1.17 (0.20) ^{ab}	1.81 (0.02) ^a	1.14 (0.07) ^a
500	1.35 (0.39) ^a	2.13 (0.07) ^a	1.41 (0.05) ^a	1.83 (0.06) ^a	1.22 (0.18) ^a

Values are expressed as log₁₀ reductions (log₁₀ N/N₀) and represent the average of three biological replicates with the standard deviations.

^{a-c}: indicate the statistically significant differences between the different nisin concentrations within the same strain (Tukey HSD test, $p < 0.05$). Means that do not share a letter are significantly different.

Supplementary Table ST7. Statistical analysis of the combined effect of HHP at 200 - 350 MPa for 10 min and nisin (100, 250, and 500 IU/mL) on five *L. monocytogenes* strains (LO28, FBR13, 10403S, L6, F2365) in **a)** BHI broth and **b)** ACES buffer.

	Pressure (MPa)	Nisin (IU/mL)	<i>L. monocytogenes</i> strains				
			LO28	FBR13	10403S	L6	F2365
a) BHI	200	0	^G 0.09 (0.03) ^b	^F 0.10 (0.07) ^b	^E 0.18 (0.06) ^b	^H 0.10 (0.03) ^b	^G 0.38 (0.16) ^a
	300	0	^{CD} 3.80 (0.26) ^a	^C 2.02 (0.40) ^b	^B 3.93 (0.25) ^a	^H 0.17 (0.06) ^d	^{EF} 1.08 (0.21) ^c
	350	0	-	-	-	^E 0.87 (0.12)	^B 4.36 (0.19) [*]
	0	100	^G 0.33 (0.11) ^a	^{EF} 0.24 (0.10) ^a	^E 0.09 (0.06) ^a	^{GH} 0.26 (0.07) ^a	^G 0.23 (0.13) ^a
	200	100	^{FG} 0.43 (0.13) ^a	^{DF} 0.33 (0.07) ^a	^{DE} 0.47 (0.11) ^a	^{FH} 0.41 (0.10) ^a	^{FG} 0.70 (0.22) ^a
	300	100	^C 4.38 (0.41) ^a	^B 2.57 (0.49) ^b	^B 4.20 (0.27) ^a	^{FG} 0.51 (0.09) ^d	^E 1.56 (0.28) ^c
	350	100	-	-	-	^D 1.64 (0.15)	^B 4.84 (0.13) [*]
	0	250	^{EF} 0.64 (0.08) ^{bc}	^{DF} 1.10 (0.26) ^a	^E 0.30 (0.09) ^c	^{EF} 0.75 (0.11) ^{ab}	^{EF} 1.11 (0.18) ^a
	200	250	^{CE} 0.76 (0.09) ^b	^{DE} 1.79 (0.17) ^a	^{DE} 0.65 (0.15) ^c	^E 0.91 (0.12) ^b	^E 1.60 (0.23) ^a
	300	250	^B 4.70 (0.37) ^a	^{AB} 3.80 (0.51) ^b	^{AB} 4.48 (0.32) ^{ab}	^E 1.04 (0.14) ^d	^D 2.31 (0.03) ^c
