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Comparative *Salmonella* Spp. and *Listeria monocytogenes* Inactivation Rates in Four Commercial Mayonnaise Products

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ABSTRACT

Salmonella spp. and *Listeria monocytogenes* strains were inoculated into four commercial mayonnaise products: sandwich spread, real mayonnaise, reduced calorie mayonnaise dressing, and cholesterol-free reduced calorie mayonnaise dressing. Products represented a broad cross-section of aqueous phase acetic acid, salt, sucrose, and other compositional factors. Results showed that *Salmonella* spp. inactivation rates were unaffected by formula composition. The organism was rapidly inactivated, decreasing ≥8 $\log_{10}$ CFU/g in ≤72 h, in each of the four products. *L. monocytogenes* inactivation rates were directly correlated with aqueous phase acetic acid concentrations as follows: sandwich spread ≥ real mayonnaise > cholesterol-free reduced calorie mayonnaise dressing > reduced calorie mayonnaise dressing. *L. monocytogenes* inactivation rate in sandwich spread and real mayonnaise was similar to *Salmonella* spp. The reduced calorie mayonnaise dressings showed gradual, incremental population declines. *L. monocytogenes* decreased 3 and 5 $\log_{10}$ CFU/g in 72 h in reduced calorie and cholesterol-free reduced calorie mayonnaise dressings, respectively. The higher anti-listerial activity in the cholesterol free formulation was attributed to egg white lysozyme. This study documented that commercial mayonnaise, including reduced calorie mayonnaise dressing varieties, represent negligible consumer safety risks.

Commercial mayonnaise and salad dressings are microbiologically shelf stable, and extremely safe processed foods. The safety of these products is directly associated with synergistic formulation components of which aqueous phase acetic acid and total formula pH level (≤4.1) are considered the most essential in inactivating foodborne pathogens such as *Salmonella* spp. and *Staphylococcus aureus* (*11,14,17,20*). The intrinsic microbial safety of properly formulated real mayonnaise and salad dressings is accepted by federal authorities (*19*). Specifically, the Food and Drug Administration (FDA) permits the use of unpasteurized eggs if the final product contains ≥1.4% aqueous phase acidity (calculated as acetic), achieves ≤4.1 pH equilibrium, and is held not less than 72 h before shipment to the trade. These criteria would also apply to products inadvertently processed with pathogen contaminated pasteurized eggs. In recent years, reduced calorie mayonnaise dressings have been introduced into the marketplace. Because of sensory requirements, their aqueous phase acetic acid levels are significantly below the 1.4% regulatory criterion. The microbial safety of these products requires investigation and documentation.

Listeria monocytogenes is an important, newly identified food pathogen (*7,15*). The organism is present in many raw foods, especially those of animal origin. Commercial liquid eggs, an important mayonnaise ingredient, has been implicated as a potential contamination source. Leasor and Foegeding (*10*) detected sporadic, low level contamination in raw liquid whole egg blend collected from eggbreaking-pasteurization plants, while Foegeding and Leasor (*5*) demonstrated that *L. monocytogenes* was ≥3 times more heat resistant than *Salmonella* spp. in pasteurized liquid whole eggs. *L. monocytogenes* is also noted for its superior physiological and environmental hardiness compared to other vegetative foodborne pathogens, including acidic pH (≥4.1) tolerance in laboratory media fortified with various inorganic and organic acids (*1,6,18*).

The purpose of this study was to determine and compare *Salmonella* spp. and *L. monocytogenes* inactivation in a cross-section of commercial real mayonnaise and reduced calorie mayonnaise dressings of varying composition.

MATERIALS AND METHODS

Microbial strain preparation

Thirteen ATCC *Salmonella* spp. strains, and one *S. enteritidis*-phage type 4 (isolated from eggs), obtained from Dr. M. P. Doyle of the Food Research Institute, Madison, WI, were used. The ATCC strains represented common food poisoning serotypes: 6960, 6962, 8326, 8388, 8400, 9270, 9607, 10722, 11511, 13076, 13311, and 13314.

Five ATCC *L. monocytogenes* strains were used: 11911, 11915, 15313, 43256, and 43257.

The *Salmonella* spp. and *L. monocytogenes* strains were individually streaked onto trypticase soy agar (Difco, Detroit, MI), and liver veal agar (LVA, Difco), respectively, and incubated at 35°C for 72 h. The surface growth from each plate was washed off with 3 to 5 ml of sterile physiological saline and combined into two separate pool inocula. The *Salmonella* spp. and *L.*

monocytogenes pool inocula were adjusted to a target 8 $\log_{10}$ CFU/ml population density.

