



***Listeria monocytogenes* survival in refrigerator dill pickles**

Jin Kyung Kim*, Elaine M. D'Sa, Mark A. Harrison*, Judy A. Harrison**, and Elizabeth L. Andress****

* Dept. of Food Science and Technology, University of Georgia, Athens

** Dept. of Foods and Nutrition, University of Georgia, Athens

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Note: This research study analyzed one particular pickling procedure that started with partially fermenting cucumbers at room temperature and then storing them in the refrigerator with no further treatment or processing. It does not represent findings or advice for any other type of refrigerator pickles.

Abstract

Listeria monocytogenes can survive and grow in refrigerated foods with pH levels of approx. 4.0-5.0 and salt concentrations of 3-4%. Home-fermented refrigerator dill pickles fit this description. Contamination of this product with *L. monocytogenes* could cause serious problems since these items are not heated prior to consumption. This study determined *L. monocytogenes* survival and growth patterns in refrigerator dill pickles at three salt levels. Pickling cucumbers were inoculated with *L. monocytogenes*, brine mixtures were added and the cucumbers were held at room temperature for one week and then refrigerated for up to 3 months. The pH, percent NaCl, percent titratable acidity and total aerobic, psychrotrophic, lactic acid bacteria and *Listeria* populations were measured at the addition of brine, at 2, 4, and 7 days during storage at room temperature and then at weekly intervals during refrigerated storage. There was a rapid decrease in pickle pH after four days at room temperature (from 6.1-6.2 to 4.4-4.6) followed by a gradual decrease. The percent NaCl in the pickles rapidly increased up to 3 weeks at refrigeration temperatures, and the percent titratable acidity in the highest salt level was significantly lower ($p < 0.05$). The initial *Listeria* population was 5.4-5.6 log cfu/cm² on the surface and 3.9-4.6 log cfu/g internally. There was approximately a 0.5-1 log increase during fermentation at room temperature followed by a population decline during refrigerator storage, with a greater decrease in the pickles with the highest NaCl content. Populations of total aerobes and lactic acid bacteria increased during room temperature storage and decreased gradually.

Introduction

- *L. monocytogenes* has been a major food safety concern since the 1st outbreak due to consumption of coleslaw in 1981 (Shlech 1983)
 - *L. monocytogenes* is widely distributed in the environment

- Isolated from various environment and food sources
- Cross-contamination with *L. monocytogenes* can occur
- Found within the food manufacturing environment
- Can attach to various manufacturing surfaces and produce a biofilm
- Adaptation of *L. monocytogenes* to environmental stresses
 - pH: can survive and/or grow as low as pH 4.4 (George 1988)
 - Salt concentration: can survive up to 14% NaCl (Farber 1992)
 - Temperature: remain metabolically active at 3°C (Walker 1990)
- Pickling (Brandt 1996)
 - Preserving vegetables, meat and fish with salt and acid
 - Cucumbers are one of the more commonly pickled foods in the U.S.
- Home-fermented refrigerator dill pickles
 - Lactic acid fermentation of cucumbers
 - Made by immersing the pickling cucumbers into a brine solution
 - Kept at room temperature for 1 week followed by storage at refrigeration temperatures during the consumption period

Objective

To determine the fate of *L. monocytogenes* on the surface and in the interior of cucumbers and in brines subjected to the different salt levels (1.3, 3.8, 7.6%)

Materials and Methods

- *Bacterial strains and inoculum preparation*
 - Five *L. monocytogenes* strains, 301, V7, LCDC, Scott A, and Brie used
 - Cultured in 10 ml of TSB and incubated for 24 h at 37°C
 - Transferred to 4,000 ml of TSB and incubated for 24 h at 37°C
 - Centrifuged at 4,550 x g for 30 min
 - Pellet was resuspended into sterile 0.1% peptone water
- *Inoculation of cucumbers*
 - Long, unwashed, pickling cucumbers (approx. 10 cm)
 - Washed with tap water and drained for 30 min
 - Immersed cucumbers into an inoculum of *L. monocytogenes* for 15 min
 - Cucumbers drained on a sterile metal grid rack for 15 min
 - 3 salt levels (1.3, 3.8, and 7.6%) with *L. monocytogenes* and a control treatment of 3.8% NaCl and no inoculum used
- *Physical Analyses*
 - pH measurement in cucumbers and brines with a pH meter
 - Percent titratable acidity: titrated 20 g of sample with 0.1N NaOH - % Acid = $(\text{mL NaOH})(N \text{ NaOH})(\text{milieq. wt of acid})(100)(\text{wt of sample}(\text{g}))^{-1}$
 - Percent NaCl: Quantab chloride titration strips with calibration table
- *Microbiological properties*
 - Sampling interval: at the addition of brine (0), 2, 4, 7 d at room temperature, 1, 2, 3, 4, 6, 8, 10, 12 weeks during refrigerated storage
 - Sampling procedure
 - Surface of cucumber: cut into 2.5 x 2.5 cm pieces using sterile knife
 - Interior of cucumber: 10 g, using sterile corer from the blossom end
 - Brine: directly diluted using peptone water
 - Microbiological count