	350	250	-	-	-	^{BC} 2.04 (0.18)	^A 5.63 (0.33) [*]
	0	500	^{DE} 0.86 (0.11) ^b	^{DE} 1.59 (0.25) ^a	^{CD} 1.22 (0.49) ^{ab}	^{CD} 1.71 (0.13) ^a	^E 1.67 (0.15) ^a
	200	500	^C 0.98 (0.16) ^b	^D 2.43 (0.33) ^a	^C 1.95 (0.55) ^a	^{BD} 1.99 (0.13) ^a	^D 2.43 (0.27) ^a
	300	500	^A 5.20 (0.32) ^a	^A 5.13 (0.37) ^a	^A 5.28 (0.44) ^a	^B 2.21 (0.26) ^c	^C 3.58 (0.30) ^b
	350	500	-	-	-	^A 3.50 (0.16)	> 7.00
b) ACES buffer	200	0	^D 0.01 (0.01) ^c	^G 0.01 (0.01) ^c	^F 0.10 (0.05) ^a	^H 0.05 (0.01) ^a	^E 0.03 (0.00) ^b
	300	0	^{CD} 0.64 (0.08) ^a	^F 0.54 (0.11) ^a	^E 0.87 (0.30) ^a	^H 0.10 (0.06) ^b	^E 0.03 (0.04) ^b
	350	0	-	-	-	^G 0.89 (0.12)	^{BD} 1.57 (0.30) [*]
	0	100	^{BC} 1.16 (0.24) ^b	^E 1.70 (0.24) ^a	^{DE} 0.98 (0.20) ^b	^F 1.48 (0.15) ^{ab}	^{CD} 1.26 (0.13) ^{ab}
	200	100	^{BC} 1.38 (0.42) ^b	^C 2.64 (0.12) ^a	^{BC} 1.72 (0.16) ^b	^{EF} 1.68 (0.14) ^b	^{CD} 1.32 (0.14) ^b
	300	100	^A 3.65 (0.40) ^a	^B 3.46 (0.21) ^{ab}	^A 2.90 (0.04) ^{bc}	^C 2.68 (0.24) ^{cd}	^B 2.01 (0.30) ^d
	350	100	-	-	-	^B 3.65 (0.18)	^A 4.46 (0.39) [*]
	0	250	^{BC} 1.24 (0.33) ^{bc}	^{DE} 1.87 (0.22) ^a	^{DE} 1.17 (0.20) ^{bc}	^{EF} 1.81 (0.02) ^{ab}	^D 1.14 (0.07) ^c
	200	250	^{BC} 1.41 (0.48) ^c	^C 2.69 (0.08) ^a	^{BC} 1.85 (0.14) ^c	^{DE} 2.01 (0.09) ^b	^{CD} 1.22 (0.09) ^d
	300	250	^A 3.20 (0.31) ^{ab}	^B 3.35 (0.18) ^a	^A 2.71 (0.24) ^b	^C 2.77 (0.07) ^b	^B 1.75 (0.11) ^c
	350	250	-	-	-	^A 5.31 (0.14) [*]	^A 4.12 (0.15)
	0	500	^{BC} 1.35 (0.39) ^{bc}	^D 2.13 (0.07) ^a	^{CD} 1.41 (0.05) ^{bc}	^{EF} 1.83 (0.06) ^{ab}	^{CD} 1.22 (0.18) ^c
	200	500	^B 1.82 (0.28) ^b	^{BC} 3.04 (0.23) ^a	^B 1.97 (0.16) ^b	^{DE} 2.02 (0.18) ^b	^{CD} 1.31 (0.11) ^c
	300	500	^A 3.21 (0.29) ^b	^A 4.03 (0.21) ^a	^A 2.74 (0.21) ^{bc}	^{CD} 2.47 (0.12) ^c	^{BC} 1.71 (0.13) ^d
	350	500	-	-	-	^A 5.58 (0.39) [*]	^A 4.00 (0.06)

