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Fate of *Salmonella* spp., *Listeria monocytogenes*, and Indigenous Spoilage Microorganisms in Home-style Salads Prepared with Commercial Real Mayonnaise or Reduced Calorie Mayonnaise Dressings

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ABSTRACT

Two home-style salads, chicken and macaroni, were prepared with three different commercial mayonnaise products: (i) real mayonnaise, (ii) reduced calorie mayonnaise dressing, and (iii) reduced calorie/reduced fat mayonnaise dressing. The salads were inoculated with 10^3/ml levels of *Salmonella* spp. or *Listeria monocytogenes* and held at 4°C (refrigeration) and 12.8°C (temperature abuse) for 10 and 2 d, respectively. Uninoculated controls were evaluated to determine the refrigerated shelf-life limit and microbial spoilage profile of both salads. *Salmonella* spp. growth occurred in the temperature-abused chicken salad, while *L. monocytogenes* grew in the temperature-abused and refrigerated chicken salad. The synergistic combination of mayonnaise and refrigeration inhibited *L. monocytogenes* outgrowth for >7 d. The microbiological shelf life of refrigerated chicken and pasta salads was 5 and 7 d, respectively. Microbial spoilage was predominantly caused by heterofermentative lactic acid bacteria, of which *Leuconostoc mesenteroides* was the most important. The organism was psychotropic and exhibited competitive inhibition against *Salmonella* spp. The latter was attributed to diacetyl formation synergistically interacting with the acidic salad environment. No microbiological safety or spoilage differences were observed between the salads prepared with real mayonnaise or reduced calorie mayonnaise dressings. Under proper refrigeration and good hygienic practices, home-style salads made with commercial real mayonnaise/mayonnaise dressings represent negligible microbial health hazard risks to consumers.

The microbiological safety of commercial mayonnaise products is well-documented and understood. Smittle (*15*) identified ≥0.25% acetic acid content and ≤4.1 pH as the critical formulation criteria for ensuring the destruction of common foodborne pathogens such as *Salmonella* spp. and *Staphylococcus aureus*. Recently, Erickson and Jenkins (*8*) and Glass and Doyle (*10*) showed that the microbiological safety profile of reduced calorie mayonnaise dressings was comparable to real mayonnaise. Though these products contained 50% lower aqueous phase acetic levels than real mayonnaise, they inactivated gross salmonellae contamination levels in ≤72 h and were lethal to *Listeria monocytogenes*.

Mayonnaise is primarily consumed as a bread spread and flavoring ingredient in prepared salads. For many years it was erroneously thought that the eggs in mayonnaise promoted spoilage and dramatically increased food poisoning risks when added to salads and sandwiches. Doyle et al. (*7*) and Swaminathan et al. (*18*) disproved mayonnaise's "growth promoting" ability in salmonellae-contaminated meat salads and turkey sandwiches, respectively.

With the emergence of the psychrotropic pathogen *L. monocytogenes*, the microbiological safety of prepared salads has come under scrutiny again. Commercially manufactured coleslaw was implicated in a Canadian listeriosis outbreak in the early 1980's (*14*), and recently, the U.S. Food and Drug Administration has recalled various commercial salad products due to *L. monocytogenes* contamination (*1-3*). Commercial product surveys (*9,14,17*) indicate that prepared salads are occasionally contaminated with low numbers of *L. monocytogenes*. However, considering the frequency and diversity associated with prepared salads consumption patterns (homes, delicatessens, restaurant-salad bars, etc.), there is no conclusive historical or epidemiological evidence (*14*) linking them to widespread or recurring microbiological health hazard risks. This suggests that mitigating factors, other than mayonnaise usage, may reduce health hazard risks even if low pathogen contamination levels are present. If this hypothesis is valid, it is important to determine that these factors function equally well in prepared salads made with the increasingly popular reduced calorie and low fat mayonnaise dressings.

The purpose of this study was to assess the microbiological safety and spoilage characteristics of "home-style", large particulated meat and pasta salads prepared with real mayonnaise, reduced calorie mayonnaise dressing, and reduced fat/calorie mayonnaise dressing stored under refrigerated (4°C) and moderate temperature-abuse (12.8°C, simulate abusive picnic handling) conditions, and identify any intrinsic or extrinsic factors that would minimize direct health hazard risks to the consumer.

