



TECHNICAL RESEARCH CENTER

November 12, 2004

Division of Dockets Management
Food and Drug Administration
5630 Fishers Lane
Room 1061
Rockville, MD 20852

Re: Docket No. 2003P-0574, Listeria Monocytogenes; Petition to Establish a Regulatory Limit

Dear Sir or Madam:

The Refrigerated Foods Association (RFA) appreciates this opportunity to provide comments on the Citizen Petition to establish a regulatory limit of 100 colony forming units per gram (cfu/g) of *Listeria monocytogenes* in foods that do not support the microorganism's growth. 69 Fed. Reg. 29564 (May 24, 2004). RFA, an organization of manufacturers and suppliers of prepared, refrigerated food products that is interested in promoting food safety, submits the following comments in support of the Petition.

RFA agrees with Petitioners that a risk based approach to *L. monocytogenes* involving a regulatory limit of 100 cfu/g of the microorganism is consistent with consumer protection. It is generally acknowledged that low levels of *L. monocytogenes* in foods that do not support its growth present little risk of harm to consumers. Furthermore, as Petitioners have noted, most cases of *L. monocytogenes* are caused by ingestion of amounts of the pathogen that far exceed 100 cfu/g.

Setting a tolerance level of *L. monocytogenes* at 100 cfu/g also provides manufacturers with more realistic requirements than the current "zero tolerance" policy. Attempting to keep *L. monocytogenes* out of a food processing facility is a constant battle. As Petitioners have mentioned, because *L. monocytogenes* is ubiquitous in the environment and can readily enter a food processing facility through raw materials, it is

nearly impossible to completely prevent contamination. Furthermore, the pathogen can sustain itself in facilities, despite stringent sanitation efforts, due to its ability to grow in cool, moist conditions.

Focusing on the concentration instead of mere presence of *L. monocytogenes* would encourage manufacturers to develop better means of detecting and controlling virulent pathogens. The current “zero tolerance” policy, as *IFT’s Expert Report on Emerging Microbiological Food Safety Issues* describes, discourages manufacturers from testing for *L. monocytogenes* due to concern over the regulatory consequences of possible detection. Studies have also suggested that some subtypes of *L. monocytogenes* have greater pathogenic potential in ready-to-eat foods than others. Therefore, as IFT has further mentioned in its *Expert Report*, it may be preferable to specifically test for virulent types of *L. monocytogenes* before rejecting food for the presence of the pathogen alone. Providing a tolerance level for *L. monocytogenes* would also allow federal agencies to focus their own limited resources and attention to areas of greater concern and to the removal of foods that pose the greatest harm to the public.

In the proposed regulation, petitioners describe “prepared foods demonstrated to not support growth of *L. monocytogenes* through competent and reliable scientific evidence, including tests, analyses, literature or research studies.” Pursuant to determining whether prepared foods support growth of the pathogen, RFA supports the use of IFT’s Microbiological Challenge Testing (Chapter 6) and Framework Developed to Determine Whether Foods Need Time/Temperature Control for Safety (Chapter 8), as outlined in its *Evaluation and Definition of Potentially Hazardous Foods*.

Providing for a *L. monocytogenes* tolerance level would encourage the use and further development of preservatives that prevent the pathogen’s growth in ready-to-eat foods. Given *L. monocytogenes*’ prevalence in the environment and the fact that it causes the greatest health risk under conditions that support its growth, one of the most logical ways to reduce the incidence of listeriosis would be to render foods less amenable to the pathogen’s growth. In fact, in a study conducted in 1993, Professor Eric Johnson found decreased levels of *L. monocytogenes* in deli salads that contained preservatives and sufficient acidity to deter the pathogen’s growth (Johnson, Eric, 1993, Control of *Listeria monocytogenes* in Fresh Salad Products (attached)). Later research has confirmed these findings (Eblen, B. S. 2002, *Listeria monocytogenes* growth values in deli salad, (unpublished data from CFSAN, FDA)).

