

ANTIMICROBIAL AGENTS AND PROCESSES FOR CONTROL OF *Listeria monocytogenes* IN READY- TO-EAT MEAT AND POULTRY PRODUCTS

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RECENT LISTERIOSIS OUTBREAKS

- **FRANKFURTERS/LUNCHEON MEAT, 1998-1999:**
 - 101 cases
 - 21 deaths (6 stillbirths/miscarriages)
 - 22 states
- **DELI POULTRY PRODUCTS, 2000:**
 - 29 cases
 - 7 deaths (3 miscarriages)
 - 10 states
- **DELI POULTRY PRODUCTS, 2002:**
 - 46 cases
 - 10 deaths (3 miscarriages/stillbirths)
 - 8 states



TOP THREE USA MEAT/POULTRY RECALLS

- Hot dogs/packaged meats
Listeria monocytogenes
17M Kg
December 22, 1998
- Various rte products
Listeria monocytogenes
17M Kg
January 22, 1999
- Fresh and frozen rte poultry products
Listeria monocytogenes
13M Kg; October 12, 2002, Company A (Drain)
2M Kg; November 2, 2002, Company B (Product)



Listeria monocytogenes

(Listeriosis)



- **Organism:** non-spore-forming, gram-positive, rods, facultative, psychrotroph
- **Illness:** infection with many severe syndromes in sensitive individuals
- **Symptoms:**
 - **Noninvasive:** mild, flu-like
 - **Invasive:** meningitis; bacteremia; endocarditis; septicemia; peritonitis; etc
 - **Pregnant women:** flu-like illness; crosses placenta; attacks fetus; abortion, stillbirth or acutely ill baby; most common in third trimester

Listeria monocytogenes

(Listeriosis)



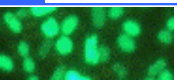
- Incubation period: >12 hours; 3 days to 3 months
- Duration of illness: short to long
- Infective dose: unknown; >100 cells/g food?
- Fatality rate: 20-30%
- Sources: soil, vegetation, humans, animals, water, silage, sewage, etc
- Foods involved: raw milk, cheeses, coleslaw, ice cream, vegetables, luncheon meats, seafood
- Control: sanitation, hygiene, proper cooking, prevent cross- or re-contamination, post-processing interventions





LISTERIA MONOCYTOGENES CONCERNS

- ➡ Survival in adverse environments: better than other nonspore-formers
- ➡ Psychrotroph: Grows at refrigeration temperatures
- ➡ Stress (acid, cold and starvation) adaptation: hardening; cross-protection; resistance
- ➡ Plant Environment: Colonize, multiply, persist; attachment and biofilms; drains, conveyors, floor mats, foot baths, freezers, coolers, equipment, chilling rooms, cutting rooms, hands, packaging
- ➡ Major Concern: post-processing contamination



SURVIVAL OF *LISTERIA MONOCYTOGENES* IN THE ENVIRONMENT

| SUBSTRATE | TEMPERATURE (°C) | DAYS |
|----------------|------------------|------------|
| Soil | 4-6 | 12 to >730 |
| Water | 2-37 | <7 to 928 |
| Animal feed | 4 to 22 | 23 to 2190 |
| Fecal material | 5 to 56 | 35 to 2190 |

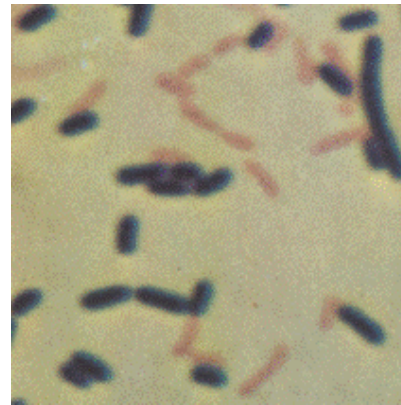


TABLE 2. Examples demonstrating that certain strains of *L. monocytogenes* can become established and persist in the food-processing environment

| Type of food produced at plant | Time of persistence ^a | Country | Implicated in illness? | Serotype(s) ^b | Reference(s) |
|---|----------------------------------|----------------|------------------------|--------------------------|--------------|
| Cheese | 4 years | Switzerland | Yes | 4b | 5 |
| Cheese, blue veined | 7 years | Sweden | No | 3b | 94 |
| Cheese, goat | 11 months | United Kingdom | Yes | 4b | 3, 63 |
| Fish, smoked | Months | Switzerland | No | Several | 6 |
| | 14 months | Finland | No | 1/2a (86%), 4b (14%) | 53 |
| | Months | United States | No | ND | 70 |
| Frankfurters | 4 months | United States | Yes | 1/2a | 16, 95 |
| Frankfurters (outbreak strain was not isolated from the plant) | Months | United States | Yes | 4b | 17 |
| Ice cream | 7 years | Finland | No | 1/2 | 66 |
| Meat, sliced lunch | 4 years | Norway | No | ND | 69 |
| Mussels, smoked | 3 years | New Zealand | Yes | 1/2 | 7 |
| Pâté (product from one plant was the source of an outbreak from 1987 to mid-1989) | 2 years | United Kingdom | Yes | 4b(x), 4b | 64, 72 |
| Pork tongue in aspic (outbreak strain recovered from the implicated plant) | Months | France | Yes | 4b | 50, 86 |
| Poultry, cooked | 1 year | Ireland | No | 1/2 | 57 |
| Poultry, cooked deli products (outbreak strain matched a strain previously isolated from the same plant (95)) | 12 years | United States | Yes | 4b | 89 |
| Salmon, cold smoked | 4 years | Denmark | No | ND | 31 |
| Salmon, smoked | 8 months | Norway | No | ND | 82 |
| Seafood, smoked salmon | Months–2 years | Norway | Possibly | 4, 1 | 81 |
| Shrimp, raw shelled frozen | NS | Brazil | No | 1, 4b | 25 |
| Trout/salmon, gravad | 1 month | Sweden | No | 4b | 60 |
| Trout, gravad and cold smoked | 11 months | Sweden | Yes (gravad) | 4b | 28 |
| Trout, smoked/salmon, gravad | >4 years | Sweden | Possibly | 1/2a | 59 |
| Trout, cold smoked | NS | Finland | No | 1/2 | 2 |

^a NS, not stated.

^b ND, not determined.



TOMPKIN



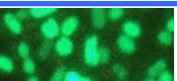
J. Food Prot., Vol. 65, No. 4

***LISTERIA MONOCYTOGENES* IN FRANKFURTERS**

| Plant # | Size | Product | Season | Pos/Tested/% |
|----------------|--------------|----------------|---------------|------------------------|
| 42 | Large | P/B | Spring | 0/2,900 |
| 94 | Large | T | Spring | 2/2,700/0.07 |
| 105 | Large | B | Fall | 0/2,800 |
| 133 | Large | T | Spring | 437/2,800/16.0 |
| 172 | Large | B | Winter | 3/2,700/0.11 |
| 236 | Small | P/B | Winter | 0/2,900 |
| 344 | Large | P/B/C | Fall | 4/2,500/0.16 |
| 367 | Small | P | Summer | 44/2,900/1.5 |
| 385 | Small | P/B | Fall | 2/2,600/0.08 |
| 399 | Large | P/B | Summer | 0/2,800 |
| 439 | Large | P/B | Spring | 51/2,300/2.2 |
| 443 | Large | P/T | Winter | 0/2,900 |
| Total | | | | 543/32,800/1.65 |

P: pork; B: beef; T: turkey

Wallace et al. 2003



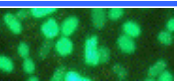


USA INCIDENCE OF *LISTERIA MONOCYTOGENES*



| Product | Number positive in CFU/g Range | | | | | |
|--------------------|--------------------------------|----|----------------------|----|------------------|----|
| | 0.04-0.1 | | >0.1-10 ² | | >10 ² | |
| | CA | MD | CA | MD | CA | MD |
| Fresh soft cheeses | 0 | 2 | 1 | 2 | 0 | 0 |
| Bagged salads | 12 | 5 | 2 | 2 | 0 | 1 |
| Blue cheeses | 13 | 5 | 4 | 1 | 0 | 0 |
| Mold cheeses | 11 | 1 | 2 | 0 | 0 | 0 |
| Seafood salads | 26 | 56 | 1 | 30 | 0 | 0 |
| Smoked seafood | 44 | 23 | 21 | 17 | 6 | 3 |
| Luncheon meats | 10 | 32 | 15 | 17 | 3 | 5 |
| Deli salads | 78 | 84 | 20 | 19 | 1 | 3 |

Gombas et al. 2003

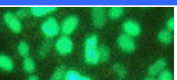




USA INCIDENCE OF *LISTERIA* *MONOCYTOGENES*

| Product | Samples Packaged (%) | | Positive Prevalence (%) | |
|-----------------------|-----------------------------|--------------|--------------------------------|--------------|
| | Plant | Store | Plant | Store |
| Luncheon meats | 77 | 23 | 0.4 | 2.7 |
| Deli salads | 48 | 52 | 1.4 | 3.6 |
| Seafood salads | 40 | 60 | 1.4 | 6.9 |

Gombas et al. 2003



USA INCIDENCE OF *LISTERIA MONOCYTOGENES*

| YEAR | POSITIVE (%) |
|-------------|---------------------|
| 2003 | 0.75 |
| 2002 | 1.03 |
| 2001 | 1.32 |
| 2000 | 1.45 |
| 1999 | 1.91 |
| 1998 | 2.54 |
| 1997 | 2.25 |
| 1996 | 2.91 |
| 1995 | 3.03 |

USDA/FSIS DATA: RTE MEAT PRODUCTS; 7,500 SAMPLES/YEAR

LISTERIA MONOCYTOGENES USA RISK ASSESSMENT

Quantitative Assessment of the Relative Risk to Public Health from Foodborne *Listeria monocytogenes* Among Selected Categories of Ready-to-Eat Foods

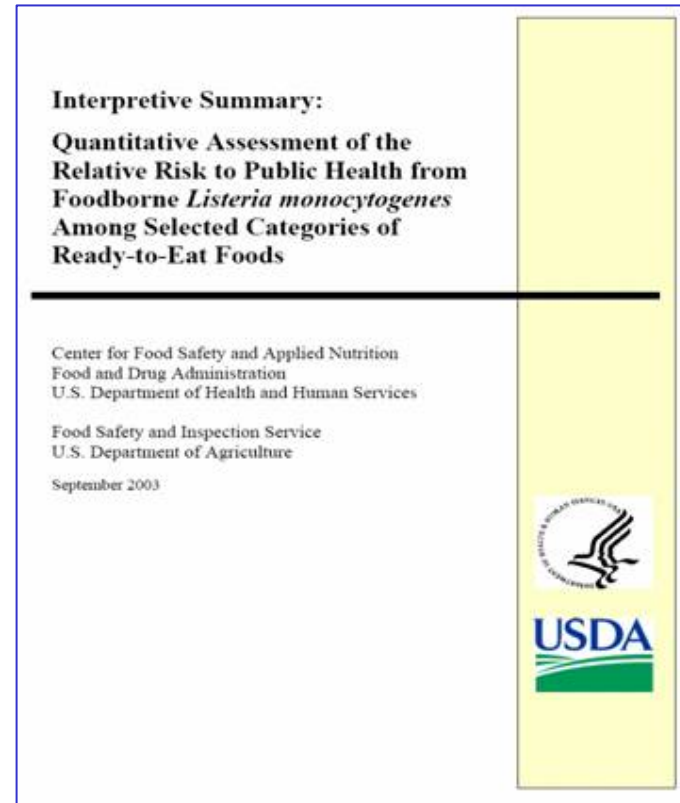
(seafood, produce, meats, dairy products, deli-type salads)

<http://www.foodsafety.gov/~dms/lmr2-toc.html>

**FDA/Center for Food Safety and Applied Nutrition
FSIS/Food Safety and Inspection Service**

CDC/Centers for Disease Control and Prevention (CDC)

September 2003



Summary Table 4. Relative Risk Ranking and Predicted Median Cases of Listeriosis for the Total United States Population on a per Serving and per Annum Basis

| Relative Risk Ranking | Predicted Median Cases of Listeriosis for 23 Food Categories | | | | | |
|-----------------------|--|---|-----------------------|------------------------------|---|--------|
| | Per Serving Basis ^a | | | Per Annum Basis ^b | | |
| | Food | Cases | | Food | Cases | |
| 1 | High Risk | Deli Meats | 7.7x10 ⁻⁸ | Very High | Deli Meats | 1598.7 |
| 2 | | Frankfurters, not reheated | 6.5x10 ⁻⁸ | High Risk | Pasteurized Fluid Milk | 90.8 |
| 3 | | Pâté and Meat Spreads | 3.2x10 ⁻⁸ | | High Fat and Other Dairy Products | 56.4 |
| 4 | | Unpasteurized Fluid Milk | 7.1x10 ⁻⁹ | | Frankfurters, not reheated | 30.5 |
| 5 | | Smoked Seafood | 6.2x10 ⁻⁹ | Moderate Risk | Soft Unripened Cheese | 7.7 |
| 6 | | Cooked Ready-to-Eat Crustaceans | 5.1x10 ⁻⁹ | | Pâté and Meat Spreads | 3.8 |
| 7 | Moderate Risk | High Fat and Other Dairy Products | 2.7x10 ⁻⁹ | | Unpasteurized Fluid Milk | 3.1 |
| 8 | | Soft Unripened Cheese | 1.8x10 ⁻⁹ | | Cooked Ready-to-Eat Crustaceans | 2.8 |
| 9 | | Pasteurized Fluid Milk | 1.0x10 ⁻⁹ | | Smoked Seafood | 1.3 |
| 10 | Low Risk | Fresh Soft Cheese | 1.7x10 ⁻¹⁰ | Low Risk | Fruits | 0.9 |
| 11 | | Frankfurters, reheated | 6.3x10 ⁻¹¹ | | Frankfurters, reheated | 0.4 |
| 12 | | Preserved Fish | 2.3x10 ⁻¹¹ | | Vegetables | 0.2 |
| 13 | | Raw Seafood | 2.0x10 ⁻¹¹ | | Dry/Semi-dry Fermented Sausages | <0.1 |
| 14 | | Fruits | 1.9x10 ⁻¹¹ | | Fresh Soft Cheese | <0.1 |
| 15 | | Dry/Semi-dry Fermented Sausages | 1.7x10 ⁻¹¹ | | Semi-Soft Cheese | <0.1 |
| 16 | | Semi-soft Cheese | 6.5x10 ⁻¹² | | Soft Ripened Cheese | <0.1 |
| 17 | | Soft Ripened Cheese | 5.1x10 ⁻¹² | | Deli-type Salads | <0.1 |
| 18 | | Vegetables | 2.8x10 ⁻¹² | | Raw Seafood | <0.1 |
| 19 | | Deli-type Salads | 5.6x10 ⁻¹³ | | Preserved Fish | <0.1 |
| 20 | | Ice Cream and Other Frozen Dairy Products | 4.9x10 ⁻¹⁴ | | Ice Cream and Other Frozen Dairy Products | <0.1 |
| 21 | | Processed Cheese | 4.2x10 ⁻¹⁴ | | Processed Cheese | <0.1 |
| 22 | | Cultured Milk Products | 3.2x10 ⁻¹⁴ | | Cultured Milk Products | <0.1 |
| 23 | | Hard Cheese | 4.5x10 ⁻¹⁵ | | Hard Cheese | <0.1 |

^aFood categories were classified as high risk (>5 cases per billion servings), moderate risk (≤5 but ≥1 case per billion servings), and low risk (<1 case per billion servings).