MATERIALS AND METHODS

Salad preparation

Two types of "home-style" salads, chicken and macaroni, were selected for the microbiological risk assessment study. The recipes were taken from a consumer information pamphlet published by a large U.S. commercial mayonnaise manufacturer. Chicken salad was prepared by mixing together the following ingredients until they were completely coated by mayonnaise: 1,020 g (wt/wt) cooked/cooled diced chicken breast, 115.32 g mayonnaise, 151.24 g chopped green onion, 6.25 g table salt, 44.22 g real lemon juice, and 1.25 g ground black pepper. Macaroni salad was prepared by mixing the ingredients together in the same manner as chicken salad: 1,575 g cooked/cooled elbow macaroni, 260.3 g mayonnaise, 84.83 g chopped yellow onion, 38.75 g yellow mustard, 36.73 g white vinegar, 302.5 g chopped celery, 302.5 g chopped green pepper, 12.5 g table salt, 10.45 g granulated table sugar, and 3.13 g ground black pepper. The mayonnaise component consisted of either real mayonnaise, reduced calorie mayonnaise dressing, or reduced fat-reduced calorie mayonnaise dressing. All the salads were prepared with ingredients purchased at local supermarkets. Perishable ingredients were stored at 4°C and used within 24 h. Raw vegetables were washed under warm tap water for 30-60 s, drained, and chopped in a standard kitchen food processor.

Culture preparation and salad inoculation

Twelve *Salmonella* spp. and five *L. monocytogenes* were used for the inoculation studies. All were ATCC strains as follows: *Salmonella* spp.-6960, 6962, 8326, 8388, 8400, 9270, 9607, 10722, 11511, 13076, 13311, 13314; and *L. monocytogenes* -15313, 19111, 19115, 42356, 42357. The 17 strains were individually inoculated into 10-ml portions of Trypticase soy-0.6% yeast extract broth (Difco, Detroit, MI) and incubated at 35°C for 18-24 h. The cultures were combined into two separate pools (*Salmonella, Listeria*), centrifuged (Mistral 3000i, Curtin Matheson Scientific, Inc., Houston, TX) at 6,000 rpm, 20°C, for 20 min, and resuspended in 10 ml of sterile physiological saline. The initial cell concentration was 10^8/ml. Each pool was diluted in physiological saline to a 10^5/ml cell concentration.

For each salad and mayonnaise type, 200-g portions were weighed into six individual plastic tubs. The tubs were covered with plastic, press-on lids. Four of the 6 tubs were inoculated, in duplicate, with 2 ml of the *Salmonella* or *Listeria*-pool cultures, and vigorously hand-mixed for 1-2 min to ensure homogeneous distribution of the microorganisms. The target contamination level was 10^3 cells per g. The remaining two tubs served as the uninoculated controls. One set of samples (2 inoculated tubs plus control) was stored at 4°C for up to 10 d in a standard laboratory refrigerator, while the second set was held at 12.8°C (moderate temperature-abuse simulation) for 2 d in an Environette incubator (Model No. 702-ASHX, Lab-Line Instruments, Inc., Melrose Park, IL).

Microbiological methods and analyses

The temperature-abused salad samples were analyzed at 1- and 2-d storage intervals, while the refrigerated samples were analyzed at 0-, 3-, 5-, 7-, and 10-d intervals. Each sample was analyzed by weighing an 11-g portion into 99 ml peptone (Difco)-Tween 80 (Fisher Chemical Co., Fairlawn, NJ), Stomacher blending for 60 s (Model No. 400, Seward Medical, London, United Kingdom), and plating 10^{-1} or 10^{-2} serial dilutions.

Microbiological analyses were performed with the hydrophobic grid membrane filtration-surface plating system (Iso-Grid®, QA Life Sciences, Inc., San Diego, CA). The samples were prepared, filtered, and surface plated per standard reference procedures (*4,5*). The pathogens were plated on the following selective media: *Salmonella* spp. -EF-18 agar (QA Life Sciences) and *L. monocytogenes* -modified Oxford agar. The plates were incubated at 35°C for 2-3 d and enumerated with the semi-automated Iso-Grid Colony Counter (QA Life Sciences). Results were scored as $\log_{10}$ CFU/g. *Salmonella* spp. -EF-18 agar was prepared and sterilized according to the manufacturer's label directions, and modified Oxford agar preparation followed the instructions of Lee (*13*). Surface colonies were randomly checked to verify that the "selective pathogen" counts were accurate and consistent. Salmonellae colonies were confirmed by inoculating lysine iron agar slants (Difco), incubating 1 d at 35°C, and observing typical positive reactions. The colonies were also screened for strong sero-positive reactions in Bacto-Poly H (Difco) antiserum. *L. monocytogenes* confirmation was based on wet mount microscopic examinations and formation of the characteristic "umbrella-shaped" motility pattern in semi-solid motility agar (Difco).