- Total aeobes: PCA, incubated at 35°C for 24 h
- Psychrotrophs : PCA, incubated at 7°C for 7 d
- Lactic acid bacteria: MRS 2X, incubated at 35°C for 48 h anaerobically
- *Listeria*: LSA (Listeria Selective Agar), incubated at 35°C for 48 h
- *Listeria* enrichment
 - 1 ml of from bag inoculated into 9 ml LEB (Listeria Enrichment Broth)
 - Incubated at 30°C for 48 h
 - Streaked onto LSA and incubated at 35°C for 48 h
 - Checked for *Listeria* colonies
- Identification and confirmation test
 - Gram stain, Catalase/Oxidase reaction
 - Umbrella-like growth in motility medium
 - Biochemical testing using Micro ID kits (Remel, Lenex, KS)
- *Statistical Analysis*
 - ANOVA (Statistical Analysis Systems Institute, Cary, NC)
 - Duncan's multiple range tests - significance value: $\alpha=0.05$

Results

Table 1. Population of *L. monocytogenes* on surface and in interior of cucumbers at three salt levels

Sampling parts	Salt levels	Storage time (days)											
		0	2	4	7	14	21	28	35	49	63	77	91
Surface (log cfu/cm ²)	1.3%	^A 5.59	^A 6.15	^A 5.98	^A 5.36	^B 3.59 ^b	^{BC} 3.16	^{BC} 2.79	^{BC} 2.69	^{BC} 3.07	^C <2.49 (3/3)	^C <2.49 (3/3)	^C <2.49 (3/3)
	3.8%	^A 5.41	^A 5.86	^A 5.57	^A 5.55	^A 5.42 ^a	^B 3.81	^{BC} 3.29	^C 2.88	^C 2.94	^C 2.59	^C <2.49 (3/3)	^C <2.49 (3/3)
	7.6%	^B 5.57	^A 5.89	^A 5.65	^B 5.22	^C 3.78 ^b	^D <2.49 (3/3)	^D <2.49 (3/3)	^D <2.49 (3/3)	^D 2.59	^D <2.49 (3/3)	^D 2.64	^D <2.49 (2/3)
Interior (log cfu/g)	1.3%	^A 4.57	^A 4.55	^A 4.41	^B 3.44	^C 2.30	^{BC} 2.92	^C <2.30 (3/3)	^C 2.46 ^a	^D <2.30 (0/3)	^D <2.30 (0/3)	^D <2.30 (0/3)	^D <2.30 (0/3)
	3.8%	^A 4.30	^{AB} 3.64	^{ABC} 3.38	^{BCD} 2.68	^{BCD} 2.93	^{CD} 2.46	^D <2.30 (2/3)	^E <2.30 ^b (0/3)	^E <2.30 (0/3)	^E <2.30 (0/3)	^E <2.30 (0/3)	^E <2.30 (0/3)
	7.6%	^A 3.88	^B 3.03	^B 3.08	^{BC} 2.70	^{BC} 2.68	^C <2.30 (1/3)	^C <2.30 (1/3)	^D <2.30 ^b (0/3)	^D <2.30 (0/3)	^D <2.30 (0/3)	^D <2.30 (0/3)	^D <2.30 (0/3)

ABCD Means with different upper case letters in a row are significantly different (p<0.05)

abc Means with different lower case letters in a column are significantly different (p<0.05)

1/3 Number of tubes showing positive result /number of replicates

- Initial population - Surface: 5.4–5.6 log cfu/cm²; Interior: 3.9–4.6 log cfu/g
- *Listeria* populations decreased the most in the highest salt content during refrigerator storage
- *Listeria* populations were not detected on surfaces or in the interiors, after 8 and 4 weeks at refrigerator temperature, respectively