^bFood categories were classified as very high risk (>100 cases per annum), high risk (>10 to 100 cases per annum), moderate risk (≥1 to 10 cases per annum), and low risk (<1 cases per annum).



Friday,
June 6, 2003

Part V

Department of
Agriculture

Food Safety and Inspection Service

9 CFR Part 430
Control of *Listeria monocytogenes* in
Ready-to-Eat Meat and Poultry Products;
Final Rule

● Post-lethality alternatives:

- (1) a. Treatment (may be antimicrobial agent) that reduces or eliminates microorganisms, **AND**
 - b. Antimicrobial agent/process that inhibits growth
- (2) a **OR** b (*more FSIS verification*)
- (3) Sanitation and microbiological testing programs (*more...more FSIS verification*)

Fed. Reg.: June 6, 2003; Vol. 68, N# 109; Pp. 34207-34254; 9 CFR Part 430

ANTIMICROBIAL ALTERNATIVES

- Processing Lethality Treatments
 - Heat Treatments
 - Chemical Formulation Treatments
 - Lactate and Diacetate
- Post-Processing/Lethality Treatments
 - Physical Treatments
 - Chemical Treatments
 - Biological Agents
 - Lactics and Bacteriocins
 - Combinations
- Combinations

ANTIMICROBIAL ALTERNATIVES

Physical Treatments

Heating

-  Pre-packaging: steam

-  Post-packaging

 -  Steam

 -  Submersion in hot water

 -  Radiant oven heat

-  Consumer re-heating

High Pressure Processing

Irradiation?

ANTIMICROBIAL ALTERNATIVES

Chemical Treatments

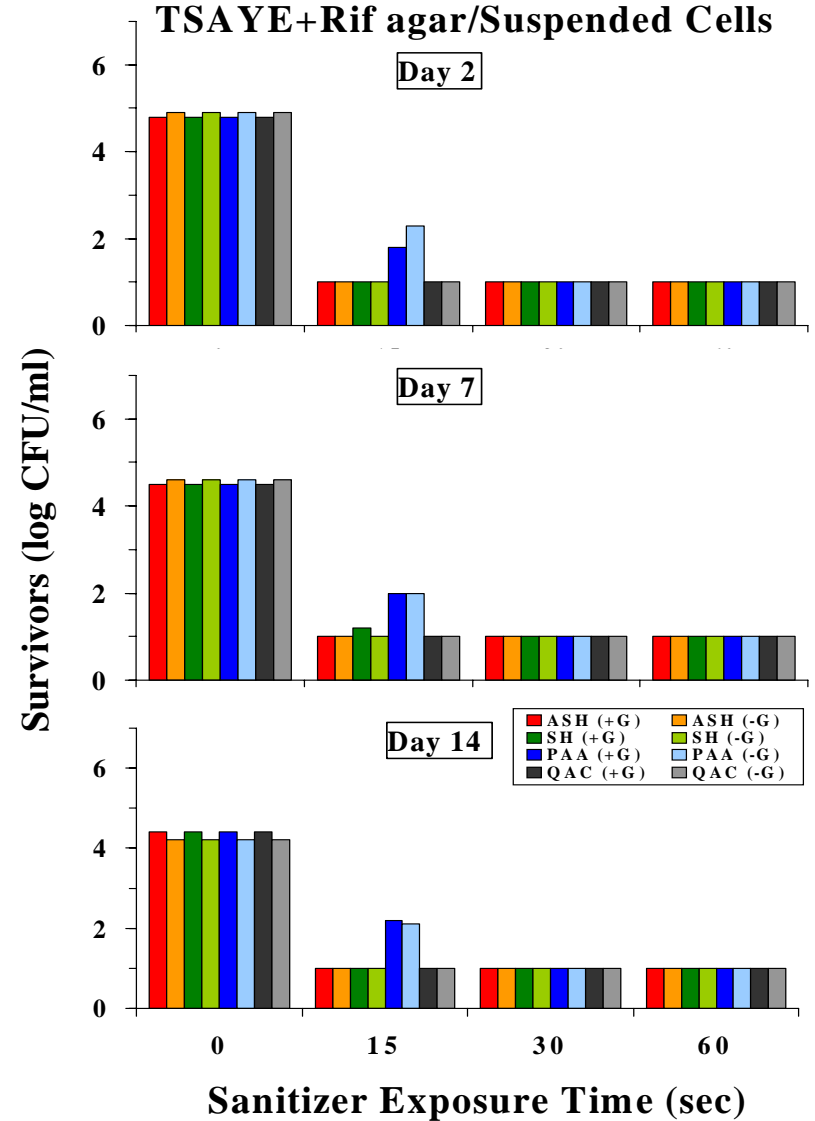
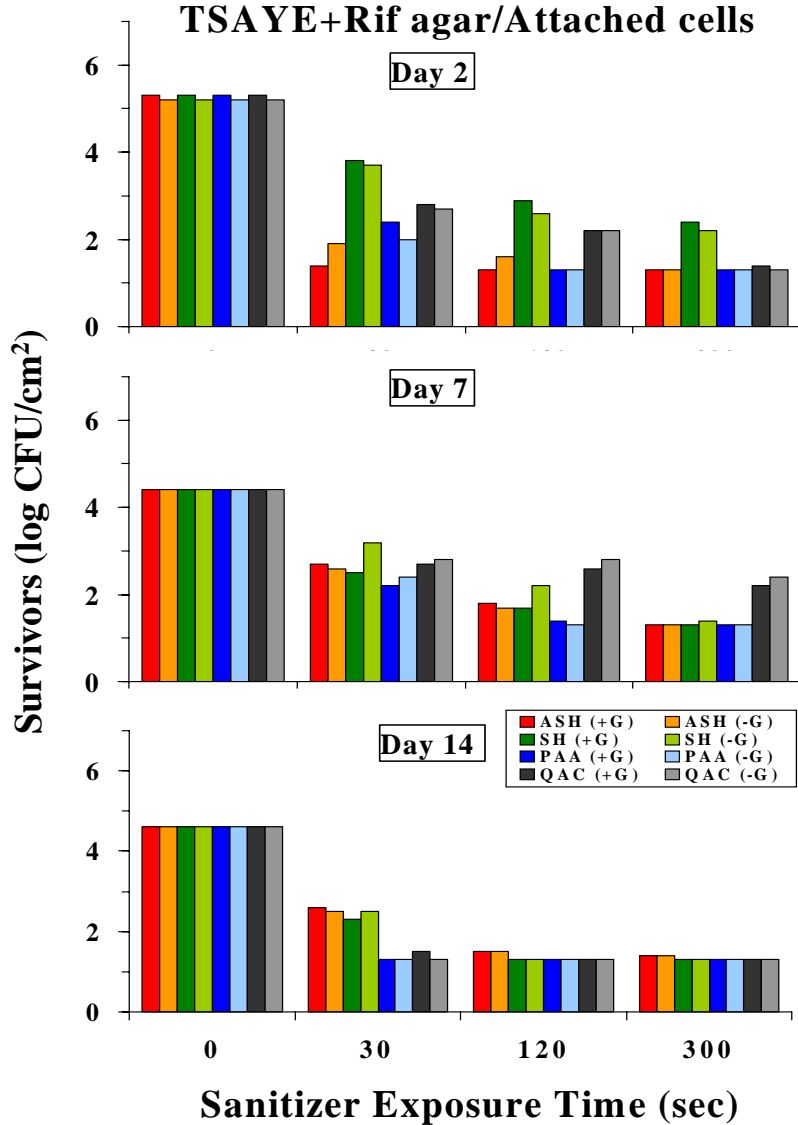
- Sodium/Potassium Lactate/Lactic Acid
- Sodium Diacetate
- Sodium Acetate/Acetic Acid
- Propionate
- Benzoate
- Sorbate
- Citrate
- Nisin
- Pediocin
- Lysozyme
- Acidic Calcium Sulfate
- Essential Oils

Applied as:

- Ingredients
- Solutions
 - Dipping
 - Spraying
- Packaging films: nisin
- Edible films and coatings
- Encapsulated: nisin, lysozyme
- Combinations

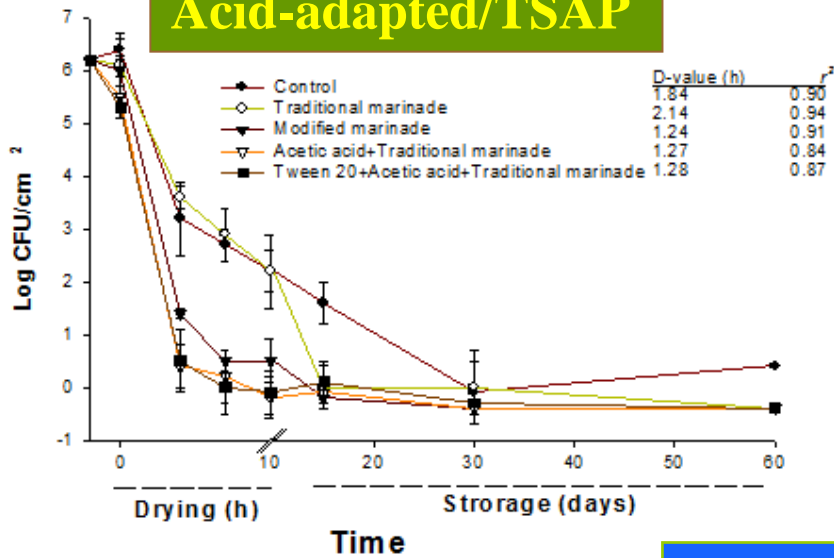
Studies on Ready-To-Eat Meat Products

- **Biofilms:** Formation, inactivation, inoculum
- **Jerky:** Enhanced inactivation
- **Frankfurters/Bologna:** Chemicals in formulations and/or as dips/sprays
- **Commercial Frankfurters and Smoked Sausage:** Chemicals in formulations and/or as dips
- **Commercial Bologna, Ham, and Turkey Breast:** Chemicals as dips or sprays
- **Frankfurters, Bologna and Ham:** Sensory quality
- **Frankfurters/Sausage/Bologna/Ham:** Acid tolerance in simulated gastric fluid