The control samples were HGMF surface plated at the same storage intervals as the pathogen inoculated samples and were analyzed for total plate count, lactic acid bacteria count, and total yeast/mold count. Total plate and total yeast/mold counts followed testing procedures listed in the Iso-Grid Methods Manual (*4*). The lactic acid bacteria count test used modified *Lactobacillus* selection agar (Becton Dickinson-BBL Microbiology Systems, Cockeysville, MD) fortified with 1% fructose (Fisher) and 0.25% Fast Green (Fisher) dye, which was incubated at 35°C for 2 d in the Gas-Pak® (Becton Dickinson) CO_2 generation system. After counting, 3-5 isolated colonies were picked off the filter membrane from each *Lactobacillus* selection plate, and identified to genus/species using the API-Rapid CH® diagnostic kit (Analytab Products, Plainview, NY).

Physicochemical analyses

The uninoculated control samples were monitored for chemical and organoleptic (odor, texture, color) changes during storage. Six hours after preparation, each salad-mayonnaise combination was tested for pH, percent of moisture, acetic acid, lactic acid, and citric acid. The percent values for the three acids were added to provide total titratable acidity. These assays were repeated on all samples held at 12.8°C for 2 d.

pH

The salads were macerated and homogenized with a sonication blender (Tissuemizer®, Tekmar Co., Cincinnati, OH) calibrated at high setting for 60-120 s. Immediately after blending, ambient temperature (25°C) pH readings were obtained on a digital laboratory pH meter (Model No. 720A, Orion Research Inc., Boston, MA).

Organic acids

Organic acids were determined in the homogenized samples by ion exclusion chromatography. A 1-g sample was weighed into a 100-ml volumetric flask and diluted to volume with deionized water. A stirring bar was placed in the 100-ml volumetric flask, and the diluted sample was heated on a hot plate (Model PC-320, Corning Science Products, Inc., Corning, NY) at a setting of low for 15-20 min with stirring. The diluted sample was cooled and allowed to settle. A 5-ml portion of the supernatant was filtered through 0.45- and 0.2-micro syringe end filters, mounted in series Acrodisc®-type low protein-binding HT tuffryn polyfulfone membrane (Gelman Sciences, Ann Arbor, MI), and reserved for injection into the ion chromatograph.

A Dionex Model 4000i HPIC System (Dionex, Inc., Marlton, NJ) was used. The system included HIPCE™ ASI column (Dionex) with anion micro membrane suppressor and conductivity detector, output range 010 US. The analyses were carried out isocratically at room temperature using 2.5 mM octane sulfonic acid (Dionex) in deionized water as the mobile phase at a flow rate 0.8 ml/min.

Regenerant solution was 5 mM tetrabutyl ammonium hydroxide (55% aqueous solution, Southern Analytical, Austin, TX) at a flow of 2.5 ml/min. The mobile phase and regenerant were vacuum degassed with sonication for 5 min before use. Injection volume was 50 µl.

Standard preparation for quantitation was performed by preparing lithium lactate (Kodak, Rochester, NY - 99% min), glacial acetic acid (Fisher, Springfield, NJ), and citric acid monohydrate (J. T. Baker Chemical Co., Phillipsburg, NJ) in water to equal 10 µg/ml concentration for each organic acid.

RESULTS

Microbiological safety profile

As expected, salmonellae did not grow in refrigerated home-style chicken and macaroni salads indicating that low (4°C) storage temperature alone was sufficient to inhibit these mesophilic organisms for extended holding periods (Fig. 1). In fact, both salads produced ≥2-$\log_{10}$ CFU/g population reductions by the end of the study (10 d). The two salads generated different inactivation patterns, where initial salmonellae contamination levels remained unchanged for 7 d in the chicken salad followed by a 2-$\log_{10}$ CFU/g decline by 10 d. Interestingly, the salmonellae decline paralleled a >3-$\log_{10}$ CFU/g increase in indigenous lactic acid bacteria contamination levels between 7 and 10 d. The macaroni salad showed more gradual but consistent salmonellae decreases over the 10 d storage period. Salmonellae inactivation rates were 0.5, 1, and 2 $\log_{10}$ CFU/g in 5, 7, and 10 d, respectively. The macaroni salad contained much higher initial lactic acid bacteria contamination levels (5- versus 2-$\log_{10}$ CFU/g) and produced slower but more continuous population increases over the 10-d holding period. By 10 d, the two salads had equivalent lactic acid bacteria contamination levels exceeding 7-$\log_{10}$ CFU/g, and both emitted pungent, undesirable buttery, or putrid off odors.