As Petitioners describe, some of the United States’ major trading partners have accepted low levels of *L. monocytogenes* in foods that do not support its growth and thus do not deem mere presence of the pathogen in food a public health risk. While Canada has not set a tolerance level, it has adopted a flexible, risk-based, three-tiered approach. Other countries such as the United Kingdom, Australia, New Zealand, and Denmark have

actually adopted regulatory limits for *L. monocytogenes*. If the United States cannot come to a similar conclusion, it may face repercussions in international trade.

In conclusion, RFA strongly supports Petitioner's request that FDA establish a regulatory limit of 100 cfu/g for *L. monocytogenes* in foods that do not support growth of the microorganism. Below please find a list of our member companies. Again, RFA appreciates the opportunity to comment on Petitioner's request. For any questions regarding these comments, please do not hesitate to contact the undersigned.

Respectfully submitted,



Martin Mitchell
Technical Director

A. S. K. Foods, Inc. (West Plant)
Boston Salads and Provisions Company
Butterfield Foods, LLC
Canyon Creek Soup Co., LTD
Country Maid Inc.
Country Queen Foods
Dawn's Foods, Inc.
Dick's Supermarkets, Inc.
E.H. Avello Mushrooms/ Queen's Produce
Excelline Foods
Future Foods, LTD/ Santa Barbara Bay Foods
Garden-Fresh Foods
Gourmet Boutique
Herkimer Foods/ IDA Mae Salads
Herold's Salads, Inc.
House of Thaller
I & K Distributors
Jongquist Family Kitchen
K.B. Specialty Foods/ The Kroger Co.
Kettle Cuisine, Inc.
Keybrand Foods, Inc.
Kozy Shack Enterprises
Lakeview Farms, Inc.
Mrs. Gerry's Kitchen, Inc.

Mrs. Grissom's Salads, Inc.
Mrs. Stratton's Salads, Inc.
Nic's Foods, Inc.
Nonna's Kitchen
Real Foods Co.
Reser's Fine Foods, Inc.
Ron's Home Style Foods, Inc., dba Texas
Kitchen Salads
Sabra/Blue & White Food Products Corp.
Sandridge Food Corporation
Saranac Brand Foods, Inc.
Schnuck Markets, Inc.
Sheri's Cookery, Inc.
SouthWest Foods
Spring Glen Fresh Foods, Inc.
St. Clair Foods, Inc.
Star Food Products, Inc.
Summer Fresh Salads, Inc.
The Suter Company, Inc.
To-Jo Food Products, Inc.
Ukrop's Super Markets, Inc.
Walker's Food Products Co.
Will's Family Favorites
Winter Gardens Quality Foods

CONTROL OF LISTERIA MONOCYTOGENES IN FRESH SALAD PRODUCTS

REFRIGERATED
FOODS
ASSOCIATION

Study by:

Dr. Eric Johnson
University of Wisconsin

Commissioned by:

Refrigerated Foods Association
Annual Conference
February 19, 1993

Survival of Listeria monocytogenes in Fresh Salad Products

Introduction

The purpose of this study was to determine if the addition of certain barrier chemicals could prevent the growth of or eliminate Listeria monocytogenes from fresh salad products that had been inoculated with low numbers of the organism.

Materials and Methods

Five salad types were tested: (1) chicken salad (shelf life 35 days), (2) seafood salad containing surimi and shrimp (38 days), (3) pasta salad (30 days), (4) potato salad (40 days), and (5) pimento cheese spread (90 days). Each was produced by a different manufacturer and 25 lb. shipments were sent by overnight delivery within 1-2 days of manufacture.

Upon arrival, the salads were assayed for pH, moisture content (5-10 g samples dried in 98-100°C air oven), and titratable acidity (titration with NaOH to phenolphthalein endpoint). The total amount of acetic acid present was also calculated from ingredient lists sent by the manufacturers.