Values are expressed as log₁₀ reductions (log N/N₀) and represent the average of three biological replicates with the standard deviations.

^{A-H}: indicate the statistically significant differences between different treatments within the same strain (Tukey HSD test, $p < 0.05$). Means that do not share a letter are significantly different.

^{a-c}: indicate the statistically significant differences between strains for each treatment combination (Tukey HSD test, $p < 0.05$). Means that do not share a letter are significantly different.

An asterisk (*) indicates statistically significant differences between L6 and F2365 at 350 MPa (t-test, $p < 0.05$).

Supplementary Table ST8. Statistical analysis of the synergistic effect of HHP at 200 - 350 MPa for 10 min and nisin (100, 250, and 500 IU/mL) on five *L. monocytogenes* strains (LO28, FBR13, 10403S, L6, F2365) in **a)** BHI broth and **b)** ACES buffer.

	Pressure (MPa)	Nisin (IU/mL)	<i>L. monocytogenes</i> strains (Synergistic effect - Log ₁₀ (N/N ₀))				
			LO28	FBR13	10403S	L6	F2365
a) BHI	200	100	^C 0.04 (0.02) ^b	^C 0.05 (0.05) ^{ab}	^B 0.20 (0.09) ^a	^C 0.05 (0.02) ^b	^C 0.09 (0.04) ^b
	300	100	^B 0.21 (0.08) ^b	^{BC} 0.45 (0.12) ^a	^B 0.19 (0.10) ^b	^C 0.08 (0.02) ^b	^{BC} 0.19 (0.11) ^b
	350	100	-	-	-	^B 0.49 (0.05)*	^{BC} 0.25 (0.11)
	200	250	^C 0.05 (0.02) ^b	^{BC} 0.48 (0.23) ^a	^B 0.17 (0.08) ^{ab}	^C 0.06 (0.02) ^b	^C 0.10 (0.03) ^b
	300	250	^B 0.32 (0.02) ^b	^B 0.68 (0.17) ^a	^B 0.25 (0.06) ^b	^C 0.12 (0.01) ^b	^{BC} 0.16 (0.04) ^b
	350	250	-	-	-	^B 0.48 (0.04)*	^{BC} 0.22 (0.10)
	200	500	^C 0.04 (0.02) ^d	^B 0.74 (0.13) ^a	^A 0.55 (0.08) ^{ab}	^C 0.18 (0.09) ^{cd}	^B 0.38 (0.09) ^{bc}
	300	500	^A 0.63 (0.05) ^b	^A 1.51 (0.29) ^a	^A 0.53 (0.10) ^b	^B 0.36 (0.19) ^b	^A 0.83 (0.05) ^b
	350	500	-	-	-	^A 0.98 (0.13)	> 1.05
	Pressure (MPa)	Nisin (IU/mL)	<i>L. monocytogenes</i> strains (Synergistic effect - Log ₁₀ (N/N ₀))				
			LO28	FBR13	10403S	L6	F2365
b) ACES buffer	200	100	^C 0.21 (0.21) ^b	^A 1.02 (0.28) ^a	^B 0.64 (0.12) ^{ab}	^D 0.15 (0.03) ^b	^D 0.05 (0.01) ^b
	300	100	^A 1.78 (0.31) ^a	^A 1.29 (0.08) ^{ab}	^A 1.06 (0.22) ^{ab}	^{BC} 1.11 (0.33) ^{ab}	^B 0.72 (0.35) ^b
	350	100	-	-	-	^B 1.28 (0.44)	^A 1.62 (0.29)*
	200	250	^C 0.16 (0.09) ^b	^A 0.81 (0.16) ^a	^B 0.57 (0.11) ^a	^D 0.15 (0.04) ^b	^D 0.07 (0.00) ^b
	300	250	^{AB} 1.18 (0.26) ^a	^A 0.97 (0.24) ^{ab}	^{AB} 0.67 (0.10) ^b	^{BC} 0.85 (0.03) ^{ab}	^C 0.58 (0.00) ^b
	350	250	-	-	-	^A 2.60 (0.21)*	^A 1.41 (0.13)
	200	500	^C 0.35 (0.19) ^b	^A 0.87 (0.29) ^a	^B 0.46 (0.09) ^{ab}	^D 0.14 (0.06) ^b	^D 0.08 (0.06) ^b
	300	500	^B 1.12 (0.33) ^a	^A 1.35 (0.19) ^a	^B 0.46 (0.19) ^b	^C 0.54 (0.11) ^b	^C 0.46 (0.09) ^b
	350	500	-	-	-	^A 2.86 (0.26)*	^{AB} 1.21 (0.09)

Values are expressed as log₁₀ reductions (log N/N₀) and represent the average of three biological replicates with the standard deviations.

^{A-D}: indicate the statistically significant differences between different treatments within the same strain (Tukey HSD test, $p < 0.05$). Means that do not share a letter are significantly different.

^{a-d}: indicate the statistically significant differences between strains for each treatment combination (Tukey HSD test, $p < 0.05$). Means that do not share a letter are significantly different.