Listeria monocytogenes (LM) behavior was more complex and recipe dependent than salmonellae (Fig. 2). In the refrigerated chicken salad LM growth was suppressed for 7 d, which was followed by a 2-$\log_{10}$ CFU/g population increase between 7 and 10 d. The final LM contamination level was >5-$\log_{10}$ CFU/g, and, unlike salmonellae, the prolific outgrowth of lactic acid bacteria after 7 d had no inhibitory effect against LM. In contrast, the more acidic (Table 1) home-style macaroni salad completely inhibited LM growth over the duration of the study. The initial 3-$\log_{10}$ CFU/g contamination level remained constant for 10 d compared to 2-$\log_{10}$ CFU/g salmonellae declines under the same storage conditions. Importantly, salmonellae and LM behavioral patterns were identical in the home-style, refrigerated salads whether commercial real mayonnaise or reduced calorie mayonnaise dressing (RCMD) was used (Fig. 1 and 2). The comparable microbiological results were directly related to the similar physicochemical profile(s) in real mayonnaise and RCMD-containing salads (Table 1). For each salad type, the crucial antimicrobial formulation factors, including pH, titratable acidity, and organic acid composition/content showed no significant quantitative variations attributable to the commercial mayonnaise ingredient used.

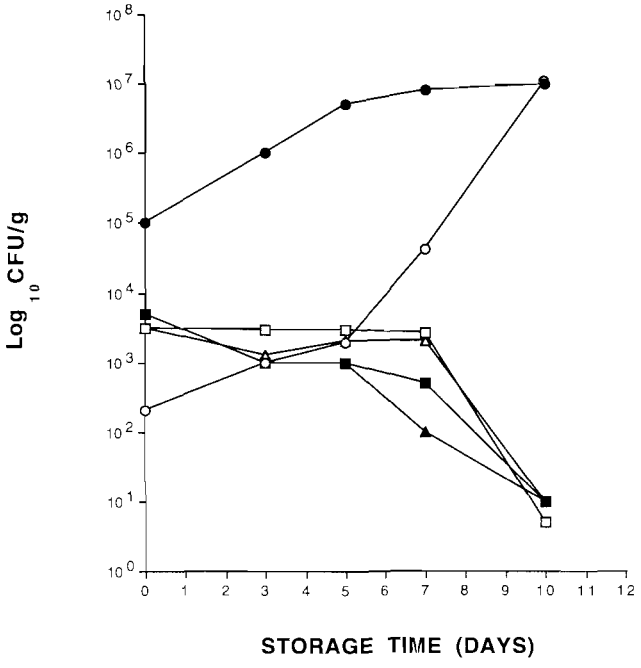

Figure 1. *Behavior of inoculated* Salmonella *spp., and indigenous lactic acic bacteria in home-style chicken and macaroni salads made with commercial real mayonnaise or reduced calorie mayonnaise dressing and held at 4°C for 10 d: Δ, real, chicken; ▲, real, macaroni; ❑, reduced calorie, chicken; ■, reduced calorie, macaroni; ○, chicken, lactic acid bacteria; ●, macaroni, lactic acid bacteria. Prepared salads made with commercial reduced fat/reduced calorie mayonnaise dressing produced microbiological responses comparable to those made with reduced calorie mayonnaise dressing.*