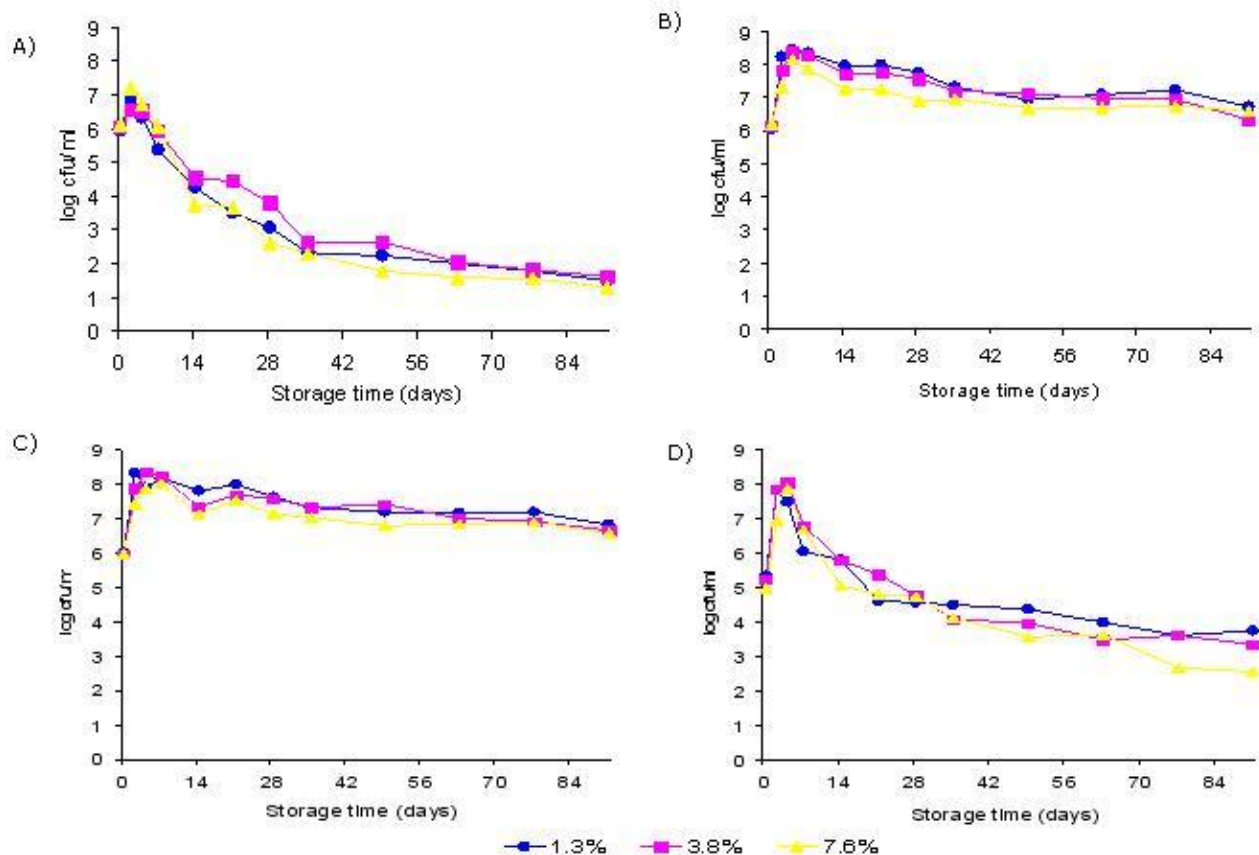


Fig. 1. Change patterns of microorganisms in the brines at three salt levels
 A) *Listeria* B) Total Aerobes C) Lactic Acid Bacteria D) Psychrotrophs

- Similar change patterns were observed for each type of microorganism
- Initial population of *L. monocytogenes*: 6–6.1 log cfu/ml
- Total aerobes increased by 3 logs after 4 d at room temperature and then, gradually decreased
- No significant difference ($p > 0.05$) in the number of lactic acid bacteria among salt levels