The bulk salads were thoroughly mixed and divided into two batches. The pH of one batch was lowered with 5 N HCl by 0.5 units. The pH of the other pH was not altered. 100 g aliquots of each batch were placed into sterile whirl-pak bags.

Barrier chemicals were added to the bags in the following concentrations:

- (1) glacial acetic acid (Fisher Scientific, Fair Lawn, NJ)
-50% of the amount already calculated to be present in the salad
- (2) 1% USP grade sodium lactate (Wilke International, Overland Park, KS)
- (3) 0.1% sodium benzoate (Mallinkrodt, Inc., Paris, KY)
- (4) 0.2% potassium sorbate (Sigma Chemical Co., St. Louis, MO)

- (5) 0.3% mixture of 5% sodium citrate (Sigma), 5% sodium ascorbate (Sigma), and 90% sodium diacetate (Intermational Sourcing, Inc., Upper Saddle River, NJ)
- (6) 2 mM EDTA (Sigma, tetrasodium salt)
- (7) 0.5% ALTA 2341 (Quest International, Sarasota, FL)
- (8) 50 ppm lysozyme (Societa Prodotti Antibiotica, Milano, Italy)
- (9) 0.15% ALTA 2001 (Quest)

ALTA 2341 and 2001 were added aseptically in powder form. The sodium lactate was too viscous to be filter sterilized, so samples were aseptically removed from a previously unopened container and added to the salads. Stock solutions of the remaining barriers were made in double distilled water and filter sterilized before adding.

ALTA 2001 was tested in pimento cheese spread only. The others were tested in all five salads. In seafood salad, lysozyme was also tested in combination with EDTA, acetic acid, or sodium lactate (same concentrations as previously). In addition, for each salad, controls were prepared with no barrier chemicals added.

The addition of acetic acid to the salads caused a drop in pH of 0.15-0.2 units. No other barrier chemical caused a change in pH.

Five strains of Listeria monocytogenes were used: Scott A, California, V7, Ohio, and LCDC 861 (isolated from coleslaw). Each was grown ~18 h at 37°C in 10 ml trypticase soy broth (Difco Laboratories, Detroit, MI), centrifuged for 10 min at 10,000 rpm, and resuspended in 10 ml 67 mM phosphate buffer (5.83 g NaH₂PO₄•H₂O, 6.74 g Na₂HPO₄•7H₂O, per liter H₂O). All five strains were combined together to make a mixed strain cocktail containing equal numbers of each strain.

After the barriers were added, the bags were inoculated at a level of about 1000 CFU/g salad with the mixed strain cocktail of L. monocytogenes. The bags were then mixed thoroughly by stomaching and other methods, and initial samples taken.

The samples were analyzed for pH, diluted in phosphate buffer, and plated on duplicate plates on Modified Oxford Agar (a selective medium for L. monocytogenes), Plate Count Agar (Difco) for total

aerobic plate counts, and Potato Dextrose Agar (Difco) for growth of yeasts and molds. The Modified Oxford Agar (MOX) plates were counted after 2 days at 37°C, and PCA and PDA plates were counted after 2-4 days at 25°C.

Some samples from chicken salad were additionally plated on Brain Heart Infusion agar (Difco), a nutrient-rich agar. L. monocytogenes counts from these plates were generally the same as counts on MOX agar.

When direct counts could not be obtained on MOX agar, enrichments were carried out by the USDA/FSIS method in UVM broth and Fraser Broth. If the enrichments were positive, a three tube MPN system in UVM broth was used to enumerate L. monocytogenes.

The salads were incubated at 4°C or 12°C for a period equivalent to one and one-half their shelf-life. Samples were removed every 1-2 weeks for pH analysis and plating on the three media. Incubations of individual samples were terminated when two consecutive samples were negative for L. monocytogenes or if obvious spoilage occurred.

Thus, the variables for each salad were (1) pH; unaltered or lowered 0.5 units, (2) barrier chemical added, and (3) incubation temperature; 4°C or 12°C. Each combination of variables was run in duplicate.