Product inoculation, storage, and sampling procedures

Four commercial mayonnaise products were used: sandwich spread (SS - formula attributes resemble spoonable salad dressings); real mayonnaise (RM); reduced calorie mayonnaise dressing (RCM); and cholesterol-free reduced calorie mayonnaise dressing (CFRCM).

Each product was inoculated with the *Salmonella* spp. or *L. monocytogenes* pool inoculum. The target initial inoculum level was 6 $\log_{10}$ CFU/g. Inoculated samples were stored at ambient temperature (26.6°C) in a constant temperature Environette® incubator (Model No. 702ASHX6, Labline, Melrose Park, IL), and analyzed at zero time (within 1 h after inoculation), daily up to 10 d, and at 14 d, if needed.

Microbiological methods

Microbial enumeration and enrichment recovery assays were performed using the Hydrophobic Membrane Filtration® technique (HGMF, QA Laboratories Ltd., Toronto, Canada), and prescribed AOAC procedures (*2*). *Salmonella* spp. and *L. monocytogenes* were enumerated on modified nonselective plating media, trypticase soy agar and LVA supplemented with 0.6% yeast extract (Difco), respectively. Both media contained 0.25% fast green dye (Sigma, St. Louis, MO) to enhance colony color contrast and ensure accurate quantitative results. An enzyme treatment was required at the 10^{-1} plating dilution to improve filtration efficiency. This consisted of adding 0.5 ml each of 10% filter sterilized trypsin (Difco) and amylase (Sigma) solutions to 1 ml of the 10^{-1} dilution; incubating 20 min in a constant temperature-shaker waterbath (Neslab Inc., Newington, NH) at 35°C; and filtering the entire contents per standard procedures. The plating media were incubated at 35°C for 96 h, removed, and counted. Results were recorded as $\log_{10}$ CFU/g.

The enrichment assays were performed on 10-g and 100-g sample portions at each testing interval. *Salmonella* spp. and *L. monocytogenes* were enriched in lactose broth (Difco) and liver veal broth (LVB), respectively. LVB was prepared by dissolving 17.5 g liver broth (Difco) and 12.5 g veal broth (Difco) into 1 L of deionized water, and sterilized by autoclaving. The sample portions were pH adjusted to 6.8-7.2 with 1 N NaOH and incubated at 35°C for 24 h. After incubation, 0.1 ml of lactose broth and LVB were HGMF filtered; plated on MacConkey agar (Difco) or selective liver veal agar; and incubated at 35°C for 48 h. Enrichment samples were scored either presumptive positive or negative (no growth). Selective liver veal agar was prepared by adding 97 g LVA (Difco), 5 g aesculin (Difco), and 10 g lithium chloride (Sigma) to 1 L of deionized water before autoclaving. The sterilized medium was cooled to 45-50°C and supplemented with colistin (Sigma), moxalactam (Sigma), acriflavin (Sigma), and cyclohexamide (Sigma). Final concentrations were 10, 20, 7.5, and 25 μg/ml, respectively. The simplified HGMF-enrichment methods (no selective enrichment step, fewer selective agars) were tested against FDA and USDA reference procedures (*2,3,12,13*), and produced equivalent or superior analytical sensitivity and precision especially in recovering acid-stressed *L. monocytogenes* cells (unpublished data).

Physical analyses

Each product was analyzed for pH and water activity (a_w) at the beginning and completion of the inoculation studies. The pH results were obtained on a digital laboratory-pH meter (Model No. EA-940, Orion Research Inc., Boston, MA); a_w analyses were conducted in the Decagon instrument (Model No. CX-1, Decagon Devices Inc., Pullman, WA).

RESULTS AND DISCUSSION

Table 1 shows that the four commercial mayonnaise products evaluated represented a broad cross-section of formulation and compositional attributes. From lowest to highest aqueous phase concentrations, acetic acid, NaCl, and sucrose levels varied 3.3-, 2.6-, and 6.8-fold, respectively. Total formula pH and a_w ranges were much narrower, varying ≤1.2-fold. Significant compositional differences also existed, including moisture-oil ratios; the type of pasteurized egg ingredient used; and the presence/absence of antimicrobial preservatives. In addition, three of 4 products (SS, RCM, CFRCM) contained starch, a potential aqueous phase acidity buffering and microbial protective factor.

TABLE 1. *Microbial related formulation attributes of four commercial mayonnaise type products inoculated with* Salmonella *spp. or* Listeria monocytogenes.