An asterisk (*) indicates statistically significant differences between L6 and F2365 at 350 MPa (t -test, $p < 0.05$).

Supplementary Table ST9. Statistical comparison between BHI and ACES buffer regarding the combined effect of HHP and nisin.

	Pressure (MPa)	Nisin (IU/mL)	<i>L. monocytogenes</i> strains				
			LO28	FBR13	10403S	L6	F2365
BHI	200	0	0.09 (0.03)*	0.10 (0.07)	0.18 (0.06)	0.10 (0.03)	0.38 (0.16)*
ACES	200	0	0.01 (0.01)	0.01 (0.01) ^{ns}	0.10 (0.05) ^{ns}	0.05 (0.01) ^{ns}	0.03 (0.00)
BHI	300	0	3.80 (0.26)*	2.02 (0.40)*	3.93 (0.25)*	0.17 (0.06)	1.08 (0.21)*
ACES	300	0	0.64 (0.08)	0.54 (0.11)	0.87 (0.30)	0.10 (0.06) ^{ns}	0.03 (0.04)
BHI	350	0	-	-	-	0.87 (0.12)	4.36 (0.19)*
ACES	350	0	-	-	-	0.89 (0.12) ^{ns}	1.57 (0.30)
BHI	0	100	0.33 (0.11)	0.24 (0.10)	0.09 (0.06)	0.26 (0.07)	0.23 (0.13)
ACES	0	100	1.16 (0.24)*	1.70 (0.24)*	0.98 (0.20)*	1.48 (0.15)*	1.26 (0.13)*
BHI	200	100	0.43 (0.13)	0.33 (0.07)	0.47 (0.11)	0.41 (0.10)	0.70 (0.22)
ACES	200	100	1.38 (0.42)*	2.64 (0.12)*	1.72 (0.16)*	1.68 (0.14)*	1.32 (0.14)*
BHI	300	100	4.38 (0.41)*	2.57 (0.49)	4.20 (0.27)*	0.51 (0.09)	1.56 (0.28)
ACES	300	100	3.65 (0.40)	3.46 (0.21)*	2.90 (0.04)	2.68 (0.24)*	2.01 (0.30) ^{ns}
BHI	350	100	-	-	-	1.64 (0.15)	4.84 (0.13)
ACES	350	100	-	-	-	3.65 (0.18)*	4.46 (0.39) ^{ns}
BHI	0	250	0.64 (0.08)	1.10 (0.26)	0.30 (0.09)	0.75 (0.11)	1.11 (0.18)
ACES	0	250	1.24 (0.33)*	1.87 (0.22)*	1.17 (0.20)*	1.81 (0.02)*	1.14 (0.07) ^{ns}
BHI	200	250	0.76 (0.09)	1.79 (0.17)	0.65 (0.15)	0.91 (0.12)	1.60 (0.23)
ACES	200	250	1.41 (0.48) ^{ns}	2.69 (0.08)*	1.85 (0.14)*	2.01 (0.09)*	1.22 (0.09)*
BHI	300	250	4.70 (0.37)*	3.80 (0.51)	4.48 (0.32)*	1.04 (0.14)	2.31 (0.03)*
ACES	300	250	3.20 (0.31)	3.35 (0.18) ^{ns}	2.71 (0.24)	2.77 (0.07)*	1.75 (0.11)
BHI	350	250	-	-	-	2.04 (0.18)	5.63 (0.33)*
ACES	350	250	-	-	-	5.31 (0.14)*	4.12 (0.15)
BHI	0	500	0.86 (0.11)	1.59 (0.25)	1.22 (0.49)	1.71 (0.13)	1.67 (0.15)
ACES	0	500	1.35 (0.39)*	2.13 (0.07)*	1.41 (0.05) ^{ns}	1.83 (0.06) ^{ns}	1.22 (0.18)*
BHI	200	500	0.98 (0.16)	2.43 (0.33)	1.95 (0.55)	1.99 (0.13)	2.43 (0.27)*
ACES	200	500	1.82 (0.28)*	3.04 (0.23)*	1.97 (0.16) ^{ns}	2.02 (0.18) ^{ns}	1.31 (0.11)
BHI	300	500	5.20 (0.32)*	5.13 (0.37)*	5.28 (0.44)*	2.21 (0.26)	3.58 (0.30)*
ACES	300	500	3.21 (0.29)	4.03 (0.21)	2.74 (0.21)	2.47 (0.12) ^{ns}	1.71 (0.13)
BHI	350	500	-	-	-	3.50 (0.16)	> 7.00
ACES	350	500	-	-	-	5.58 (0.39)*	4.00 (0.06)