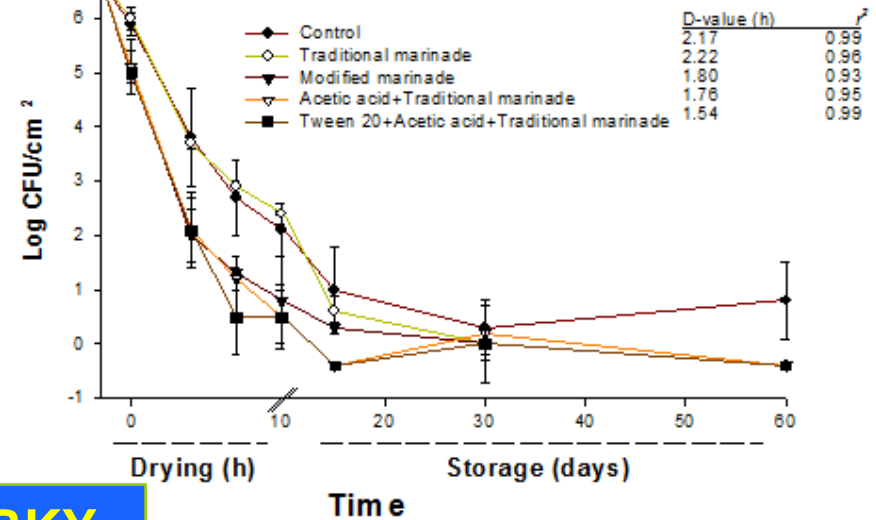


Listeria monocytogenes

Acid-adapted/TSAP

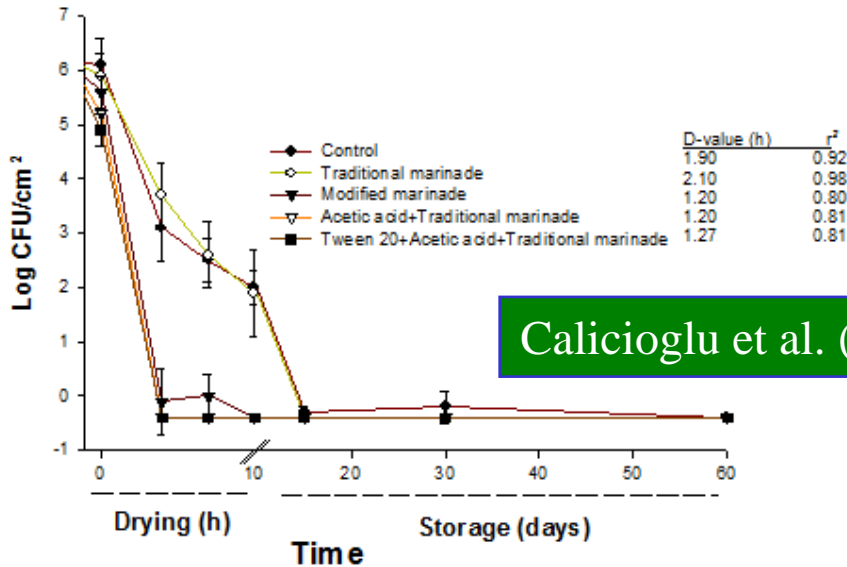


Nonacid-adapted/TSAP

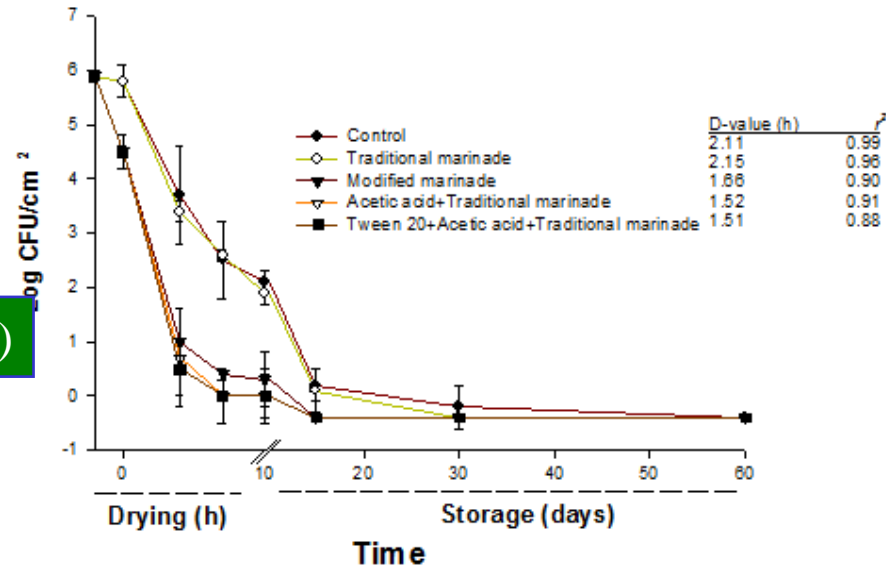


BEEF JERKY

Acid-adapted/PALCAM



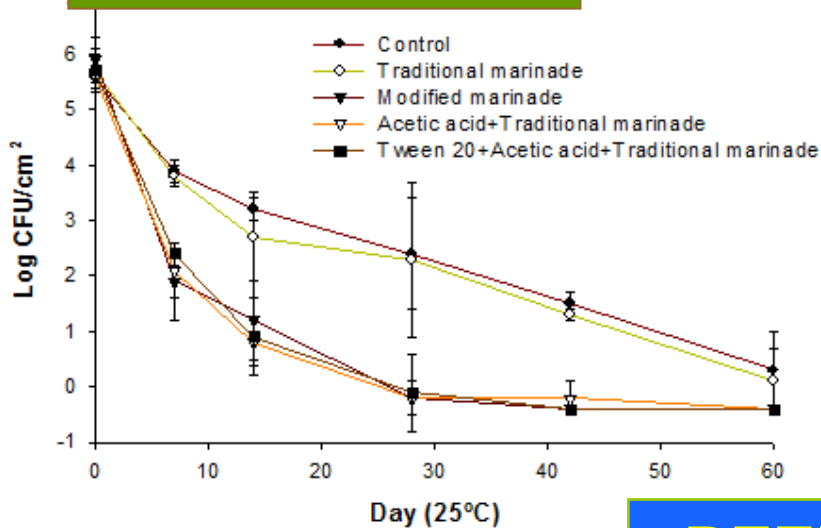
Nonacid-adapted/PALCAM



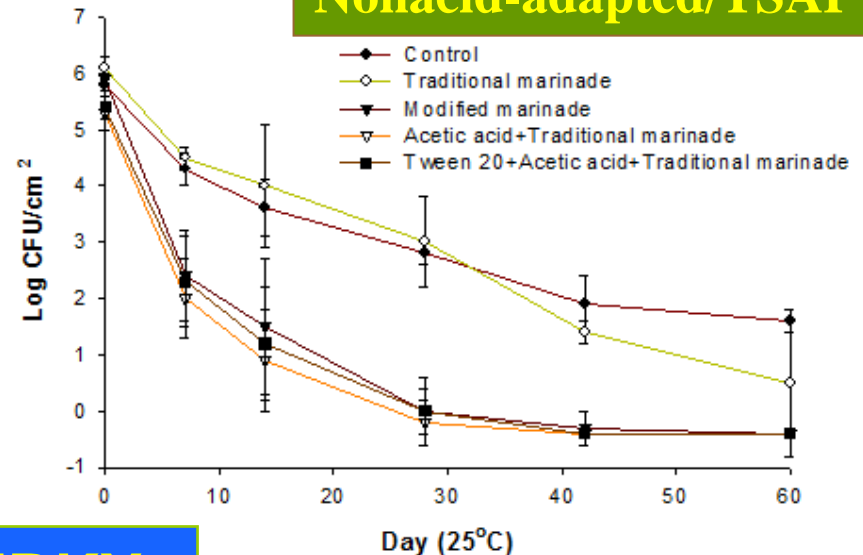
Calicioglu et al. (2002)

Listeria monocytogenes

Acid-adapted/TSAP



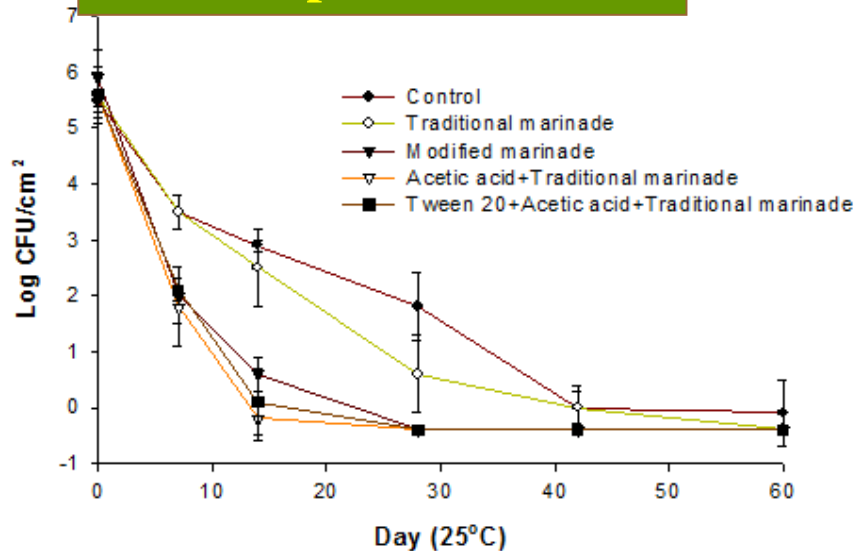
Nonacid-adapted/TSAP



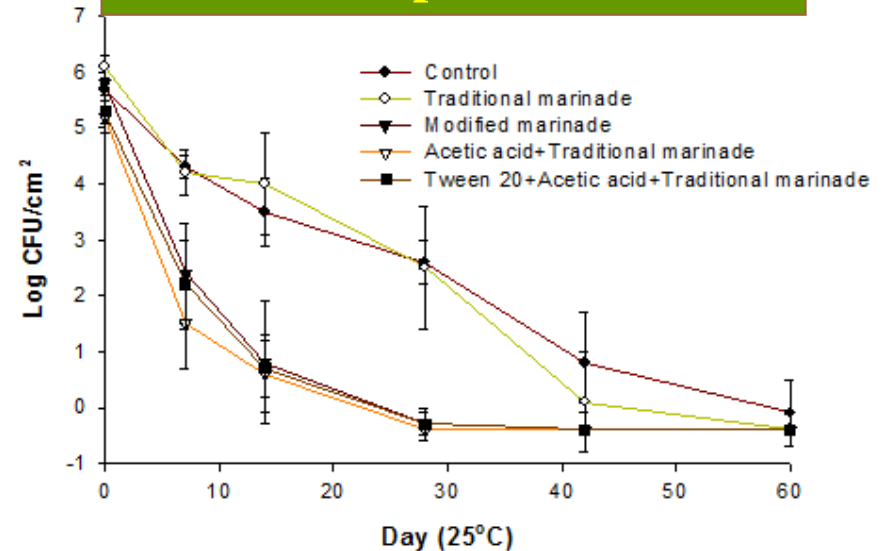
Calicioglu et al. (2002)

BEEF JERKY

Acid-adapted/PALCAM



Nonacid-adapted/PALCAM



SUBMERSION HEATING

Surface inoculated

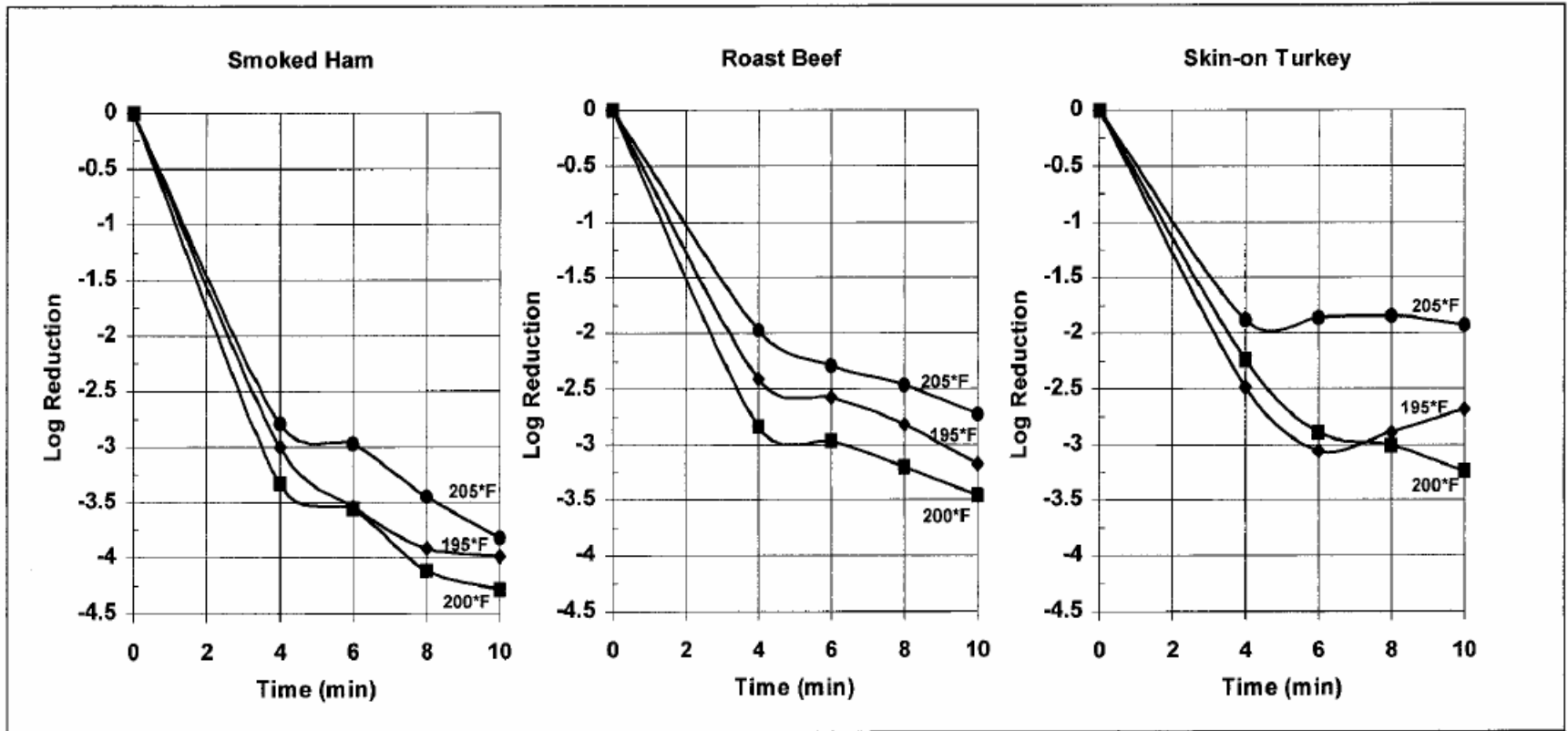


FIGURE 3. Reduction of *L. monocytogenes* on RTE deli-style smoked ham, roast beef, and turkey from manufacturer A at 195°F (90.6°C), 200°F (93.3°C), and 205°F (96.1°C).

Radiant Heat Surface Pasteurization

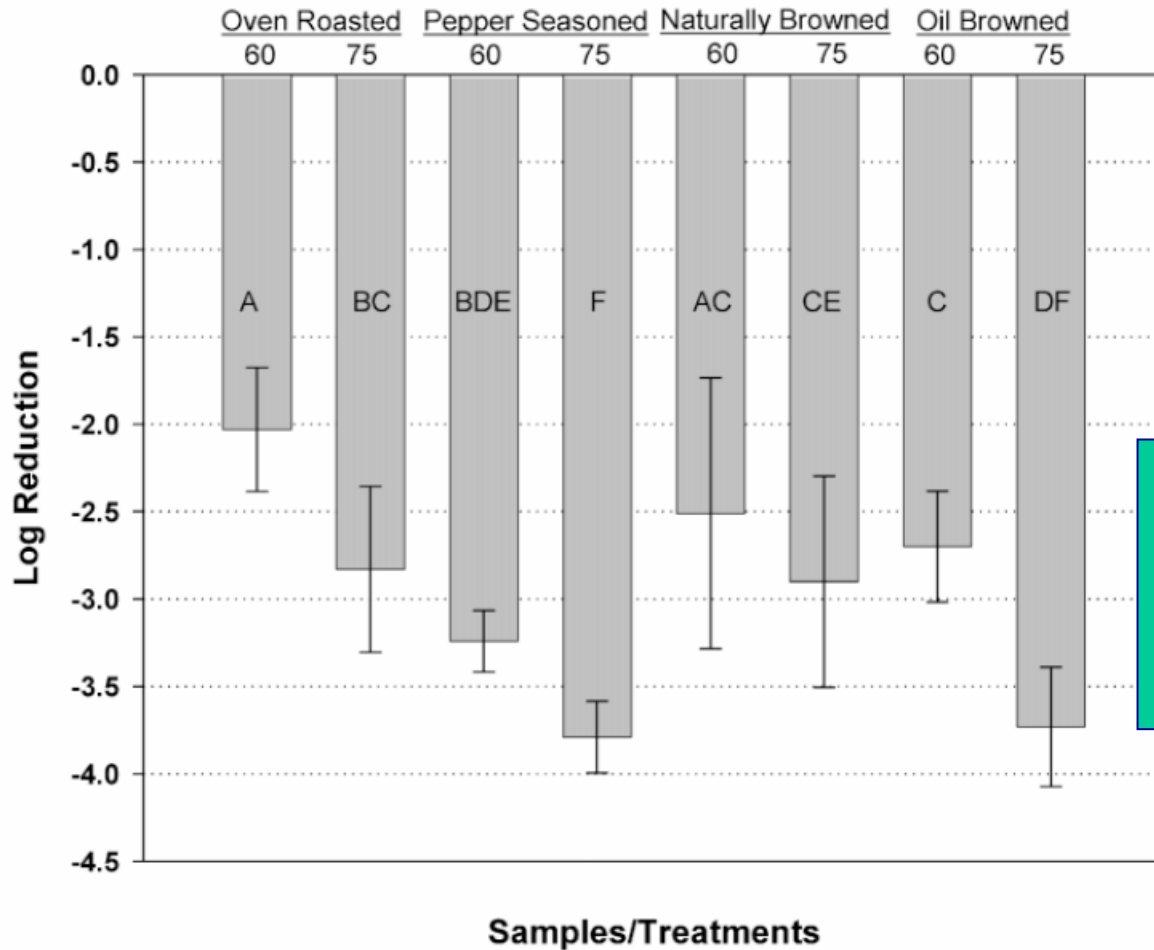


FIGURE 2. Prepackage surface pasteurization using a radiant heat oven on four types of deli turkey (oven roasted, pepper seasoned, naturally browned or skin on, and oil browned) from manufacturer A. Oven dwell time was either 60 or 75 s, as indicated. Error bars represent \pm SD of the means of three replicates ($n = 6$). Bars with the same letter are not significantly different ($P > 0.05$).