Product type	Pasteurized egg ingredient	% Moisture (TF)	% Acetic acid (AP)	% NaCl (AP)	% Sucrose (AP)	% Antimicrobial preservatives (TF)	pH (TF)	A_w (TF)
Sandwich spread (SS)	NaCl whole egg blend	40	2.2	6.1	25	---	3.3	0.95
Real mayonnaise (RM)	NaCl whole egg blend	18	1.8	9	7.4	---	3.9	0.94
Reduced calorie mayonnaise dressing (RCM)	NaCl egg yolk	57	0.67	3.5	3.7	0.1% Potassium sorbate	3.9	0.98
Cholesterol-free reduced calorie mayonnaise dressing (CFRCM)	Unsalted egg white	57	0.67	3.5	3.7	0.1% Potassium sorbate	3.9	0.99

(TF) = Total formula value.
(AP) = Aqueous phase concentration $\frac{\%\text{ ingredient} \times 100.}{\%\text{ moisture}}$

Salmonella spp. inactivation rates were unaffected by aqueous phase formulation attributes, and compositional factors (Fig 1). In SS and RM, *Salmonella* spp. declined >6, >7, and >8 $\log_{10}$ CFU/g in 24, 48, and 72 h, respectively. Despite much lower aqueous phase acetic acid, NaCl, and sucrose levels, *Salmonella* spp. inactivation rates were comparable to SS and RM in the two reduced calorie mayonnaise dressings. Initial lethality was 5 $\log_{10}$ CFU/g in 24 h. By 48 and 72 h, both RCM and CFRCM achieved the identical cumulative inactivation rate as SS and RM. Our findings confirmed and expanded previously reported *Sal-*

monella spp. safety profile data for commercial and homemade mayonnaise products. Wethington and Fabian(*20*) reported 5 to 6 $\log_{10}$ CFU/g daily inactivation rates in several commercial mayonnaise and salad dressing formulations. Perales and Garcia (*16*) demonstrated that vinegar usage (substituted for lemon juice), proper pH control (≤4.0), and ambient temperature storage mitigated *S. enteritidis*-phage type 4 safety risks in homemade mayonnaise recipes. Glass and Doyle (*8*) documented rapid *Salmonella* spp. destruction in experimentally prepared reduced calorie mayonnaise dressings made with 0.7% aqueous phase acetic acid and corresponding 3.8-3.9 total formula pH levels. Extended survival rates were detected at ≤0.5% aqueous phase acetic acid, and higher pH (4.2) values. We duplicated their results in commercial reduced calorie mayonnaise dressing containing 0.67% aqueous phase acetic acid and 3.9 total formula pH attributes.

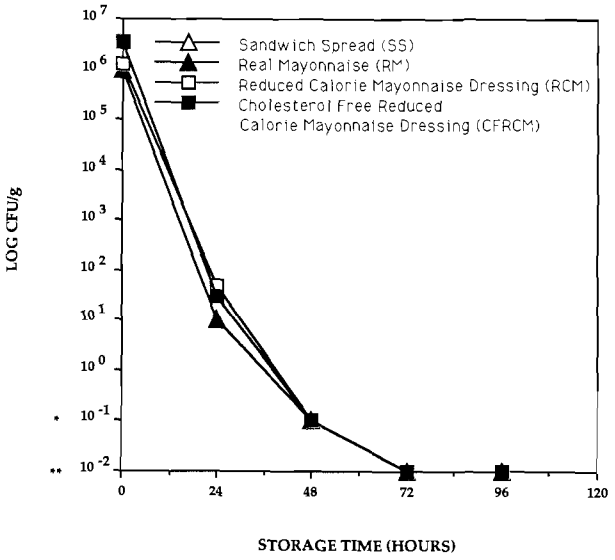

Figure 1. *Behavior of 14-strain* Salmonella *pool inoculum in four commercial mayonnaises products held at 26.6°C. Counts represent an average of three replicate runs, each utilizing a freshly manufactured production code. SS and RM inactivation rates overlapped.*

Unlike *Salmonella* spp., *L. monocytogenes* inactivation rates (Fig. 2) were directly correlated with aqueous phase acetic acid concentrations as follows: SS ≥ RM > CFRCM > RCM. This was consistent with the published literature. Chung and Goepfert (*4*) reported that acetic acid prevented *Salmonella* spp. growth in laboratory media at pH 5.5. In comparison, Farber et al. (*6*) and Sorrels et al. (*18*) evaluated *L. monocytogenes* behavior in laboratory media supplemented with various inorganic and organic acids. They detected growth at pH as low as 4.1 but identified acetic acid as the most effective anti-listerial acidulant. Ahamad and Marth (*1*) reported similar findings. Acetic acid suppressed and inactivated *L. monocytogenes* at 0.2 and ≥0.3%, respectively, which corresponded to 4.4-4.6 and 3.8-4.3 pH values. In SS and RM, *L. monocytogenes* inactivation rates