Results

The results of the initial analysis of each salad are as follows:

<u>Salad type</u>	<u>pH</u>	<u>Titratable Acidity</u>	<u>Moisture</u>
chicken	4.65	0.50%	65.1%
pimento cheese	4.9-5.0	0.69%	55.4%
potato	4.35	0.28%	73.9%
pasta	4.1	0.37%	68.5%
seafood	4.4	0.20%	74.0%

Titratable acidity was calculated as % acetic acid, although other acids were present in small amounts in some of the salads.

The initial total aerobic plate counts (APC's) of all of the salads ranged between 10^3 and 10^4 CFU/g. These numbers decreased in most samples. However, in many samples kept at 12°C , APC's rose dramatically (up to as high as 10^9 CFU/g), indicating probable spoilage. This rapid increase in APC's were almost always accompanied by a drop in pH of around 0.5-1.5 units. None of the samples at 4°C exhibited similar spoilage.

Spoilage occurred in 12°C samples as follows:

- chicken- half of samples starting ~14 days
- pimento cheese- a few samples starting 20-70 days
- potato- most samples starting 7-21 days
- pasta- a few samples starting 20-27 days
- seafood- most samples starting 7-14 days.

Yeast and mold counts were low in all salads (between <10 and 100 CFU/g) and stayed relatively constant during incubation. None of the barrier chemicals seemed to have an effect on yeast/mold survival.

Except for samples showing obvious spoilage, the pH of the samples stayed relatively constant.

Overall, L. monocytogenes (L.m.) did not survive well in the salads, even in control samples with no barrier chemicals added. (See graphs). Populations of L.m. decreased steadily in all of the salads, but slower in seafood salad (lowest titratable acidity and highest moisture of all salads). Populations decreased slightly quicker in pimento cheese spread (highest titratable acidity and lowest moisture) and pasta salad (lowest pH).

In all salads, L.m. populations decreased slower in samples kept at normal pH than in salads with lowered pH. L.m. populations decreased slower in samples of chicken salad, pimento cheese spread, and seafood salad kept at 4°C , and in samples of potato salad kept at 12°C .

Populations of L.m. were very low or gone by 53 days (in chicken salad, 60 days (in pimento cheese spread), 42 days (in potato salad), and 13 days (in pasta salad).

The addition of most barrier chemicals did not appear to have significant antilisterial effects, except for acetic acid, which caused numbers of L.m. to decrease faster in chicken salad, potato salad, pasta salad, and seafood salad. Other barrier chemicals that may have had very slight antilisterial effects include:

- chicken-ALTA 2341; mixture of sodium citrate, ascorbate, and diacetate
- pasta-ALTA 2341
- seafood-acetic acid & lysozyme; mixture of sodium citrate, ascorbate, and diacetate

To determine if some L.m. were developing resistances to barrier chemicals (for instance, acetic acid or lysozyme), L.m. isolates were taken from seafood salad samples containing either acetic acid or lysozyme. Isolates were also taken from control samples of seafood salad. These isolates, as well as the five separate L.m. strains, were grown in trypticase soy broth (TSB), TSB + 0.5% acetic acid, and TSB + 50 ppm lysozyme. The tubes were incubated at 30°C, and the optical density read over time to indicate growth of L.m.

The isolates all showed growth patterns similar to at least one of the L.m. strains. The isolate from the acetic acid sample was not more resistant to acetic acid. The isolate from the lysozyme sample was not more resistant to lysozyme.