**PRE-PACKAGE
RADIANT HEATING
Surface inoculated**

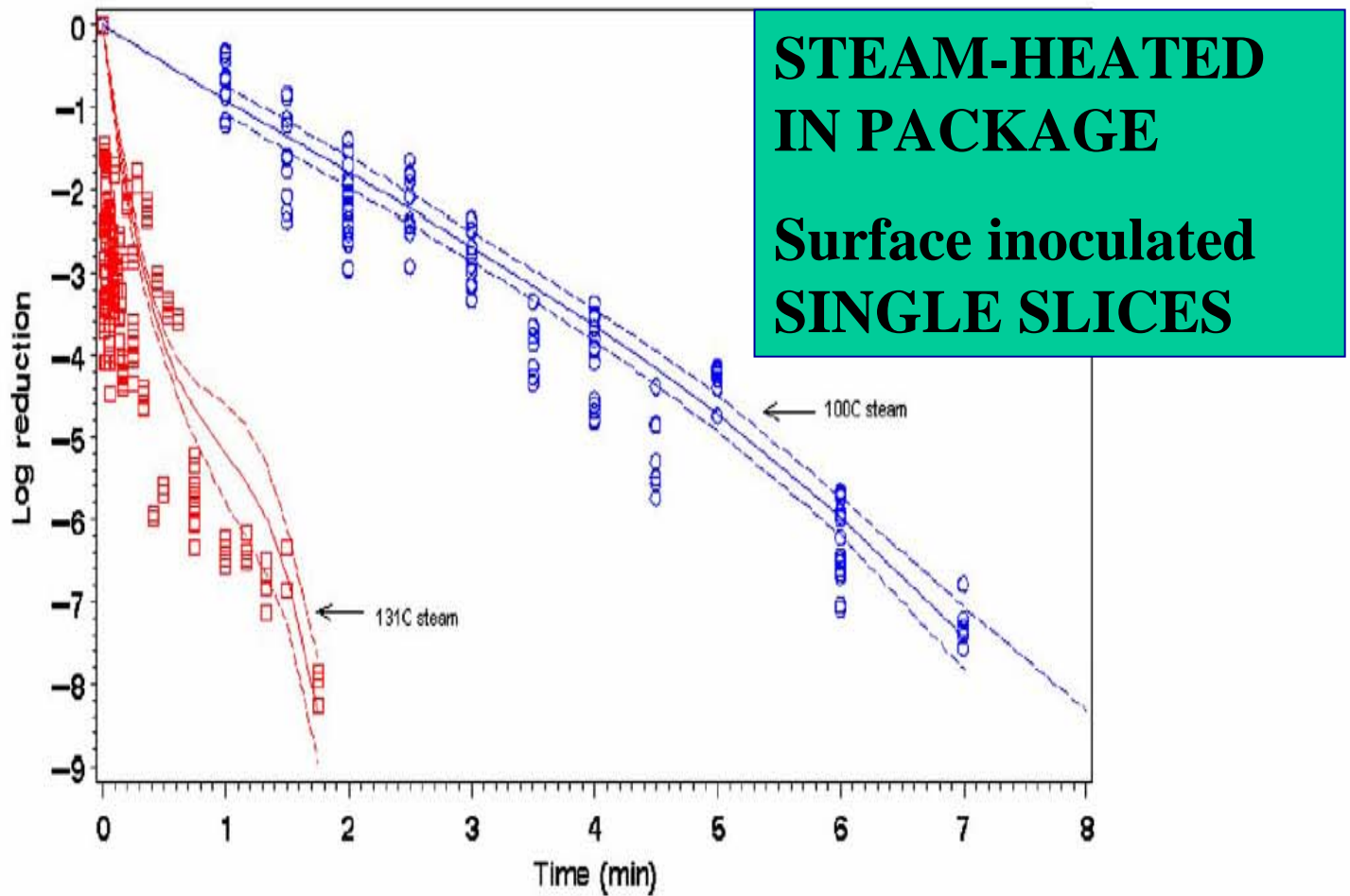
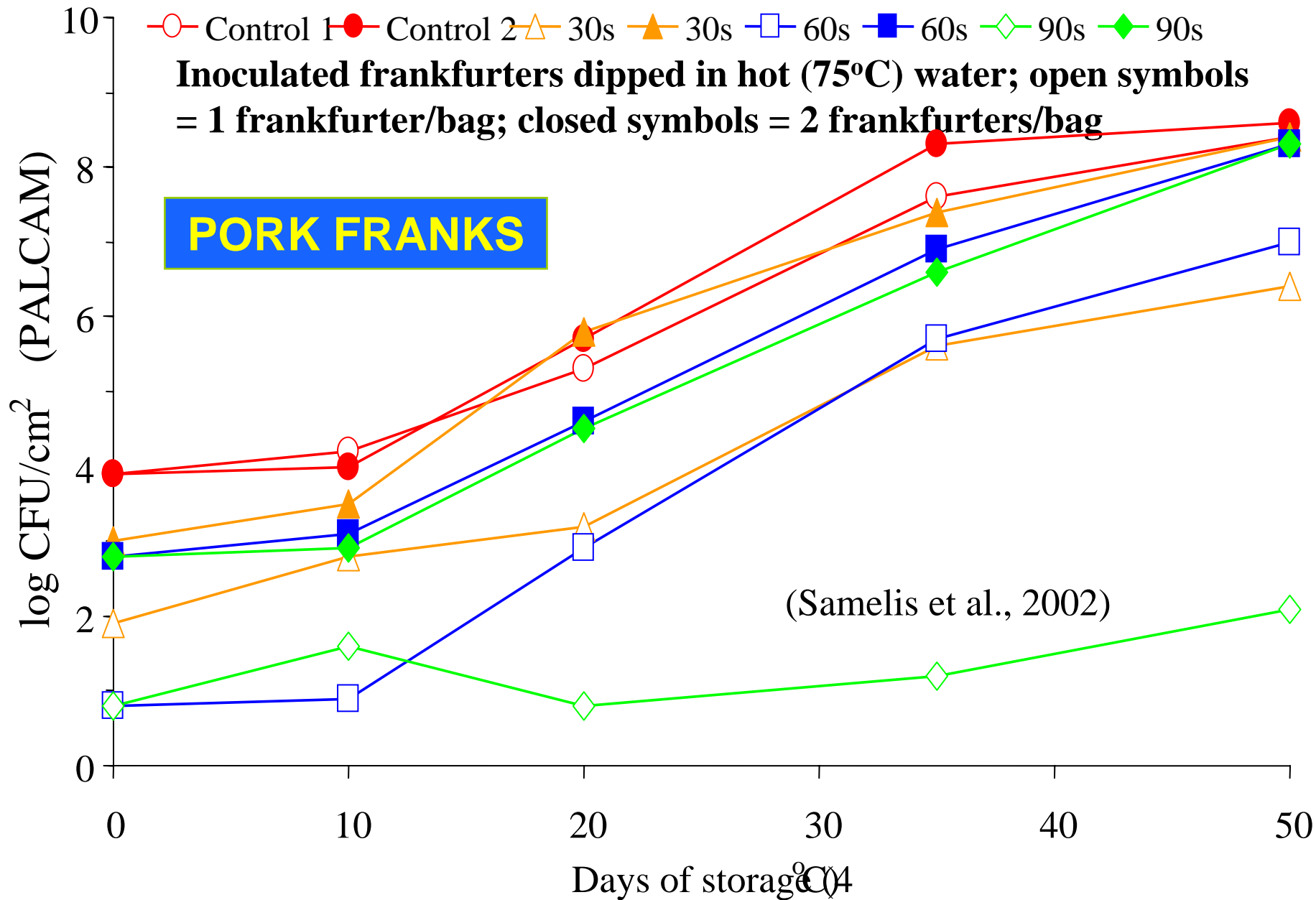


Fig. 1. Log reduction of *L. monocytogenes* in fully cooked bologna (14 cm diameter \times 1.5 cm thickness) pasteurized by ambient steam at 100 °C or pressurized steam at 131 °C. Symbols were experimental data and the lines were model predictions with 95% confidence limits (dashed lines).

R. Y. Murphy et al. / Food Microbiology 22 (2005) 359–365



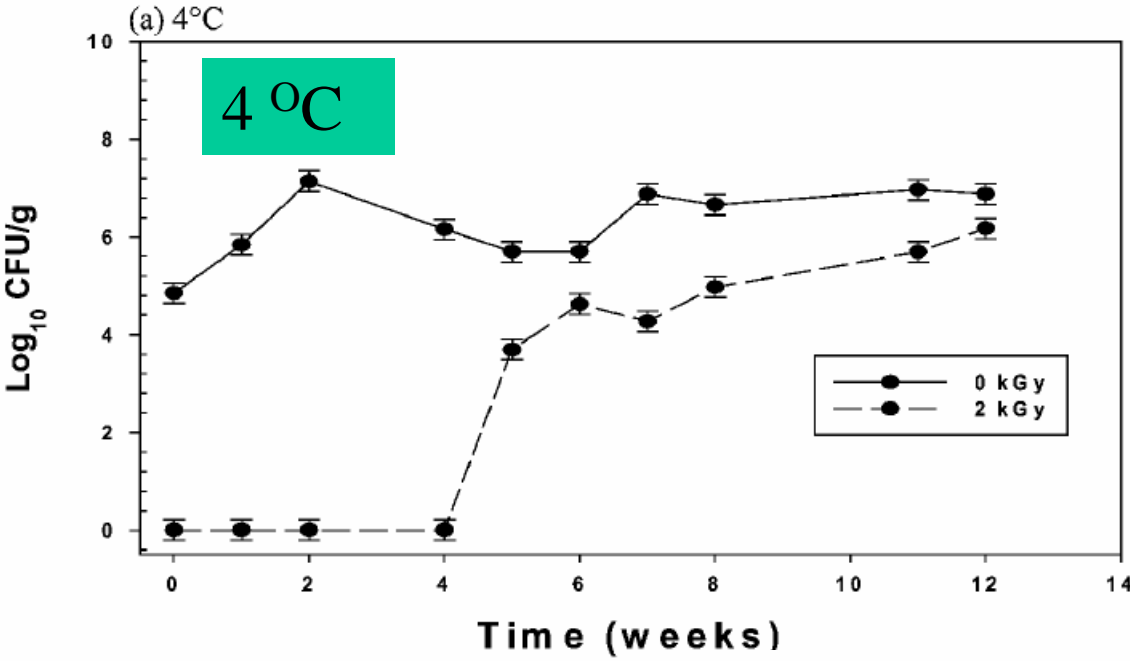
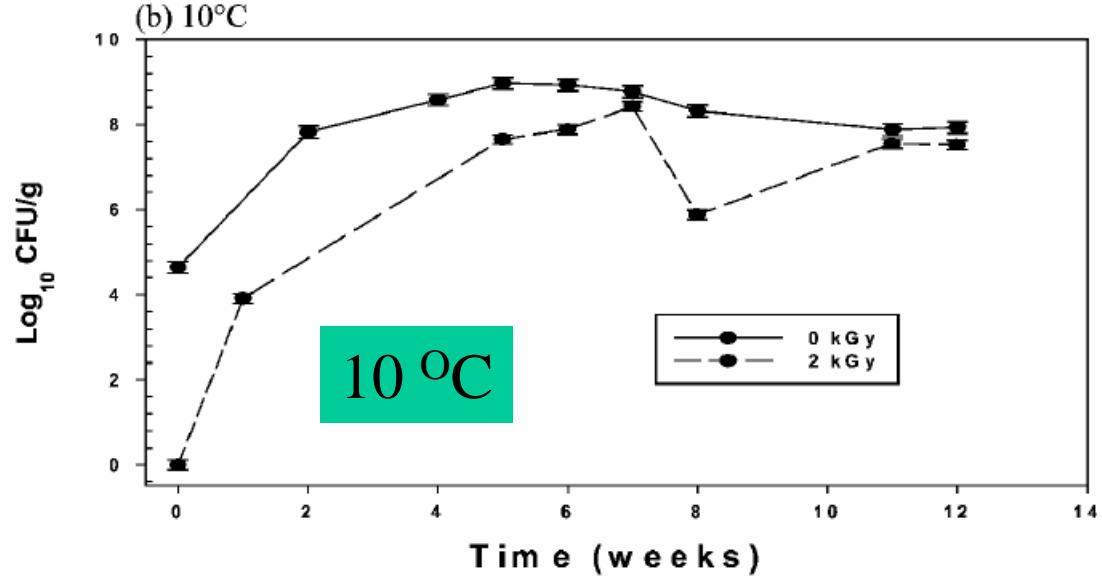
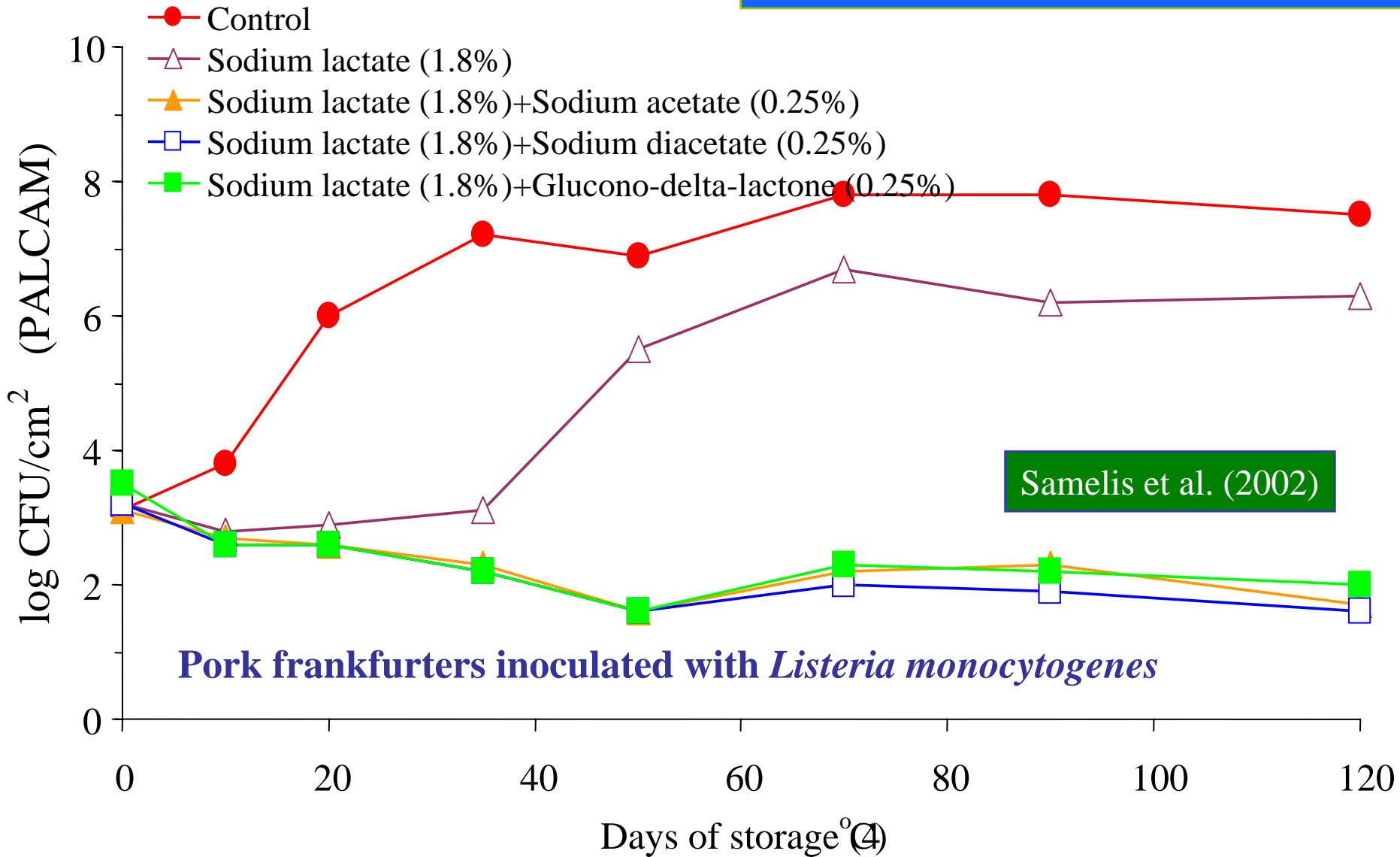


FIGURE 4. Survival and growth of *L. monocytogenes* on bologna.