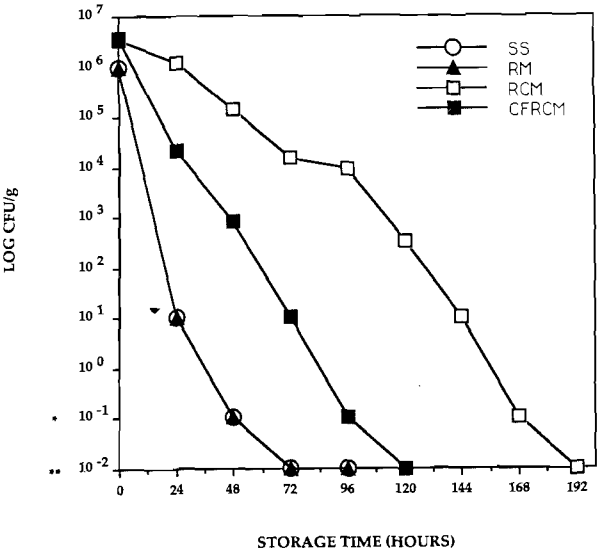

Figure 2. *Behavior of 5-strain* L. monocytogenes *pool inoculum in four commercial mayonnaise products held at 26.6°C. Counts represent an average of three replicate runs, each utilizing a freshly manufactured production code. SS and RM inactivation rates overlapped.*

were equivalent to *Salmonella* spp. Initial contamination levels decreased ≥6 $\log_{10}$ CFU/g in 24 h and were reduced to >8 $\log_{10}$ CFU/g in ≤72 h. In contrast, *L. monocytogenes* inactivation rates were gradual and incremental in the reduced calorie mayonnaise dressings. Also, significant inactivation profile differences were observed between the two reduced calorie mayonnaise dressings. In RCM, *L. monocytogenes* decreased <1 $\log_{10}$ CFU/g during the initial 24 h. This was followed by daily 1 $\log_{10}$ CFU/g decreases up to 96 h. Between 144 and 192 h, the inactivation rate accelerated until no viable *L. monocytogenes* was detected, which corresponded to a ≥4 $\log_{10}$ CFU/g population decrease over the final 48 h holding time. *L. monocytogenes* was more rapidly and efficiently inactivated in CFRCM. The organism decreased >5 $\log_{10}$ CFU/g in 72 h compared to 3 $\log_{10}$ CFU/g in RCM and was totally eliminated in ≤120 h. The only discernible difference between RCM and CFRCM was the egg ingredient used (Table 1). It appeared that hen egg white lysozyme synergistically interacted with acetic acid and pH to enhance CFRCM anti-listerial activity. Glass and Doyle (*8*) observed similar *L. monocytogenes* inactivation rates in experimentally prepared CFRCM. In the 0.7% aqueous phase acetic acid formula (pH 3.9), *L. monocytogenes* decreased 4 $\log_{10}$ CFU/g in 72 h. As with *Salmonella* spp., *L. monocytogenes* inactivation rates were moderated at ≤0.5% aqueous phase acetic acid levels. Hughey et al. (*9*) documented that hen egg white lysozyme produced anti-listerial activity in vegetable based foods, but its effectiveness diminished in proteinaceous foods such as cheese and cooked sausage meat. This was probably caused by lysozyme-protein binding interference or neutralization effects in the highly buffered cheese and meat menstra. Obviously, CFRCM contained insufficient protein levels to depress lysozyme anti-listerial efficacy. Also, the antago-

nistic (acidity) properties of CFRCM, or higher total lysozyme concentrations, may have amplified individual *L. monocytogenes* cell sensitivity compared to lysozyme effectiveness in the dairy and meat based foods investigated by Hughey and coworkers.

This research conclusively proved that the microbial safety of commercial real mayonnaise and reduced calorie mayonnaise dressings is equivalent for *Salmonella* spp. Massive contamination levels (>6 $\log_{10}$ CFU/g), which would not be encountered in commercial situations, are completely inactivated in ≤72 h. In practical terms, this represents fail-safe consumer protection at 52.2% lower aqueous phase acetic acid concentrations than currently required by federal regulations (*19*). The study also documented that *L. monocytogenes* health hazard risks are negligible in commercial mayonnaise products. The organism is inactivated in real mayonnaise and closely related formulations as rapidly as *Salmonella* spp. Because of slower lethality in reduced calorie mayonnaise dressings, it is prudent to use pasteurized eggs and stringent sanitation programs to obviate in-process and post-process *L. monocytogenes* contamination risks. However, in the unlikely event of finished product contamination, consumer safety risks remain negligible. Commercial reduced calorie mayonnaise dressings do not support *L. monocytogenes* growth or extended survival and are capable of inactivating low to moderate (≤1 $\log_{10}$ CFU/g) contamination levels in ≤72 h.

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