Additional Experiments

Another researcher in our laboratory tested the antilisterial effects of six barrier chemicals against L. monocytogenes Scott A in seafood salad only. Each chemical was tested alone and in combination with 100 ppm lysozyme (Societa Prodotti Antibiotica). The chemicals tested were:

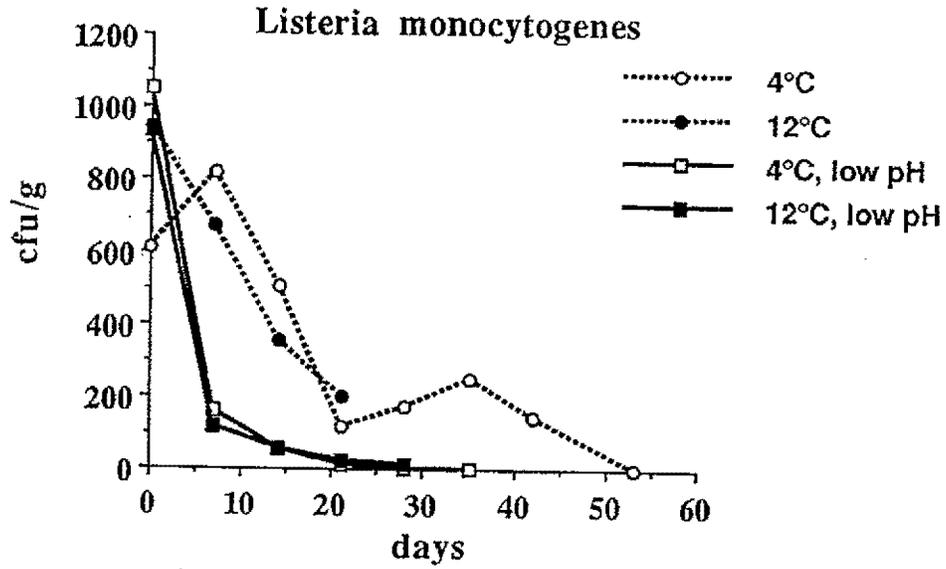
- (1) 2 mM EDTA (Sigma, tetrasodium salt)
- (2) 0.27% sodium diacetate (International Sourcing, Inc.)
- (3) 0.5 mg/g conalbumin peptide (prepared from conalbumin in our lab)
- (4) 0.17% *Mayonet* (Feindost Ingredient Co.)

- (5) 1000 ppm 1-monolauroyl-rac-glycerol (Sigma, C12:0)
- (6) 0.03% tripolyphosphate (Sigma, pentasodium salt)

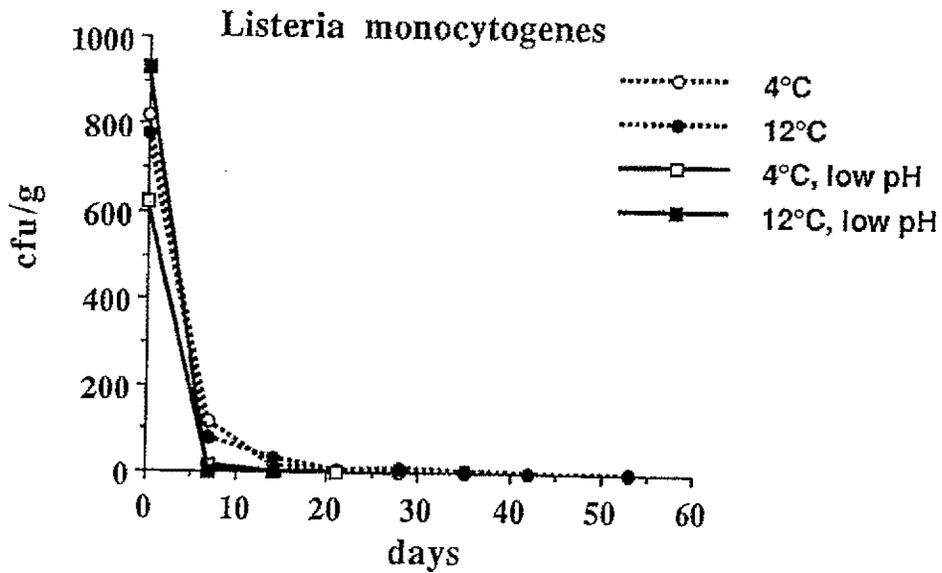
Experimental design was the same as in the previous experiment, except that samples were prepared in triplicate, and the samples were only plated on MOX for enumeration of L. monocytogenes.