IRRADIATION
Bologna



SALTS IN FORMULATION

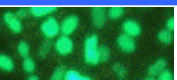
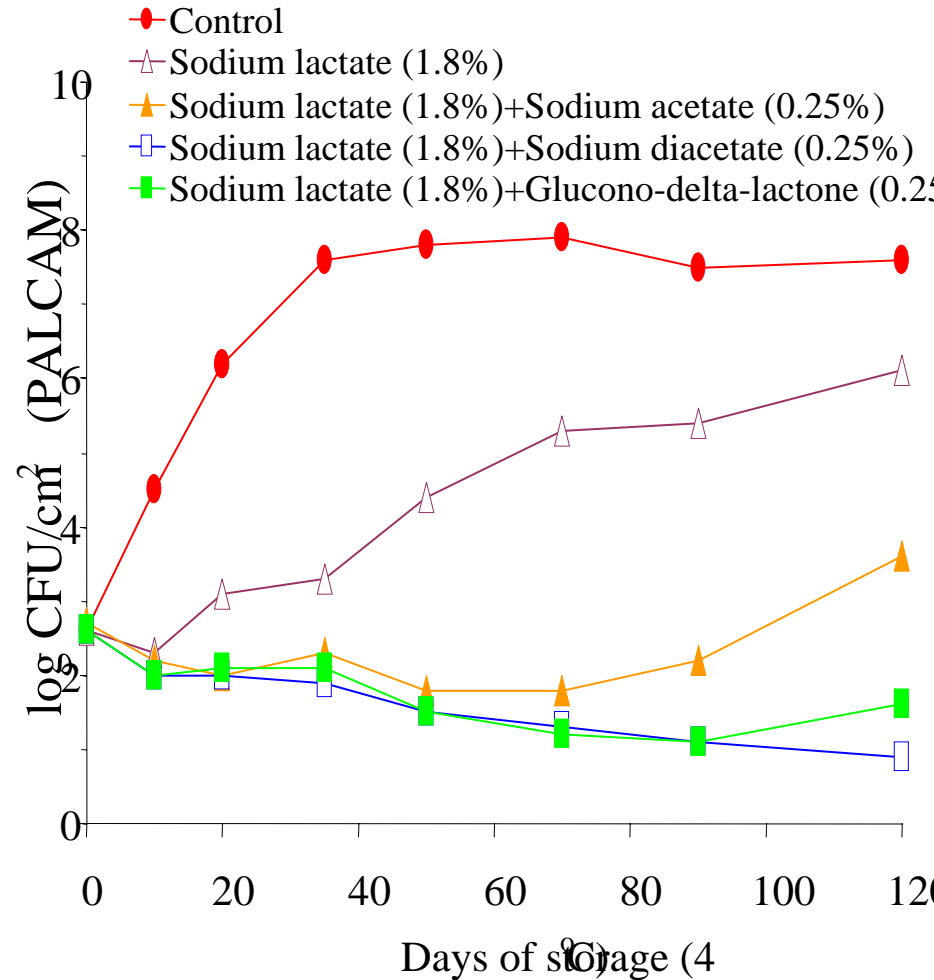
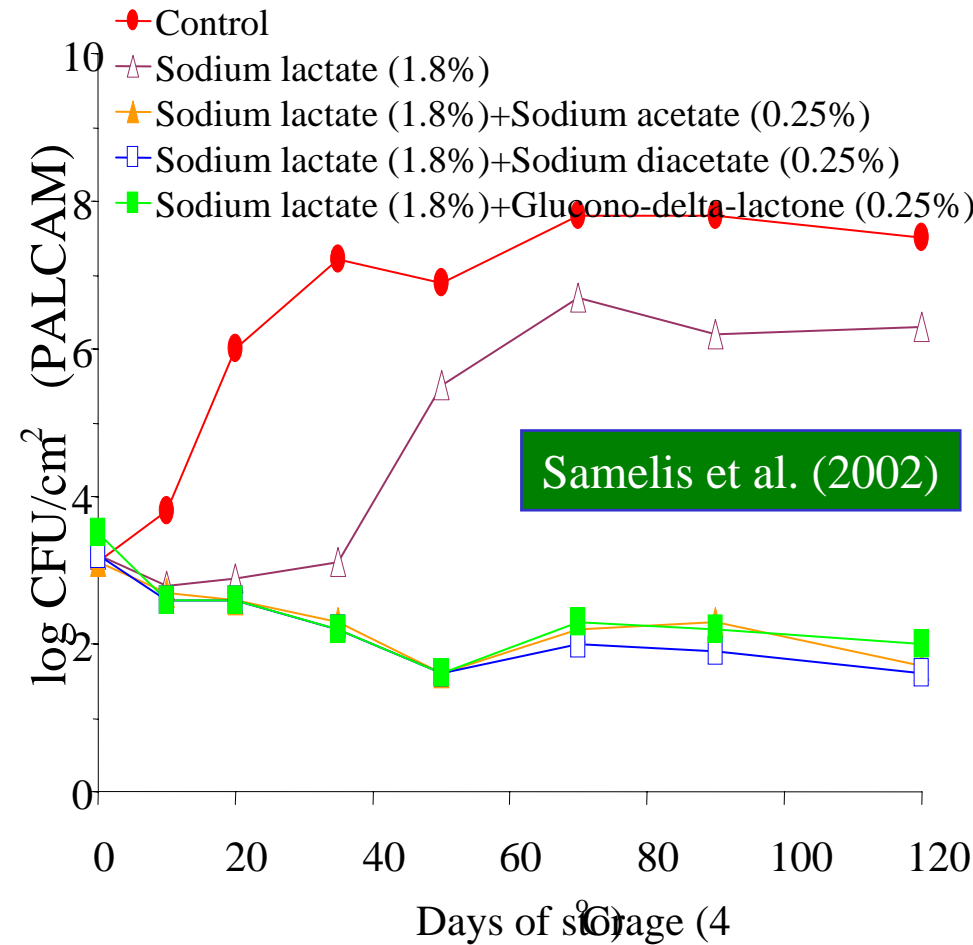


Pork frankfurters inoculated with *Listeria monocytogenes*

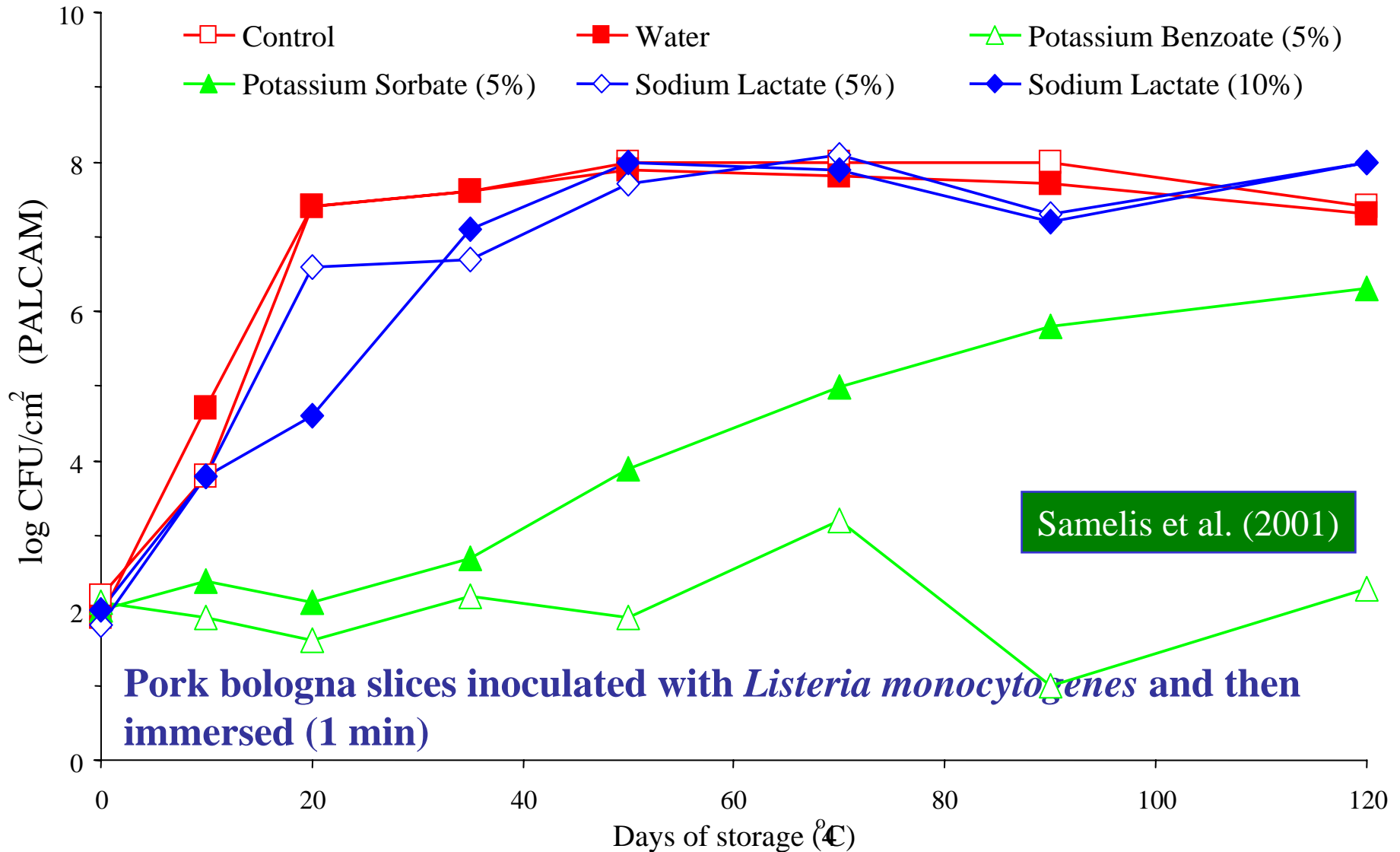
SALTS IN FORMULATION + HEAT

Not dipped

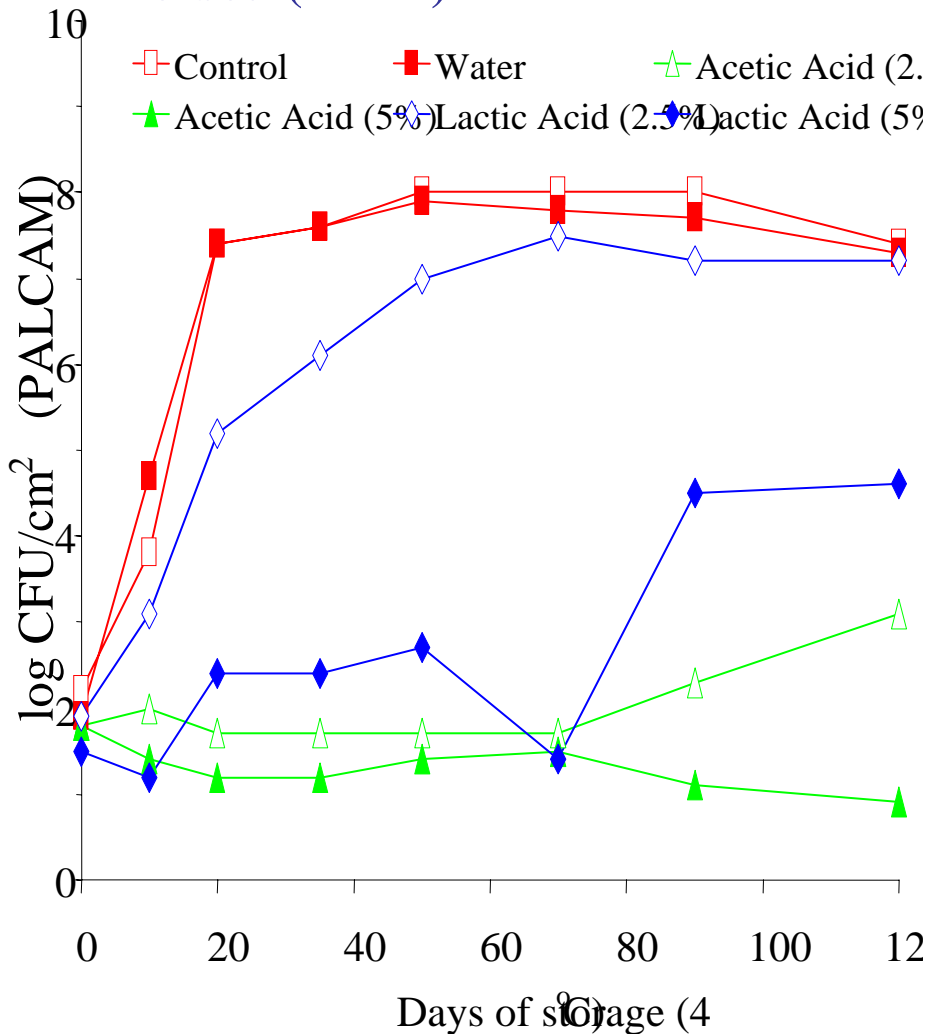
Dipped in hot (80°C) water



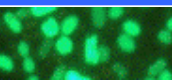
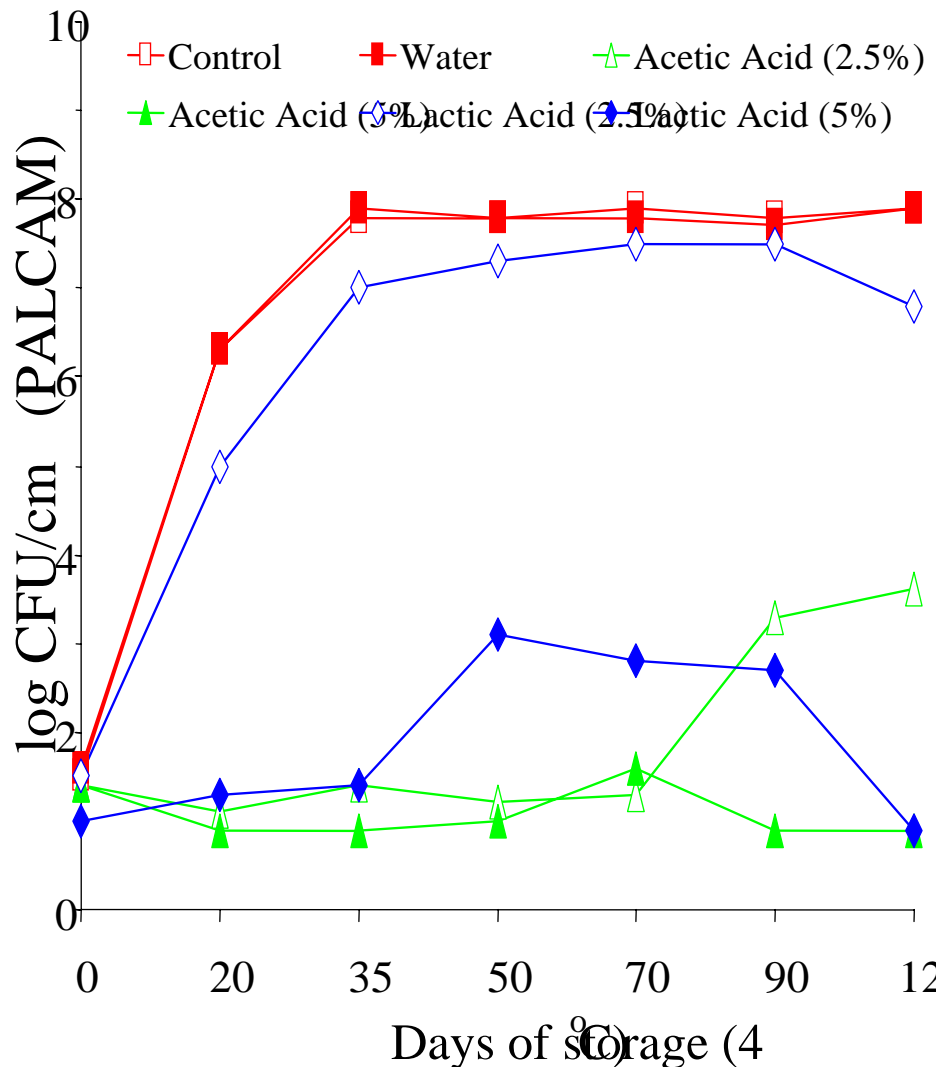
DIPPING SALT SOLUTIONS