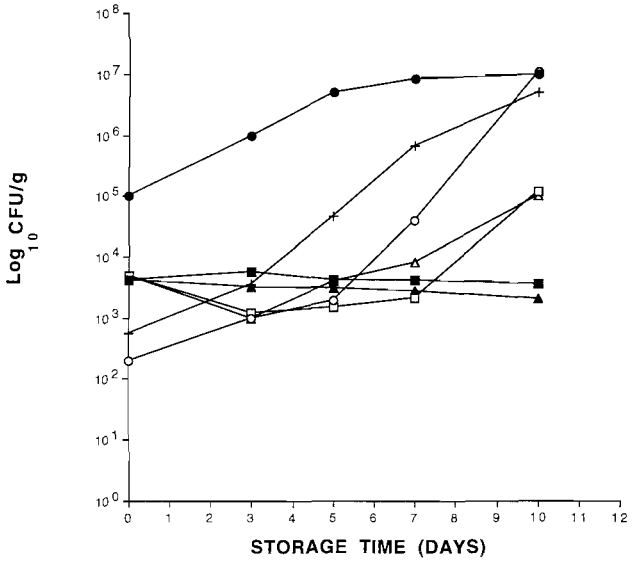

Figure 2. *Behavior of inoculated* L. monocytogenes, *and indigenous lactic acic bacteria in home-style chicken and macaroni salads made with commercial real mayonnaise or reduced calorie mayonnaise dressing and held at 4°C for 10 d: Δ, real, chicken; ▲, real, macaroni; ❑, reduced calorie, chicken; ■, reduced calorie, macaroni; ○, chicken, lactic acid bacteria; ●, macaroni, lactic acid bacteria; +, chicken, no mayonnaise added. Prepared salads made with commercial reduced fat/reduced calorie mayonnaise dressing produced microbiological responses comparable to those made with reduced calorie mayonnaise dressing.* L. monocytogenes *growth in mayonnaise-free chicken salad is provided for comparison.*

TABLE 1. *Physicochemical profile and changes in home-style prepared salads made with three different types of commercial mayonnaise and held at 12.8°C for 2 d.*

Salad[a]	Mayonnaise type	Storage temperature/time: OT[b] pH	OT[b] T.A.[d] (%)	OT[b] Acetic (%)	OT[b] Lactic (%)	12.8°C/2d[c] pH	12.8°C/2d[c] T.A.[c] (%)	12.8°C/2d[c] Acetic (%)	12.8°C/2d[c] Lactic (%)
Chicken	Real	5.65	0.66	0.02	0.51	5.23	0.90	0.16	0.61
	Reduced calorie	5.78	0.61	0.03	0.45	5.36	0.88	0.13	0.62
	Reduced fat/ reduced calorie	5.65	0.62	0.02	0.47	5.38	0.88	0.14	0.62
Macaroni	Real	4.62	0.15	0.14	0.006	3.94	0.37	0.24	0.12
	Reduced calorie	4.54	0.15	0.14	0.005	4.02	0.33	0.23	0.09
	Reduced fat/ reduced calorie	4.56	0.15	0.14	0.007	4.11	0.34	0.28	0.05

[a] Percent moisture ranged from 73-75%.
[b] All physicochemical analysis performed on refrigerated salads, 6 h after preparation.
[c] Similar physicochemical profiles were obtained with chicken and macaroni salads held at 4°C for 10 d.
[d] T.A. = Titratable acidity - the sum of three organic acids quantified (citric acid data not listed).

Synergistic interaction between refrigeration (4°C) and the commercial mayonnaise ingredient caused a delay in LM growth. When commercial real mayonnaise or RCMD was omitted from the refrigerated chicken salad recipe, LM grew >50% faster (3 versus 7 d) and attained 1-$\log_{10}$ CFU/g higher (6-$\log_{10}$ CFU/g) contamination levels (Fig. 2). Not unexpectedly, gross visible spoilage also occurred at a 50% faster rate.

Temperature abuse (12.8°C) results further identified pathogen growth risk variations between the home-style chicken and macaroni salads (Fig. 3). The chicken salad supported 1-$\log_{10}$ and ≥3-$\log_{10}$ CFU/g pathogen increases in 1 and 2 d, respectively, and salmonellae/LM growth rates were equivalent. Conversely, the macaroni salad generated >2-$\log_{10}$ CFU/g salmonellae decreases in <2 d, which was a 3-fold faster inactivation rate than observed with refrigerated salad samples. No LM growth was detected in the macaroni salad, but the organism survived the 2-d holding period due to its innate ability to tolerate harsh and dynamic changes in environmental conditions. The chicken salad supported rapid salmonellae and LM population increases because it was weakly acidic (pH 5.65 to 5.78) and contained extremely low amounts of acetic acid (0.02 to 0.03%). Despite >5-$\log_{10}$ CFU/g lactic acid bacteria increases in ≤2 d, salmonellae inactivation was not observed, strongly suggesting that the types of lactic acid bacteria present were more important than the total population levels reached. The absence of pathogen growth in the macaroni salad was mainly due to its acidic pH (4.54-4.64), which decreased >0.5 units over the 2-d storage time. As seen with the refrigerated salads, the substitution of commercial real mayonnaise with RCMD had absolutely no effect on pathogen behavior under temperature-abuse conditions (Fig. 4).