Conalbumin peptide showed possible slight antilisterial effects, both alone and in combination with lysozyme. Monolauroyl showed definite antilisterial effects, both alone and in combination with lysozyme.

Chicken salad; Control

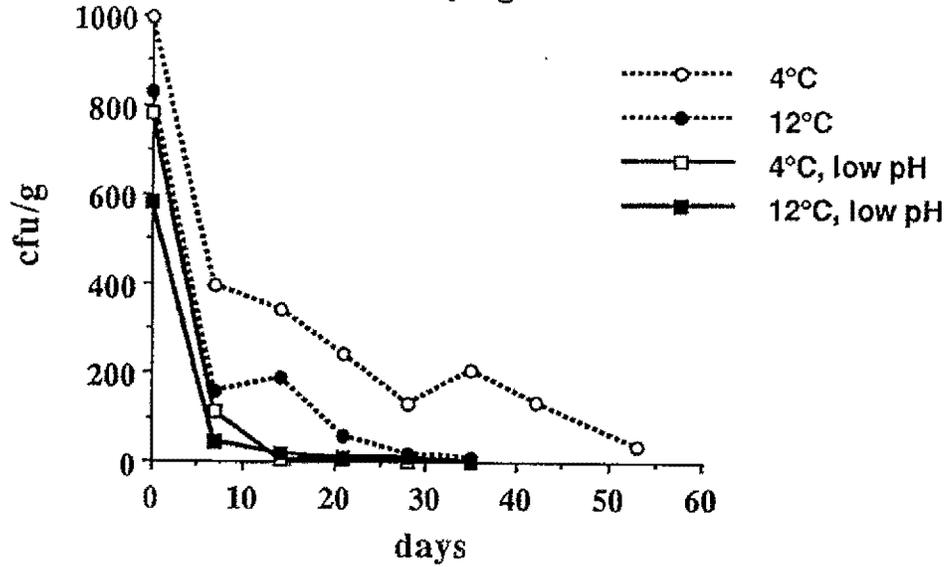


Chicken salad; Acetic acid