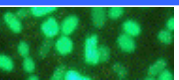
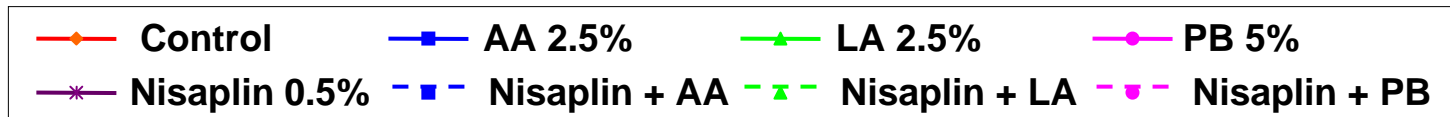
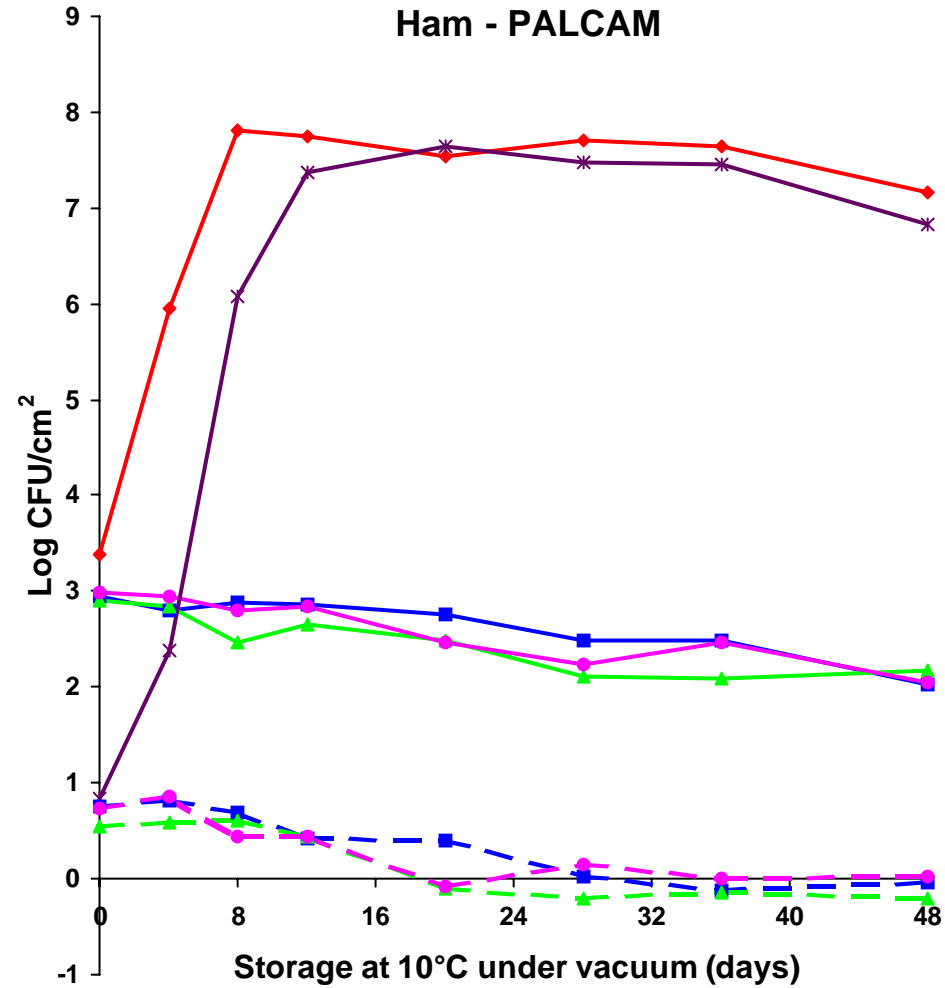
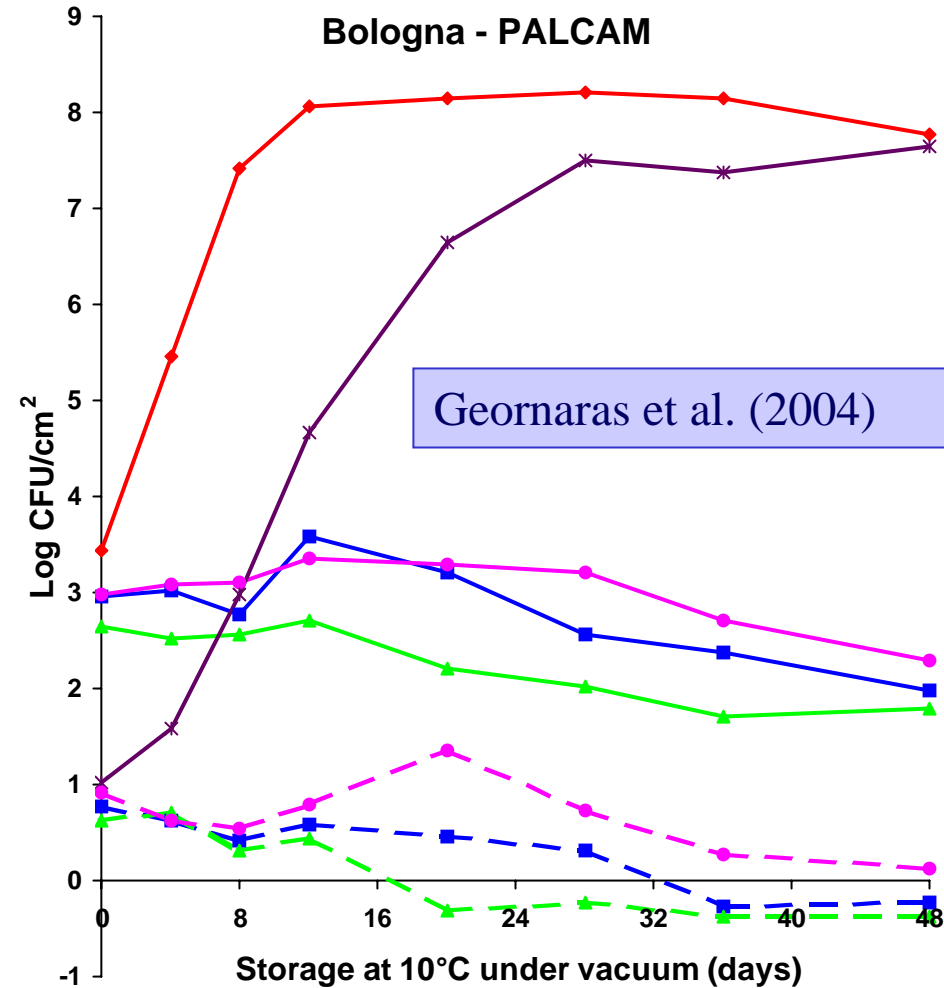
Pork bologna slices inoculated with *Listeria monocytogenes* and then immersed (1 min)



Pork bologna slices immersed (1 min) and then inoculated

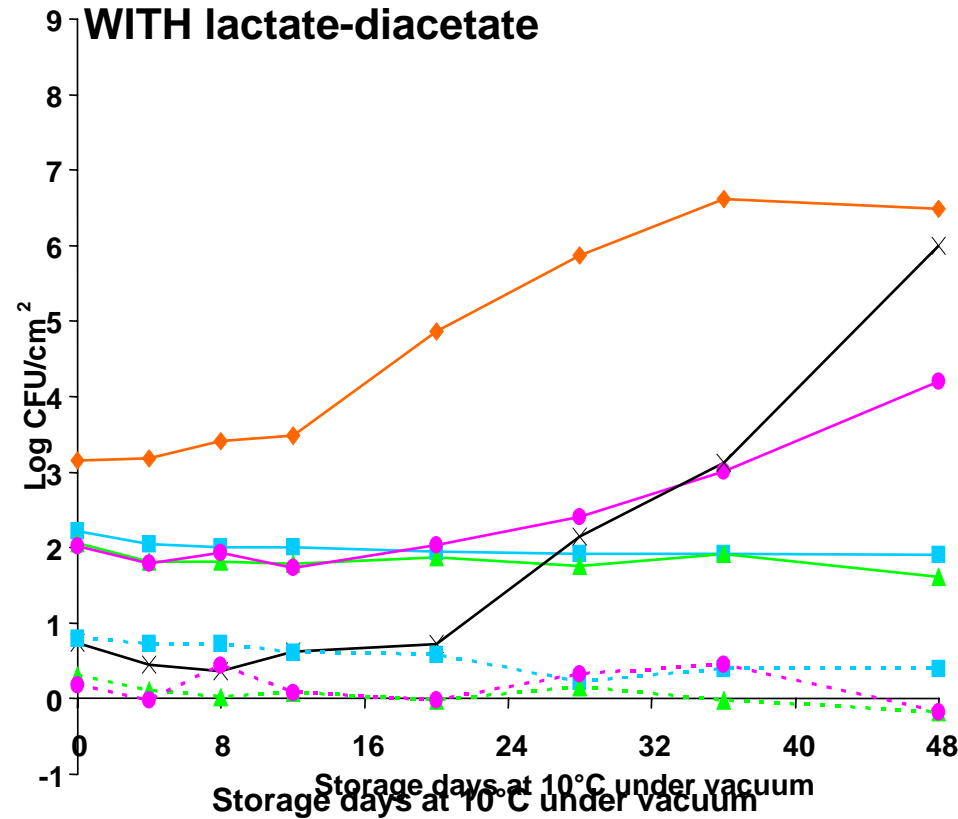
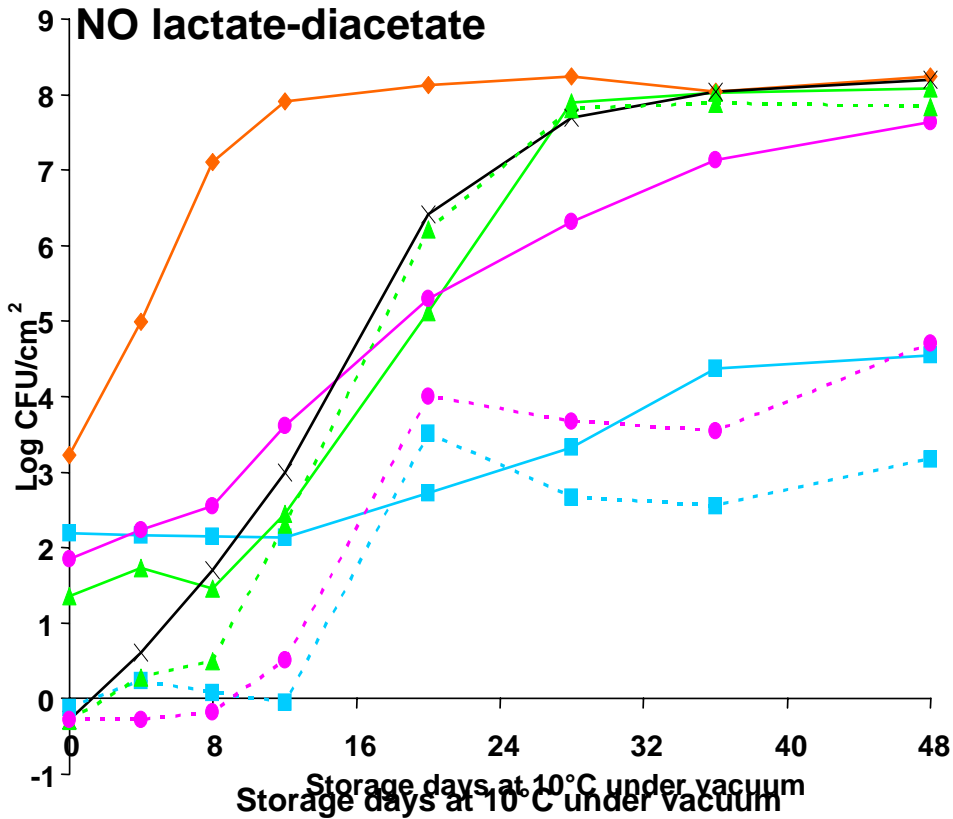


DIPPING (2 min) ANTIMICROBIALS; COMBINATIONS

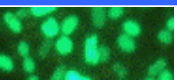


ANTIMICROBIALS IN FORMULATION AND DIPPING (2 min)

FRANKFURTERS

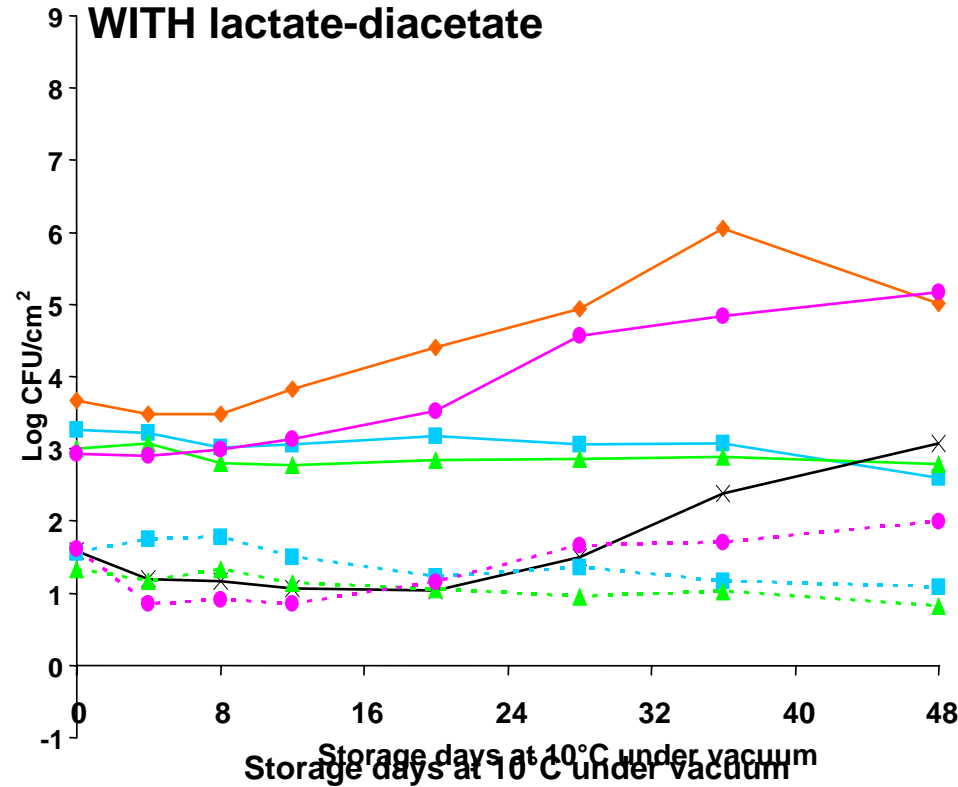
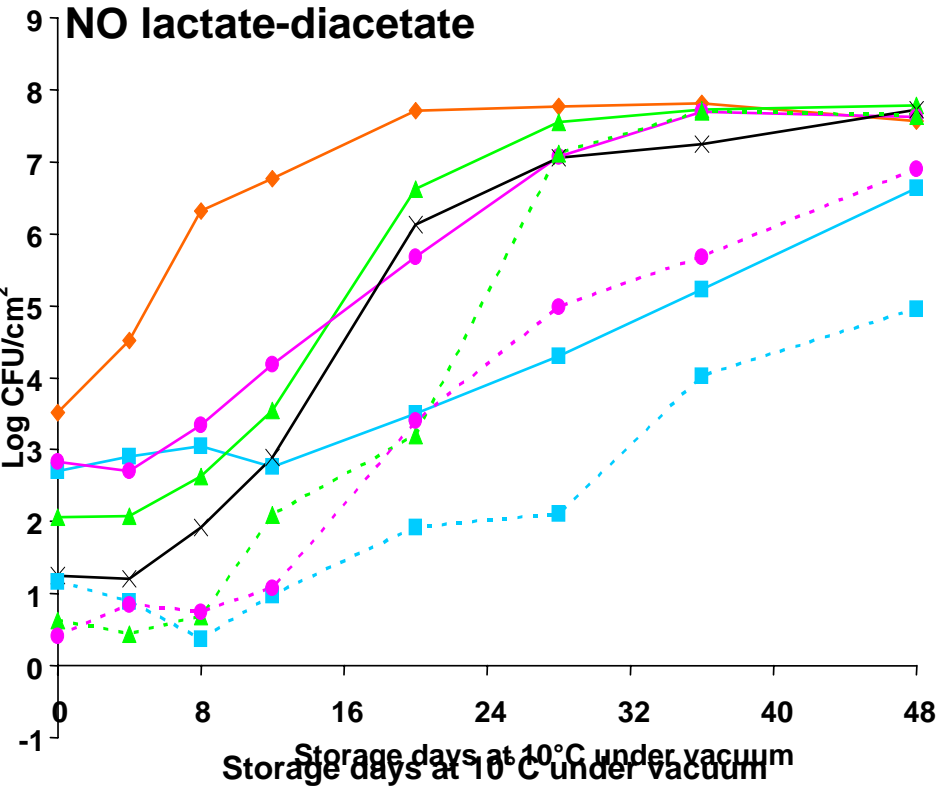