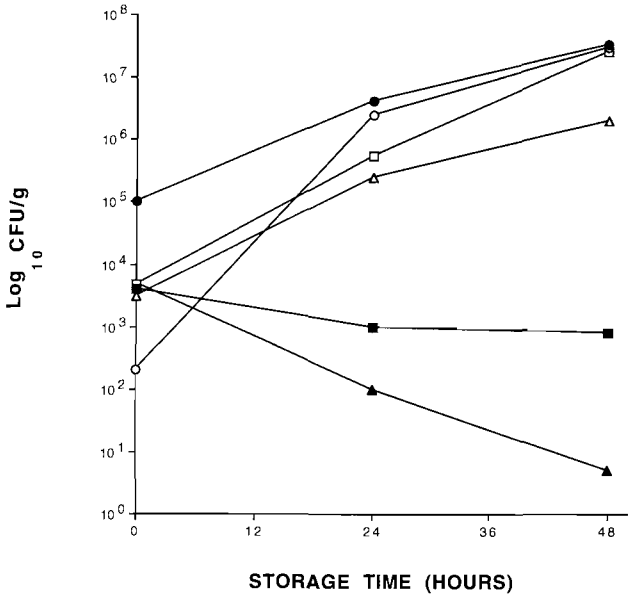

Figure 3. *Behavior of inoculated* Salmonella *spp.,* L. monocytogenes, *and indigenous lactic acic bacteria in home-style chicken and macaroni salads made with commercial real mayonnaise and held at 12.8°C for 2 d: Δ, chicken,* Salmonella*; ▲, macaroni,* Salmonella*; ❑, chicken,* L. monocytogenes*; ■, macaroni,* L. monocytogenes*; ○, chicken, lactic acid bacteria; ●, macaroni,, lactic acid bacteria.*

Microbiological spoilage profile

The home-style prepared salads had limited (7-10 d) refrigerated shelf lives, illustrating their "semiperishable" nature. Lactic acid bacteria were the most ubiquitous and versatile spoilage microorganisms encountered. In the temperature-abused salads, lactic acid bacteria increased more rapidly than salmonellae and LM (Fig. 3 and 4). This was

especially pronounced in the chicken salad where 1-d lactic acid bacteria counts increased 4-$\log_{10}$ CFU/g compared to 2-$\log_{10}$ CFU/g for the two pathogens. Under refrigeration (Fig. 2), lactic acid bacteria increases occurred 50% earlier than LM and reached 2-$\log_{10}$ CFU/g higher contamination levels by 10 d. The composition of the microbial spoilage population was influenced by holding temperature and salad type (Table 2). In the temperature-abused salads, mixed culture spoilage occurred involving lactobacilli, *Leuconostoc mesenteroides*, coliforms, and yeasts. Coliforms exclusively grew in chicken salad, while yeast growth was detected in both salads. The refrigerated salads were predominantly spoiled by *L. mesenteroides*, a psychrotropic, heterofermentative strain. The physicochemical data provided corroborating evidence that heterofermentative lactic acid bacteria were the primary spoilage microorganisms. The temperature-abused salads exhibited ≥0.5 pH decreases, 2- to 100-fold acetic/lactic acid increases, and overt physical/sensory decomposition changes (gas formation, off-odors). The co-production of organic acids and gaseous by-products was especially indicative of heterofermentative metabolic activity. As demonstrated with pathogen behavior, the commercial mayonnaise type did not influence or alter spoilage patterns. Hence, each salad supported the same microbial populations and produced comparable spoilage rates and sensory defects whether commercial real mayonnaise or RCMD was used.

