Fate of *Listeria monocytogenes* on Citric Acid-Treated Hard-Cooked Eggs

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Abstract

Commercially-prepared hard-cooked eggs are available for food service and to the public in retail grocers. Potential contamination *with Listeria monocytogenes* after the cooking and peeling steps is of concern since this pathogen can proliferate at refrigeration temperatures. This study evaluated the efficacy of citric acid treatment of hard-cooked peeled eggs to reduce the population levels of *L. monocytogenes*. Fresh eggs were boiled for 12 min, cooled to 4°C, peeled, and stored at 4°C for 24 h prior to experiments. Hard-cooked eggs were dip inoculated with a 4-strain cocktail of rifampicin-resistant *L. monocytogenes* resulting in either 4 (low) or 8 (high) log CFU/egg. Eggs were air dried 10 min, followed by treatment with pH 2.5 citric acid (3.84 g/L) at 5 or 25°C for 24 h. *L. monocytogenes* populations were enumerated at intervals up to 24 h by homogenization of eggs with BLEB and cultivation on BHI/rifampicin agar. Triplicate eggs were assessed for each timepoint, and three independent trials were conducted. At a low inoculation level, the *L. monocytogenes* population on the eggs was 3.95±0.69 and 3.74±0.76 log CFU/egg prior to treatment with 5 or 25°C citric acid, respectively. After 1 h, *L. monocytogenes* was reduced by 0.56 and 0.18 log CFU/egg when treated at 5 or 25°C, respectively; after 24 h, reductions were 0.73 and 0.86 log CFU/egg. At a high inoculation level, the *L. monocytogenes* population on the eggs was 7.93±0.26 and 8.04±0.47 log CFU/egg prior to treatment with 5 or 25°C citric acid, respectively. After 1 h, *L. monocytogenes* was reduced by 0.48 and 0.67 log CFU/egg when treated at 5 or 25°C, respectively; after 24 h, reductions were 1.23 and 1.16 log CFU/egg. The data obtained in this study can be used to determine the efficacy of citric acid against *L. monocytogenes* during treatment of hard cooked eggs.

Plain language summary: Retail hard-cooked eggs may be contaminated with *Listeria monocytogenes* during processing and packaging. Citric acid can be used to reduce this risk, but it is not understood how well it works. We inoculated eggs with *Listeria* and treated them with citric acid, but only 10% of the cells were killed during treatment.

Introduction

An outbreak of listeriosis associated with hard-cooked and peeled eggs occurred in the U.S. in 2019 which resulted in eight illnesses, four hospitalizations, and one death (1). The hard-cooked eggs had been treated with citric acid, which is a common preservative used in the food industry. However, little information exists in the published literature regarding how well citric acid may reduce *L. monocytogenes* levels on treated hard-cooked eggs. Therefore, the purpose of this project was to evaluate the efficacy of a pH 2.5 citric acid treatment at either 5 or 25°C to reduce the population levels of *L. monocytogenes* on hard-cooked eggs.

Materials and Methods

Preparation of hard-cooked eggs

- Large Grade A eggs were boiled for 12 min, followed by submersion in ice water
- Eggs were stored at 4°C for 24 h prior to peeling
- Peeled eggs were stored at 4°C and used within 24 h

Dip inoculation

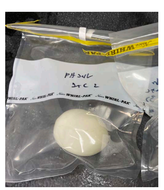
- 24 eggs were submerged in 1.6 L of a 9 or 6 log CFU/mL *L. monocytogenes* four-strain rifampicin-resistant cocktail for 30 min
- Eggs were removed from the inoculum and dried on wire racks for 10 min

Citric acid treatment

- pH 2.5 citric acid was prepared and stored at either 5 or 25°C
- Eggs were submerged in a 2× volume of citric acid at either 5 or 25°C for 24 h
- During treatment, the pH of the citric acid, egg white, egg yolk, and whole egg was measured

Enumeration of *L. monocytogenes*

- Triplicate eggs removed from the citric acid at 0 (no treatment), 5, 15, 30, 60 min, and 6 and 24 h and placed individually into 18 oz. stomacher bags
- Eggs (~50 g) were homogenized 1:1 with Buffered *Listeria* Enrichment Broth (BLEB) in a stomacher for 1 min
- Serial dilutions of the homogenate were plated onto Brain Heart Infusion (BHI) agar supplemented with rifampicin
- BHI agar plates were incubated at 37°C for 24-48 h