- ◆— Control
- 2.5% AA
- ▲— 2.5% LA
- 5% PB
- ×— 0.5% Nisaplin
- -■- - Nisaplin + AA
- -▲- - Nisaplin + LA
- -●- - Nisaplin + PB

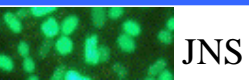


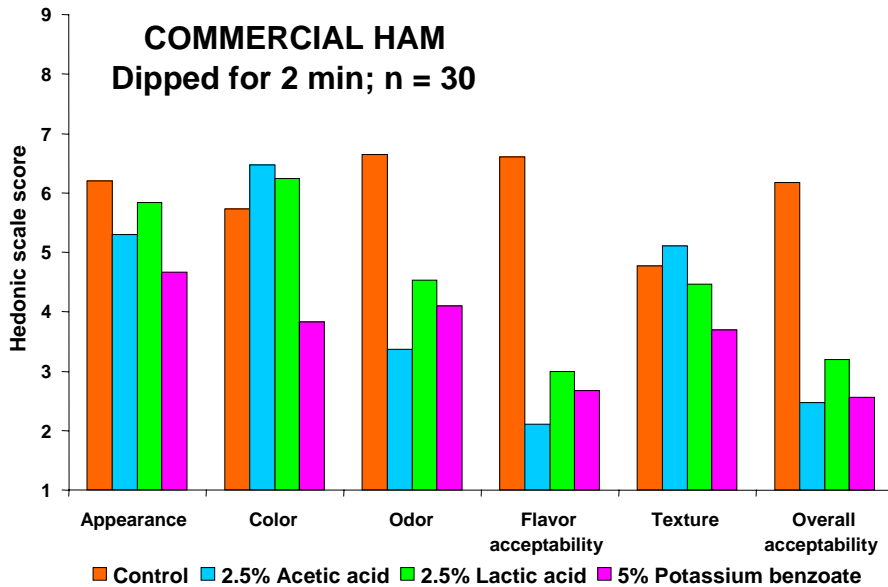
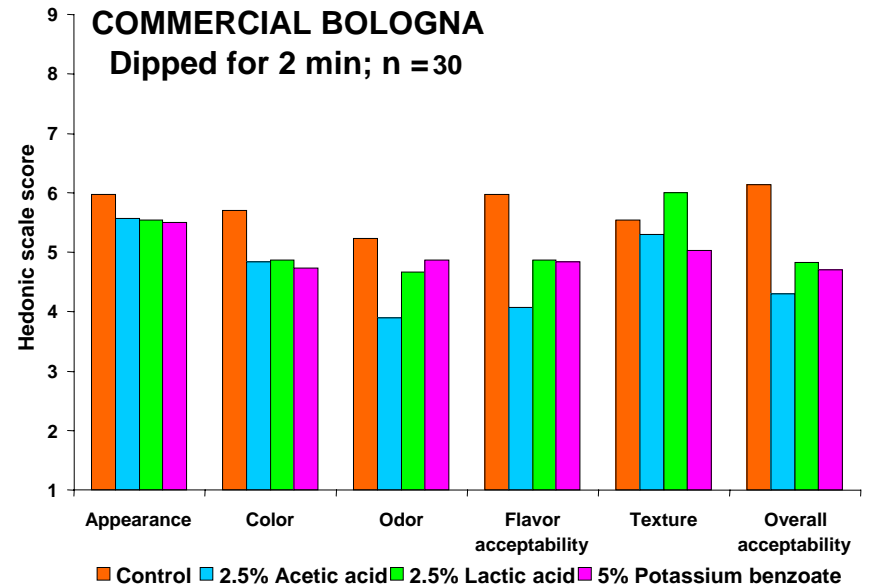
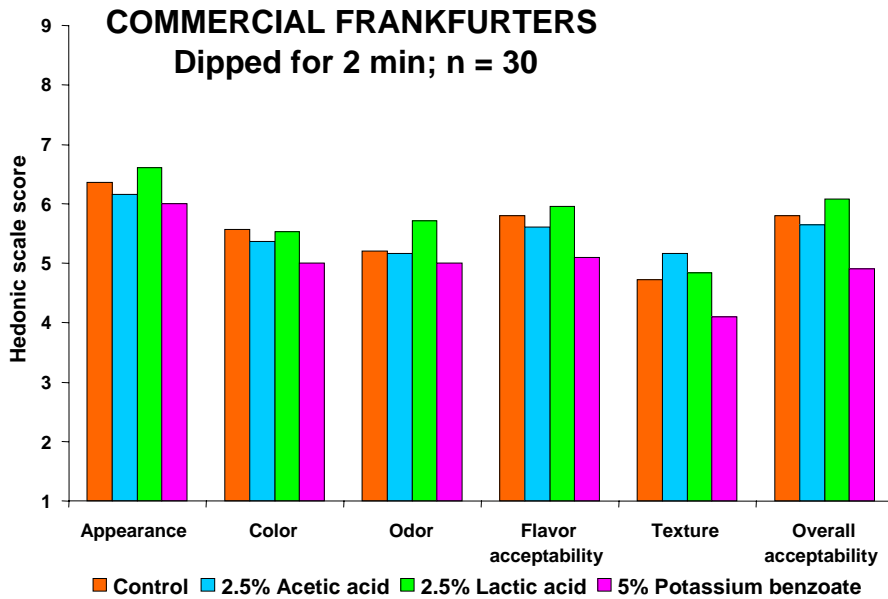
ANTIMICROBIALS IN FORMULATION AND DIPPING (2 min)

SMOKED SAUSAGE



- ◆ Control
- 2.5% AA
- ▲ 2.5% LA
- 5% PB
- × 0.5% Nisaplin
- Nisaplin + AA
- ▲ Nisaplin + LA
- Nisaplin + PB





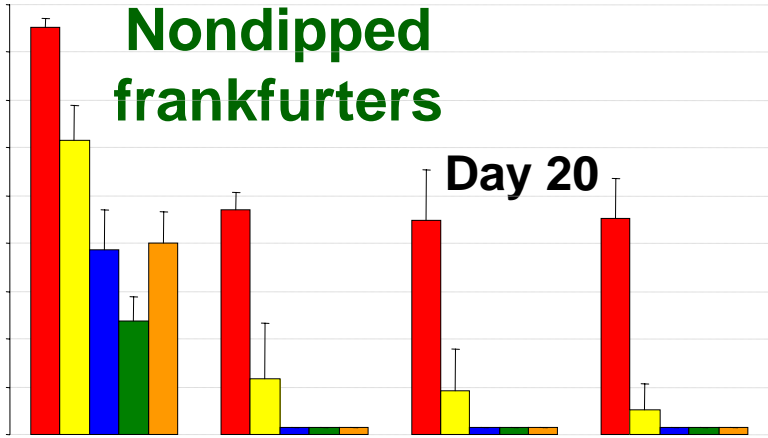
SENSORY QUALITY

Geornaras et al. (2004)

Nondipped frankfurters

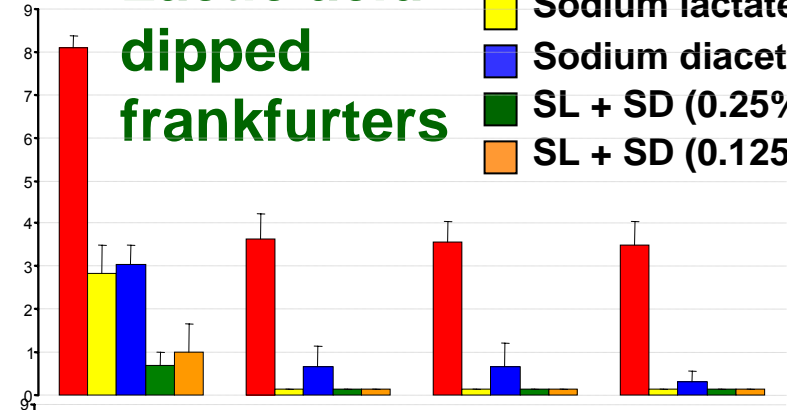
Day 20

log CFU/cm²



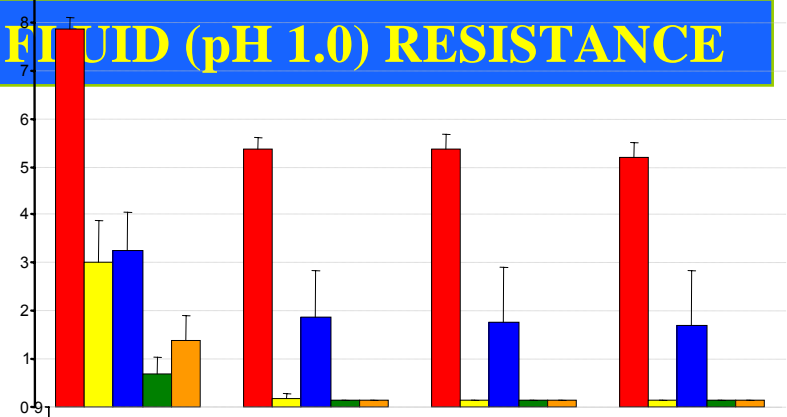
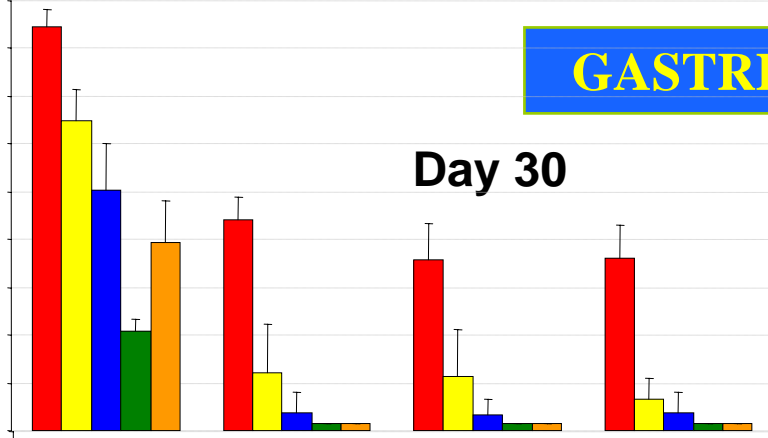
Lactic acid-dipped frankfurters

- █ Control
- █ Sodium lactate (SL)
- █ Sodium diacetate (SD)
- █ SL + SD (0.25%)
- █ SL + SD (0.125%)

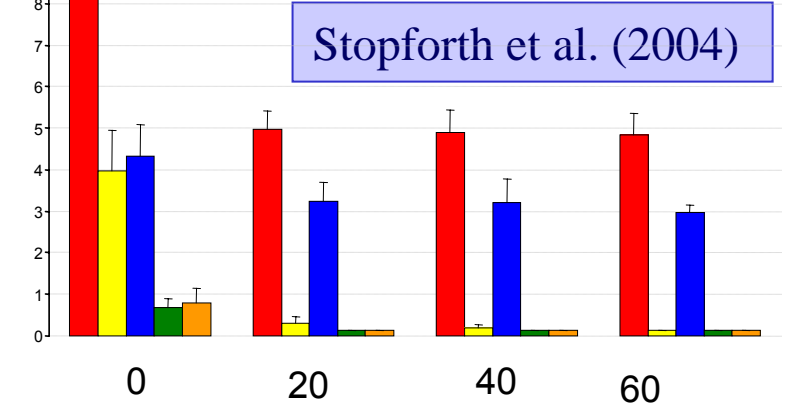
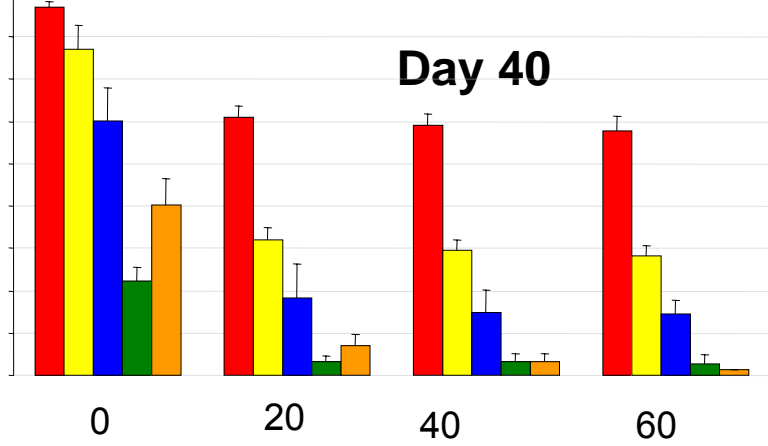


GASTRIC FLUID (pH 1.0) RESISTANCE

Day 30

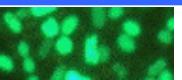


Day 40



Stopforth et al. (2004)