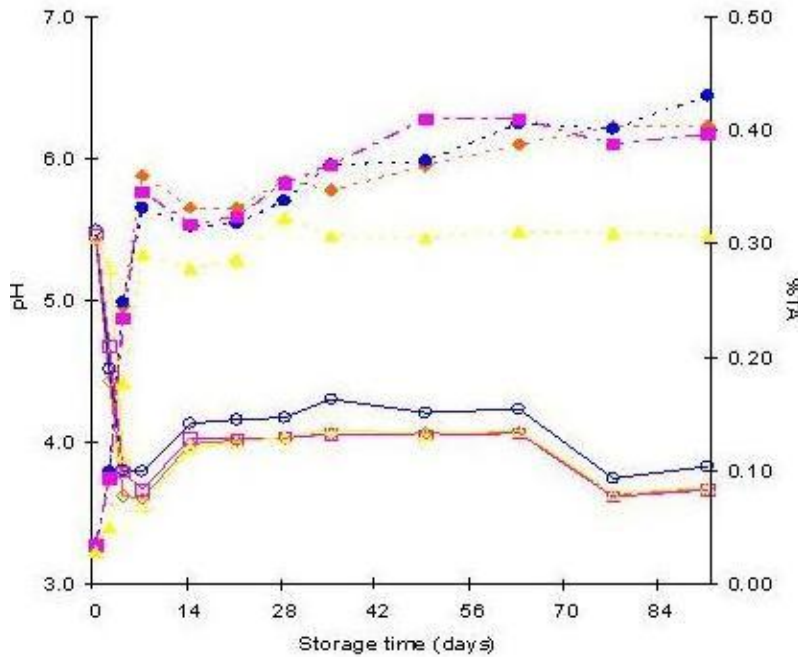
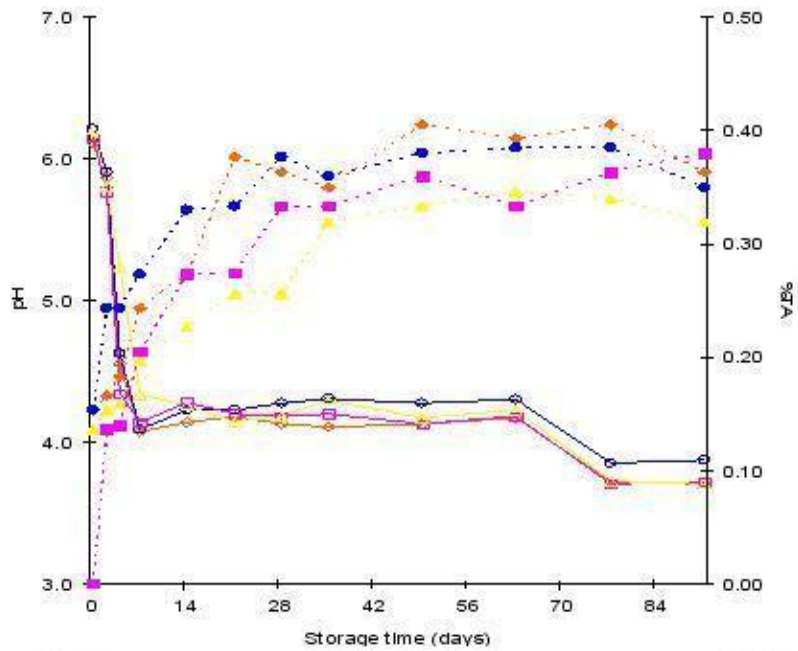
Table 2. Population of total aerobes on surface and in interior of cucumbers at three salt levels

Sampling parts	Salt levels	Storage time (days)											
		0	2	4	7	14	21	28	35	49	63	77	91
Surface (logcfu/cm ²)	1.3%	BCD6.51	ABC7.27	A7.97 ^a	AB7.44	ABC6.92	BCD6.68	CD6.22 ^a	BCD6.36	DE5.89 ^a	DE6.01 ^a	E5.03	E4.95
	3.8%	BCDE6.59	ABC7.02	A7.56 ^a	AB7.29	ABC7.01	ABCD6.77	CDEF6.22 ^a	DEF5.98	EF5.77 ^a	FG5.32 ^a	F5.57	G4.66
	7.6%	BC5.75	AB6.25	A6.82 ^a	A6.74	AB6.50	AB6.42	CD5.03 ^a	CD5.00	CD4.87 ^a	D4.79 ^a	D4.83	D4.12
Interior (logcfu/g)	1.3%	BCD4.74	AB6.73 ^a	A6.95	AB6.66	ABC6.55 ^a	ABCD5.48	ABCD5.20 ^a	ABCD5.20 ^a	BCD4.76 ^a	CD4.60 ^a	BCD4.82 ^a	D4.30 ^a
	3.8%	CD4.65	BC5.04 ^b	A6.01	A5.96	AB5.79 ^a	AB5.62	AB5.52 ^a	DE4.16 ^b	EF3.77 ^a	FG3.03 ^b	G2.54 ^b	FG3.15 ^b
	7.6%	CD3.69	BC4.53 ^b	AB4.83	A5.63	BC4.07 ^b	CD3.82	DE3.17 ^b	DE2.93 ^b	E<2.30 ^b	E<2.30 ^b	E2.54 ^b	E2.46 ^b

