Pseudomonas Survive Thermal Processing and Grow during Vacuum Packaged Storage in an Emulsified Beef System

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Summary with Implications

New research has suggested the ability of Pseudomonas*, a common spoilage microorganism, to grow in cooked beef products stored under vacuum which challenges the traditional understanding of the role of* Pseudomonas *during cooked beef spoilage. Understanding the mechanisms of survival and growth of* Pseudomonas *in these products is crucial for improving shelf life. The objective of this experiment was to determine* Pseudomonas *survival in a thermally processed, emulsified cooked beef model system. After eight weeks of refrigerated storage,* Pseudomonas *was recovered from cooked emulsified beef, indicating the potential for* Pseudomonas *to survive thermal processing and cause spoilage in cooked vacuum packaged beef products.*

Introduction

Some *Pseudomonas* species, like *P. fragi, P. lundensis*, and *P. fluorescens*, are considered the predominant microbial spoilers of aerobically stored raw meat products, such as meat in overwrap packaging, with minor roles in vacuum packed meat product spoilage. Lactic acid bacteria have traditionally been understood to be the primary bacterial spoilers of vacuum packaged cooked meat products. Additionally, traditional understanding has been that *Pseudomonas* are not capable of growing in anaerobic environments. However, recent findings have challenged this principle and opened new avenues for research on the role of *Pseudomonas* in the spoilage of thermally processed vacuum packaged meat. Spoilage *Pseudomonas* can be found at all stages of animal agriculture and food processing suggesting the natural animal environment and contamination from the food processing environment could both contribute to the *Pseudomonas* presence in vacuum packed cooked beef product. Thermal processing in the meat industry is implemented to achieve product safety by reducing the pathogenic bacteria present in the raw meat and typically is not used to completely sterilize a product. Given the potential thermal resistance of *Pseudomonas*, populations that survive cooking may also be responsible for product spoilage. Therefore, an experiment was conducted to determine whether spoilage *Pseudomonas* can survive thermal processing and grow anaerobically through refrigerated storage in an emulsified model beef system.

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Table 1. Concentration of *Pseudomonas* (log_{10} cfu/g ± SE) in emulsified beef during thermal processing and 39° F refrigerated storage (P < 0.01)

Sampling time	Uncooked Control	130° F Cooked	160° F Cooked
Inoculated raw beef	4.93 ± 0.05 [a]	5.01 ± 0.04[a]	5.06 ± 0.04[a]
After cooking or emulsifying (control)	4.75 ± 0.07[a]	0.18 ± <0.01[c]	0.18 ± <0.01[c]
14 days storage	3.73 ± 0.06[b]	0.44 ± 0.09[cde]	0.39 ± 0.09[cde]
28 days storage	3.81 ± 0.10[b]	0.23 ± 0.04[dc]	0.69 ± 0.21[e]
56 days storage	3.55 ± 0.17[b]	0.57 ± 0.25[de]	0.67 ± 0.24[e]

[a-e]Means within the table with different superscripts differ ($P < 0.05$)

Procedure

Three *Pseudomonas* isolates collected from spoiled meat were grown individually in Luria-Bertani broth for 48 hours at 89° F (32° C) and combined to create an inoculation cocktail (approx. 8 log_{10} colony forming units (cfu)/g). Coarse ground beef (4.4 lbs.) was inoculated by directly adding inoculation cocktail to the meat to approximately 5 log_{10} cfu/g of *Pseudomonas* and emulsified to form a frankfurter-like meat batter with ice, salt, sodium nitrite, sodium erythorbate, black pepper, and garlic in a Hobart Food Processor. Batter samples (ca. 20 grams, approx. 2 by 2 inches, and < 0.6 inch thickness) were vacuum packaged individually and packages were allocated into three treatments: two cooked treatments (heated to final temperatures of 160° F held for one second or 130° F held for 121 minutes) and one uncooked treatment. Samples were cooked in water baths using sous vide units to target internal temperatures and then chilled in an ice bath for 15 minutes. For the 130° F treatment, samples were placed in a 130° F water bath and upon reaching 130° F, held for 121 minutes. For the 160° F treatment, samples were placed in 145° F water bath for one hour, then moved to a 155° F water batch for 30 minutes, and then held in a 175° F water bath until reaching 160° F. Time-temperature combinations for cooking treatments were based on common thermal processing schedules used in the meat industry. After cooking, samples from all treatment groups were split into refrigerated storage at 39 and 50° F. *Pseudomonas* concentrations were determined after inoculation, after chilling for cooked samples and after emulsifying for uncooked samples, and at 14, 28, and 56 days of storage. At each sampling time, 10 grams of an individually packed sample were stomached with 20 grams of buffered peptone water. Homogenates were serially diluted and plated onto *Pseudomonas* Agar Base plates supplemented with Cetrimide-Fucidin-Cephalosporin Selective Supplement to solely determine the concentration of *Pseudomonas*. The experiment was

Table 2. Concentration of *Pseudomonas* (log_{10} cfu/g ± SE) in emulsified beef during thermal processing treatments and 50° F refrigerated storage (P < 0.01)

Sampling time	Uncooked Control	130° F Cooked	160° F Cooked
Inoculated raw beef	5.07 ± 0.04[a]	5.03 ± 0.03[a]	4.99 ± 0.02[a]
After cooking or emulsifying (control)	4.69 ± 0.04[a]	0.18 ± <0.01[d]	0.18 ± <0.01[d]
14 days storage	4.09 ± 0.19[b]	0.18 ± <0.01[d]	0.58 ± 0.17[ef]
28 days storage	3.75 ± 0.06[bc]	0.28 ± 0.04[de]	0.49 ± 0.04[def]
56 days storage	3.41 ± 0.16[c]	0.70 ± 0.27[f]	0.58 ± 0.23[ef]

[a-f]Means within the table with different superscripts differ ($P < 0.05$)

conducted in three independent replications with duplicate samples. Data were reported as log_{10} cfu/g and analyzed using the GLIMMIX procedure with LSD mean separation in SAS 9.4.

Results

Pseudomonas concentrations in uncooked treatments decreased by 1.39 log_{10} CFU/g ($P < 0.05$) during 39° F refrigerated storage (Table 1) and by 1.66 log_{10} CFU/g ($P < 0.05$) during 50° F refrigerated storage after 56 days (Table 2). In both cooked treatments at both storage temperatures, *Pseudomonas* concentrations were reduced below the detection limit (0.18 log_{10} CFU/g) immediately following cooking ($P < 0.05$). Those populations increased to > 0.5 log_{10} CFU/g after 56 days of storage ($P < 0.05$) in each cooking, storage temperature treatment combination. These results suggest that spoilage *Pseudomonas* may not be strictly aerobic and are potentially capable of causing spoilage in thermally processed beef products continuously stored in vacuum packaging when stored beyond 56 days. Additionally, final cooking temperature did not have an impact on the growth of *Pseudomonas*, indicating the ability of *Pseudomonas* to survive a range of thermal treatment processes used in the meat industry. As the emphasis to reduce food loss and waste increases in importance, the spoilage potential of *Pseudomonas* in vacuum packaged meat products must be considered.

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