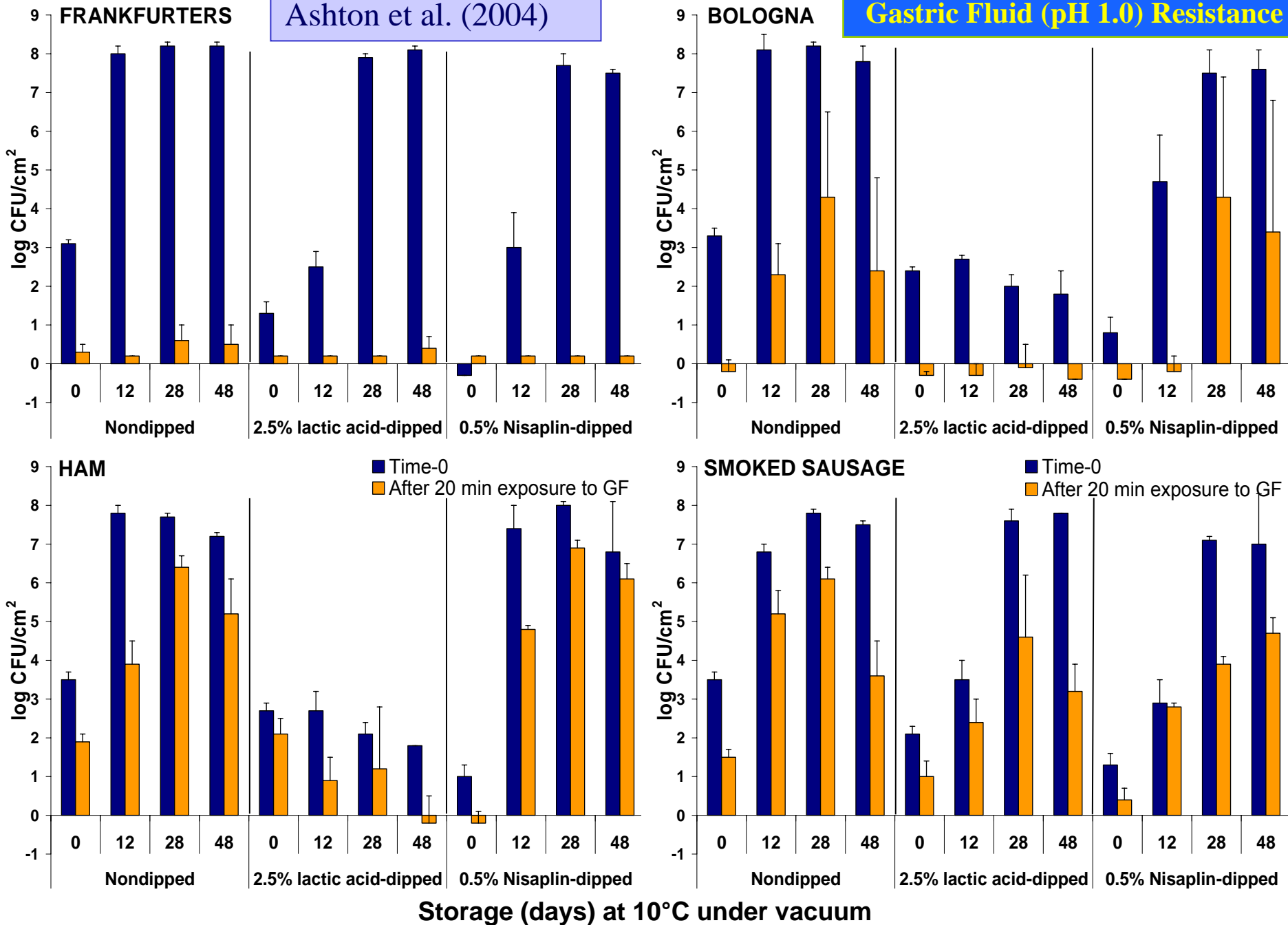
0 20 40 60 Exposure time (min)



COMMERCIAL PRODUCTS NO LACTATE/DIACETATE INGREDIENTS

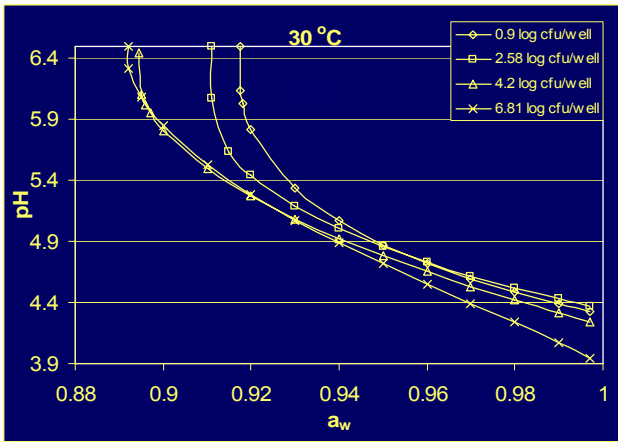
Ashton et al. (2004)

Gastric Fluid (pH 1.0) Resistance

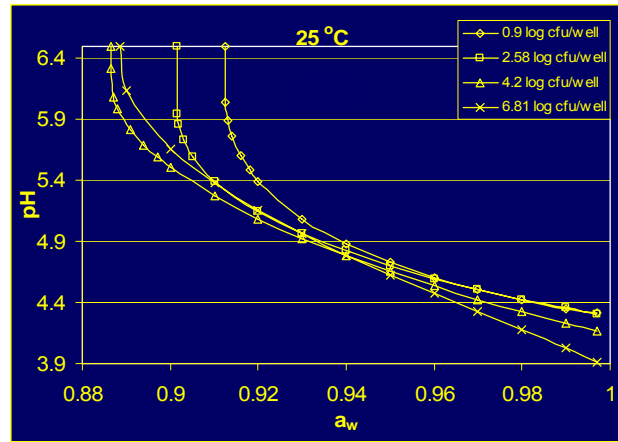


Effect of Inoculum Level, Temperature, pH and a_w on the Growth/No Growth Limits of *Listeria monocytogenes*

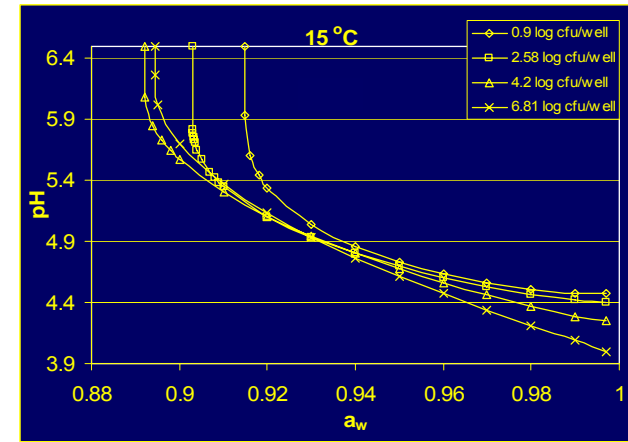
30 °C



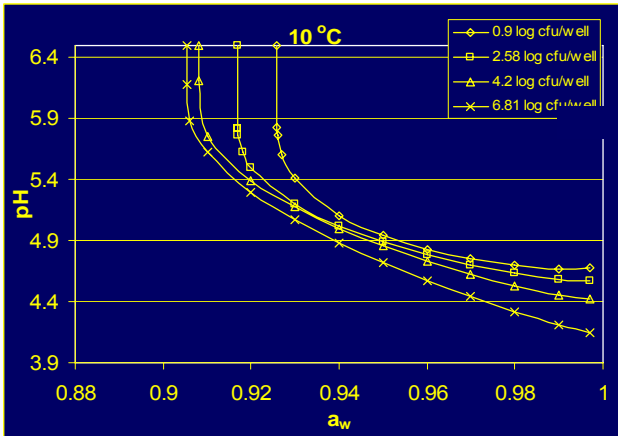
25 °C



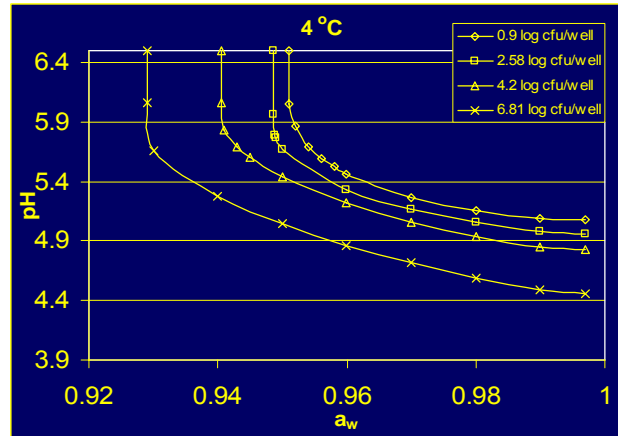
15 °C



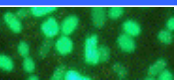
10 °C



4 °C



Koutsoumanis et al., 2004



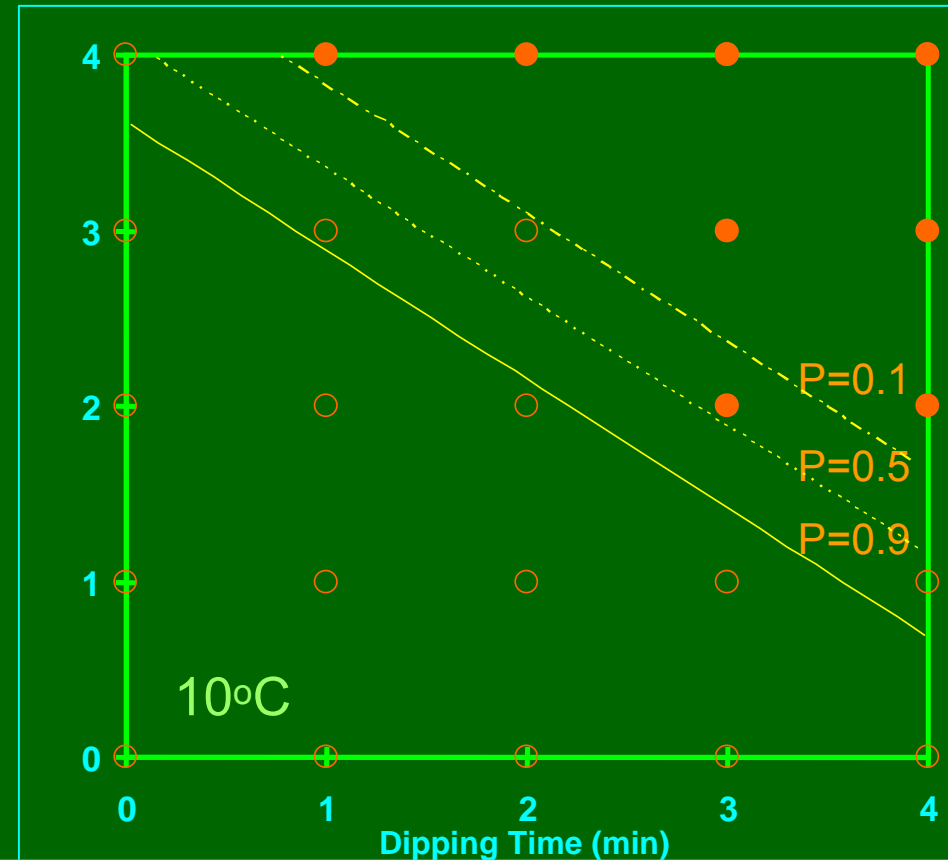
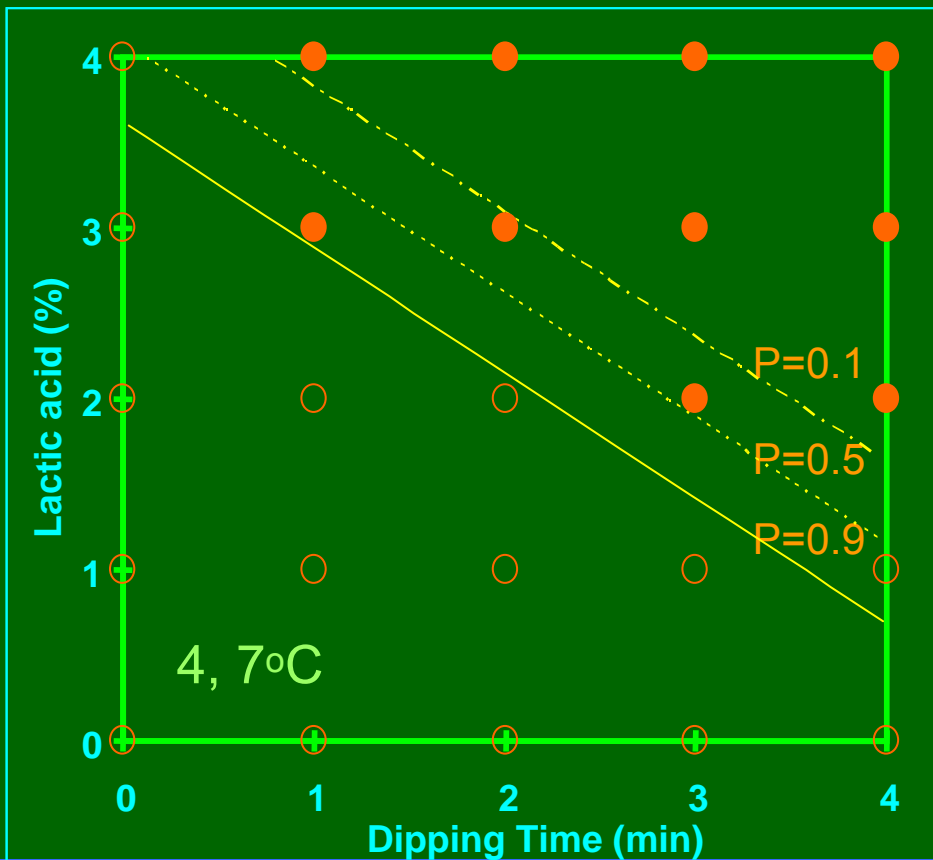
TURKEY BREAST

$$\text{Logit}(P) = a_0 + a_1 \cdot T + a_2 \cdot LA + a_3 \cdot DT + a_4 \cdot T \cdot LA + a_5 \cdot T \cdot DT + a_6 \cdot LA \cdot DT + a_7 \cdot T^2 + a_8 \cdot LA^2 + a_9 \cdot DT^2$$

$P \leq 0.05$

● Growth: ○ No Growth

$$\text{Logit}(P) = 18.9039 - 4.6142 \cdot LA + -3.3843 \cdot DT$$



SUMMARY

- ◆ Need to control *L. monocytogenes* in RTE foods
- ◆ Effective antimicrobials available
 - ⊕ Individually or in combinations
 - ⊕ As ingredients and/or dips/sprays
 - ⊕ In combination with physical hurdles
- ◆ Optimize combinations of hurdles
 - ⊕ Inhibition or even death of pathogen
 - ⊕ Under variable conditions
 - ⊕ In various types of products
 - ⊕ Without adverse effects on product quality
 - ⊕ No resistance development



INDUSTRY CONTROLS FOR *LISTERIA*



- Proper risk and hazard analysis
- Proper documentation and verification
- Manage supplier quality
- Environmental sampling/testing/monitoring
- Effective and documented sanitation/Prevent niches
- Good manufacturing practices (GMP) and HACCP
- Proper and effective worker training/SOP
- Upgrade older plants (drains, traffic, etc)
- Control rework and product reprocessing
- Effective product lotting/Limit the size of product lots
- Effective coding and traceability/recall plans
- Use lethal processes (steam, hot water, pressure, etc)
- Use chemicals



CONSUMER EDUCATION FOR CONTROL OF LISTERIOSIS



- ▀ Thorough cooking of animal foods
- ▀ Thorough washing of raw vegetables
- ▀ Separation of uncooked from ready-to-eat foods
- ▀ Washing hands, cutting boards, knives, etc.
- ▀ At-risk individuals:
 - ▀ Avoid or cook risky foods
 - ▀ Avoid raw/unpasteurized foods

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