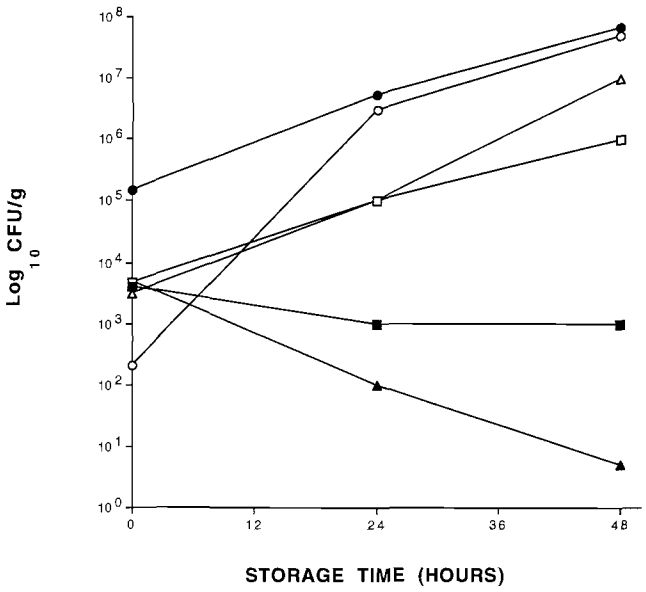

Figure 4. *Behavior of inoculated* Salmonella *spp.*, L. monocytogenes, *and indigenous lactic acic bacteria in home-style chicken and macaroni salads made with commercial reduced calorie mayonnaise dressing and held at 12.8°C for 2 d:* Δ, *chicken,* Salmonella; ▲, *macaroni,* Salmonella; ❑, *chicken,* L. monocytogenes; ■, *macaroni,* L. monocytogenes; ○, *chicken, lactic acid bacteria;* ●, *macaroni,, lactic acid bacteria. Prepared salads made with commercial reduced fat/reduced calorie mayonnaise dressing produced microbiological responses comparable to those made with reduced calorie mayonnaise dressing.*

DISCUSSION

Some forms of commercially manufactured salads, such as those sold at gourmet supermarkets and restaurant salad bars, are moving closer to home-style salads by using larger particulates, reducing acid content, and decreasing chemical preservative usage. Van Schothorst (*19*) linked the successful production of commercial salads to ingredient pasteurization, acid soaking large particulates, and adding liberal amounts of mayonnaise or salad dressing to reduce the pH as low as possible. He identified lactobacilli and yeasts as the most common commercial salad spoilage microorganisms. Brocklehurst and Lund (*6*) investigated the microbiological quality of commercial mayonnaise-based salads sold in the United Kingdom. The mayonnaise content and pH of the salads ranged from 35-50% and 3.4-4.4, respectively. Under moderate temperature abuse (10°C), the salads were spoiled by yeasts, *Geotrichum* spp., and lactobacilli. Minimal spoilage risks were detected in refrigerated (5°C) salads held up to 28 d. In comparison, the home-style salads contained 11.25-11.60% mayonnaise and correspondingly less acidic pH levels. Due to the use of fresh, unprocessed vegetable ingredients, home-style salads also have much higher bioloads than commercial counterparts. For example, the initial lactic acid bacteria contamination level in the macaroni salad was 5-$\log_{10}$ CFU/g. The observation by Van Schothorst (*19*) that lactic acid bacteria were the predominant spoilage microorganisms in prepared salads was confirmed even when the pH was above 5.5. Specifically, *L. mesenteroides* was identified as the most important spoilage bacterium in home-style salads, primarily due to its ability to grow under refrigerated storage conditions. Rather than being strictly a quality detriment, *L. mesenteroides* growth may provide significant safety benefits. The spoiled salads emitted strong buttery off-odors consistent with the formation of diacetyl, a lactic acid bacteria metabolite. Jay (*11,12*) reported that diacetyl was inhibitory to gram-negative bacteria, and its antibacterial activity was amplified in acidic environments. A strong correlation was observed between the increase of *L. mesenteroides* and decrease of salmonellae under refrigeration, suggesting that the continuous buildup of diacetyl partially contributed to the ≥2-$\log_{10}$ CFU/g salmonellae inactivation rates detected in the two home-style salads. The anti-*Salmonella* activity also disappeared in the temperature-abused chicken salad where lactobacilli "out-competed" *L. mesenteroides*.