Values are expressed as log₁₀ reductions (log N/N₀) and represent the average of three biological replicates with the standard deviations (SD). Asterisk (*) indicates statistically significant differences between BHI and ACES buffer within the same pressure and nisin concentration for each *L. monocytogenes* strain, while "ns" denotes no significant difference (t-test; $p < 0.05$).

Supplementary Table ST10. Statistical comparison between BHI and ACES buffer regarding the synergistic effect of HHP and nisin.

	Pressure (MPa)	Nisin (IU/mL)	<i>L. monocytogenes</i> strains (Synergistic effect - Log ₁₀ (N/N ₀))				
			LO28	FBR13	10403S	L6	F2365
BHI	200	100	0.04 (0.02)	0.05 (0.05)	0.20 (0.09)	0.05 (0.02)	0.09 (0.04)
ACES	200	100	0.21 (0.21) ^{ns}	1.02 (0.28)*	0.64 (0.12)*	0.15 (0.03)*	0.05 (0.01) ^{ns}
BHI	300	100	0.21 (0.08)	0.45 (0.12)	0.19 (0.10)	0.08 (0.02)	0.19 (0.11)
ACES	300	100	1.78 (0.31)*	1.29 (0.08)*	1.06 (0.22)*	1.11 (0.33)*	0.72 (0.35)*
BHI	350	100	-	-	-	0.49 (0.05)	0.25 (0.11)
ACES	350	100	-	-	-	1.28 (0.44)*	1.62 (0.29)*
BHI	200	250	0.05 (0.02)	0.48 (0.23)	0.17 (0.08)	0.06 (0.02)	0.10 (0.03)
ACES	200	250	0.16 (0.09) ^{ns}	0.81 (0.16) ^{ns}	0.57 (0.11)*	0.15 (0.04) ^{ns}	0.07 (0.00) ^{ns}
BHI	300	250	0.32 (0.02)	0.68 (0.17)	0.25 (0.06)	0.12 (0.01)	0.16 (0.04)
ACES	300	250	1.18 (0.26)*	0.97 (0.24)*	0.67 (0.10)*	0.85 (0.03)*	0.58 (0.00)*
BHI	350	250	-	-	-	0.48 (0.04)	0.22 (0.10)
ACES	350	250	-	-	-	2.60 (0.21)*	1.41 (0.13)*
BHI	200	500	0.04 (0.02)	0.74 (0.13)	0.55 (0.08)	0.18 (0.09)	0.38 (0.09)*
ACES	200	500	0.35 (0.19) ^{ns}	0.87 (0.29) ^{ns}	0.46 (0.09) ^{ns}	0.14 (0.06) ^{ns}	0.08 (0.06)
BHI	300	500	0.63 (0.05)	1.51 (0.29)	0.53 (0.10)	0.36 (0.19)	0.83 (0.05)*
ACES	300	500	1.12 (0.33) ^{ns}	1.35 (0.19) ^{ns}	0.46 (0.19) ^{ns}	0.54 (0.11) ^{ns}	0.46 (0.09)
BHI	350	500	-	-	-	0.98 (0.13)	> 1.04
ACES	350	500	-	-	-	2.86 (0.26)*	1.21 (0.09)

Values are expressed as log₁₀ reductions (log N/N₀) and represent the average of three biological replicates with the standard deviations (SD). Asterisk (*) indicates statistically significant differences between BHI and ACES buffer within the same pressure and nisin concentration for each *L. monocytogenes* strain, while "ns" denotes no significant difference (t-test; $p < 0.05$).