Results and Discussion

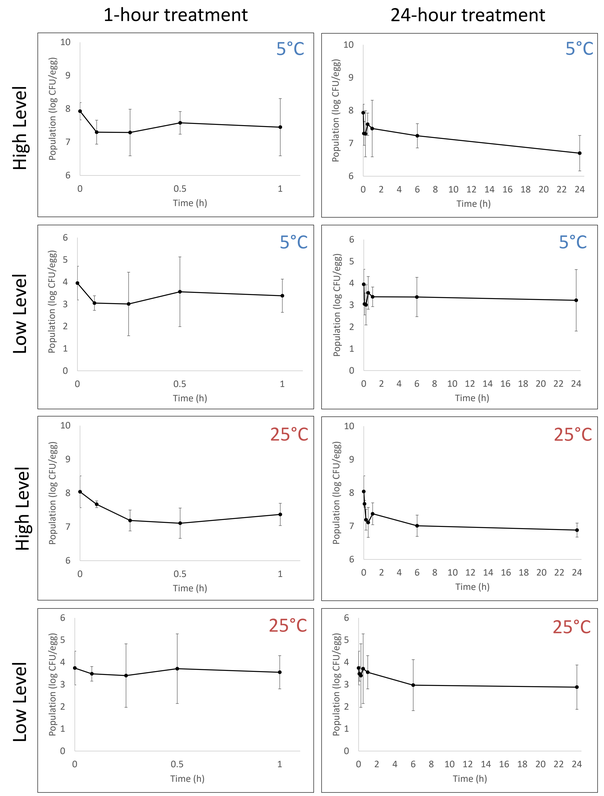


Figure 1. Population of *L. monocytogenes* (log CFU/egg) on hard-cooked eggs during citric acid treatment at 5 or 25°C when eggs were inoculated with either 8 log CFU/egg (high level) or 4 log CFU/egg (low level).

Table 1. The pH of the citric acid liquid, the egg white, the egg yolk, and the whole egg during 5 or 25°C citric acid treatment.

Time (h)	5°C Treatment				25°C Treatment			
	Liquid	Egg white	Egg yolk	Whole egg	Liquid	Egg white	Egg yolk	Whole egg
0	2.25	7.83	6.98	7.13	2.1	7.87	6.82	7.37
1	2.57	5.51	6.21	6.72	2.44	3.2	5.64	6.97
6	2.69	5.64	5.67	6.81	2.65	3.7	6.48	6.64
24	2.65	4.94	6.77	6.56	2.71	3.66	6.64	6.28

Table 2. *L. monocytogenes* reductions (log CFU/egg) on hard-cooked eggs during 5 or 25°C citric acid treatment when eggs were inoculated with either 8 log CFU/egg (high level) or 4 log CFU/egg (low level).

Time (h)	5°C Treatment		25°C Treatment	
	High	Low	High	Low
1	0.48	0.56	0.67	0.18
6	0.70	0.58	1.04	0.77
24	1.23	0.73	1.16	0.86

Conclusion

- *L. monocytogenes* reductions on hard-cooked eggs inoculated with 8 log CFU/egg were 0.48 and 0.67 log CFU/egg after 1 h and 1.23 and 1.16 log CFU/egg after 24 h treatment at 5 and 25°C, respectively.
- *L. monocytogenes* reductions on hard-cooked eggs inoculated with 4 log CFU/egg were 0.48 and 0.67 log CFU/egg after 1 h and 0.73 and 0.86 log CFU/egg after 24 h treatment at 5 and 25°C, respectively.
- The largest *L. monocytogenes* reduction on hard-cooked eggs was observed after 24 h when eggs were treated at 5C and when inoculated at a high level (1.23 log CFU/egg reduction).
- Overall, this study provides information on the efficacy of a pH 2.5 citric acid treatment for the reduction of *L. monocytogenes* on hard-cooked eggs.
- Future research could explore different treatment types, including a lower pH citric acid or other organic acids.

Reference

(1) Centers for Disease Control and Prevention (CDC). 2020. Outbreak of Listeria infections linked to hard-boiled eggs. Final update. Available at https://www.cdc.gov/listeria/outbreaks/eggs-12-19/index.html.

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