LM growth risks were previously examined in commercial salads and fresh vegetables. George and Levett (*9*) assessed LM growth in commercially purchased coleslaw adjusted to pH 4, 5, 6, and 7.0 and held at 5-25°C. They demonstrated that LM growth was prevented at ≤5.0 pH regardless of the holding temperature, whereas it grew at pH 6 and 7.0 in coleslaw samples held at 25 and 4°C, respectively. Steinbruegge et al. (*17*) observed prolific LM growth in ready-to-serve lettuce held at 5-25°C for 14 d. Both studies also observed eventual decreases in viable LM populations or erratic growth responses, which were attributed to the accumulation of fermentation by-products and corresponding pH declines produced by unidentified com-

TABLE 2. *Indigenous lactic acid bacteria profile and sensory spoilage characteristics of home-style prepared salads held at 12.8°C and 4°C.*[a]

	12.8°C/2 d			4°C/10 d		
Salad[a,b]	Microbial spoilage characteristics	Predominant lactic acid bacteria	Other spoilage microorganisms[d]	Microbial spoilage characteristics[c]	Predominant lactic acid bacteria	Other spoilage microorganisms[d]
Chicken	Putrid odor, severe meat discoloration, slimy film on meat surface	*L. plantarum* *L. confusus*	Coliforms, yeasts	Buttery odor, putrid, slimy film	*L. mesenteroides*	None
Macaroni	Strong buttery odor, gas buildup (lid distended), mayonnaise separation; slimy film on elbow pieces	*L. mesenteroides* *L. confusus*	Yeast	Strong buttery odor, gas, slimy film	*L. mesenteroides*	None

[a] 25-g portions were enrichment tested for indigeneous pathogen contamination at 2 d (12.8°C), and 5 and 10 d (4°C). All samples were *Salmonella* and *L. monocytogenes* negative.
[b] Observed same microbial spoilage populations and organoleptic deterioration rates, regardless of mayonnaise variety used.
[c] Chicken salad inedible by 7th d.
[d] Contamination level reached > 6-$\log_{10}$ CFU/g.

peting microorganisms. This study verified George and Levett's findings (*9*) that LM growth was completely inhibited in prepared salads containing an equilibrium pH of 5.0 or below. Conversely, the chicken salad recipe supported LM growth at refrigerated temperature and lower pH values (5.8 versus 7.0) than reported in their study. This was probably due to formulation differences, in which the commercial coleslaw contained significantly higher mayonnaise and acetic acid content than the home-style chicken salad. The results also determined that the antagonistic, competing microorganisms mentioned by George and Levett (*9*) and Steinbruegge et al. (*17*) were lactic acid bacteria, specifically *L. mesenteroides* and lactobacilli. This study reconfirmed and expanded the findings of Doyle et al. (*7*) and Swaminathan et al. (*18*) by demonstrating that mayonnaise-free chicken salad was more susceptible to refrigerated LM growth compared to the mayonnaise-containing product. Thus, even relatively low concentrations of commercial mayonnaise still are inhibitory against a wide range of gram-negative and gram-positive pathogens.

The type of commercial mayonnaise used in home-style prepared salads did not affect or alter microbiological safety and refrigerated shelf-life characteristics inherent to the specific recipes. With respect to the latter, we recommend 5 d maximum refrigerated shelf life for home-style meat-based salads and 7 d maximum for pasta/vegetable-based salads. When higher quantities of mayonnaise and other acidulating agents are used, the shelf life can be increased.

In toto, the experimental results provide a convincing explanation as to why home-style (and commercial), mayonnaise-containing salads are rarely the cause of small-cluster or large-scale food poisoning outbreaks. A 5-tier microbiological safety barrier system is involved as follows (listed in order of importance): (i) refrigerated storage; (ii) inhibitory recipes/formulations; (iii) natural competitive inhibition-lactic acid bacteria enhances protection through pH reduction, organic acid increases, and formation of specific antimicrobial metabolites; (iv) severe visual and sensory changes caused by microbial growth which deters consumption; and (v) consumer awareness of the semiperishable nature of prepared salads leading to their consumption within a few days of preparation or purchase. However, we completely concur with the precautions and recommendations of Schuchat et al. (*16*) as to the most effective means of reducing foodborne pathogen exposure risks in susceptible populations, including the immunosuppressed, debilitated elderly, and pregnant women. Individuals from these high risk groups, who may be extremely predisposed to foodborne illness, should avoid eating ready-to-eat-processed foods that may naturally or inadvertently contain low level pathogen contamination; particularly if the foods are capable of supporting rapid microbial growth under recommended or abusive storage conditions. Our results conclusively demonstrate that home-style salads prepared with either commercial real mayonnaise or reduced calorie mayonnaise dressings possess sufficient antimicrobial properties and perishability indicators to minimize health hazard risks, as long as they are prepared properly, handled hygienically, and not subject to severe storage abuse.

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