ABCD Means with different upper case letters in a row are significantly different ($p < 0.05$)

abc Means with different lower case letters in a column are significantly different ($p < 0.05$)

- Aerobic populations were significantly higher ($p < 0.05$) at 1.3 % salt levels
- Aerobic populations were greatest at 4 (surface) or 7 (interior) d at room temperature
- Aerobic populations decreased gradually during refrigeration temperature



◆ 3.8%, uninoc.,pH ● 1.3%,pH ■ 3.8%,pH ▲ 7.6%,pH
◆ 3.8%, uninoc.,%TA ● 1.3%,%TA ■ 3.8%,%TA ▲ 7.6%,%TA

Fig. 2. The pH and percent titratable acidity at three salt levels - A) Cucumbers B) Brines

- There was a rapid pH decrease from 6.1–6.2 to 4.4–4.6 (cucumbers), from 5.5 to 3.6–3.9 (brines), except the highest salt level (from 6.2 to 5.2), by the 4th day.
- Significantly lower ($p < 0.05$) percent titratable acidity for brines in 7.6% salt level

Table 3. Population of psychrotrophs on surface and in interior of cucumbers at three salt levels

Sampling parts	Salt levels	Storage time (days)												
		0	2	4	7	14	21	28	35	49	63	77	91	
Surface (log cfu/cm ²)	1.3%	BC 6.09	A 7.92 ^a	B 6.68	B 6.50	BCD 5.59	CD 5.06	DE 4.48	DE 4.38	EF 3.67	EF 3.61	F 2.73	F 2.58	
	3.8%	AB 6.30	A 7.03 ^a	A 7.28	A 6.91	AB 6.73	BC 5.79	CD 5.26	CD 4.95	D 4.29	E 2.94	E 2.99	E 2.79	
	7.6%	AB 5.61	AB 5.80 ^b	A 6.52	A 6.38	AB 5.87	BC 4.69	CD 3.65	CD 3.57	CD 3.19	CD 3.09	D 2.99	D 2.77	
Interior (log cfu/g)	1.3%	B 4.72	A 6.54	A 6.41	AB 5.73	AB 5.30 ^a	C 3.03	C 2.46	C 3.01	C <2.30	C 3.11	C <2.30	C <2.30	
	3.8%	BC 4.23	AB 5.17	A 5.52	A 5.68	A 5.62 ^a	D 3.05	CD 3.25	D 2.71	D 2.40	D 2.46	D <2.30	D 2.46	
	7.6%	AB 4.53	B 4.33	A 4.80	A 4.78	C 3.45 ^b	D <2.30	D <2.30	D 2.40	D 2.40	D 2.46	D <2.30	D <2.30	

ABCD Means with different upper case letters in a row are significantly different (p<0.05)

abc Means with different lower case letters in a column are significantly different (p<0.05)

- Psychrotrophic populations increased 1-2 logs at room temperature and then decreased
- Psychrotrophic populations decreased 2-3.5 logs at the end of storage time
- For each sampling day there was no significant difference (p<0.05) in psychrotrophic populations in the pickle interiors among salt levels

Conclusion

- Based on old recommendations, consumption of refrigerator dill pickles could typically occur anytime after 3 days of refrigerated storage
- Since *L. monocytogenes* may still be viable well after this point, there is a food safety risk involved
- Recommendations to prepare this product in the home should not be distributed

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Contact:

National Center for Home Food Preservation
208 Hoke Smith Annex
The University of Georgia
Athens, GA 30602-4356

Tel: (706) 542-3773

Fax: (706) 542-1979

Web: <http://www.homefoodpreservation.com>

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