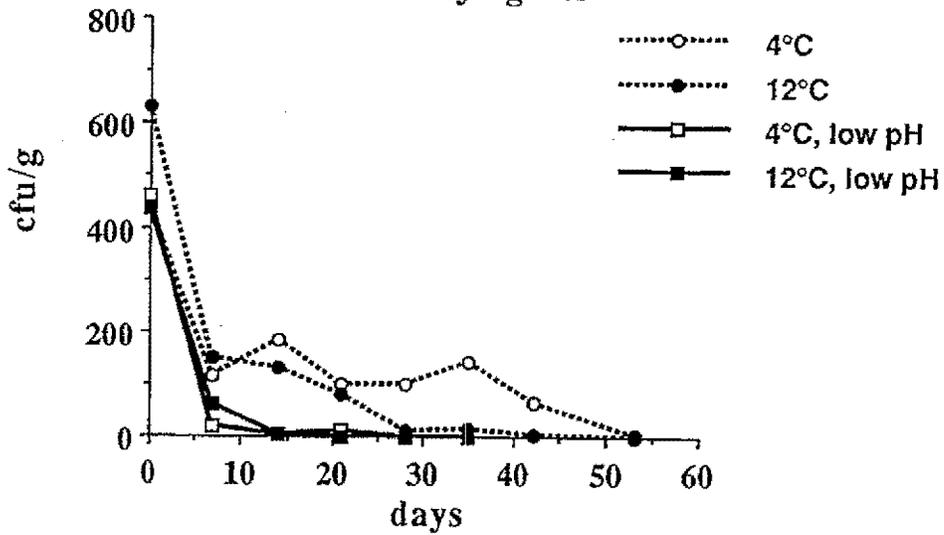
Chicken salad: Sodium lactate

Listeria monocytogenes



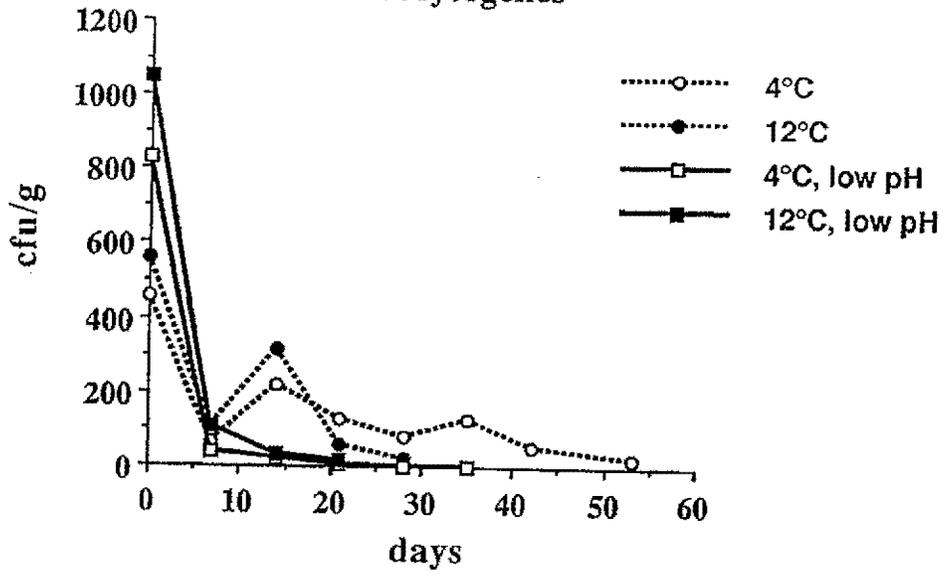
Chicken salad; Mixture of Sodium citrate, ascorbate, and diacetate

Listeria monocytogenes



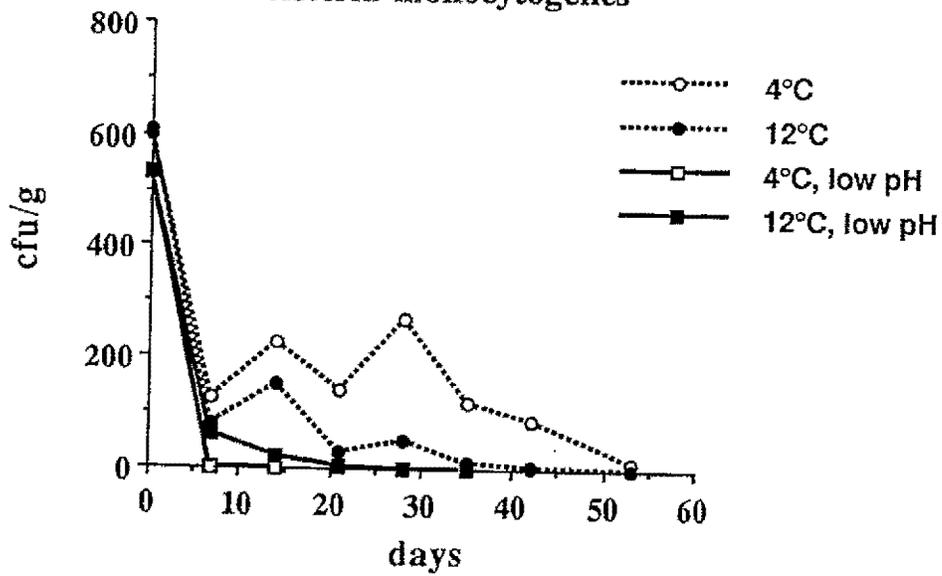
Chicken salad; Sodium benzoate

Listeria monocytogenes



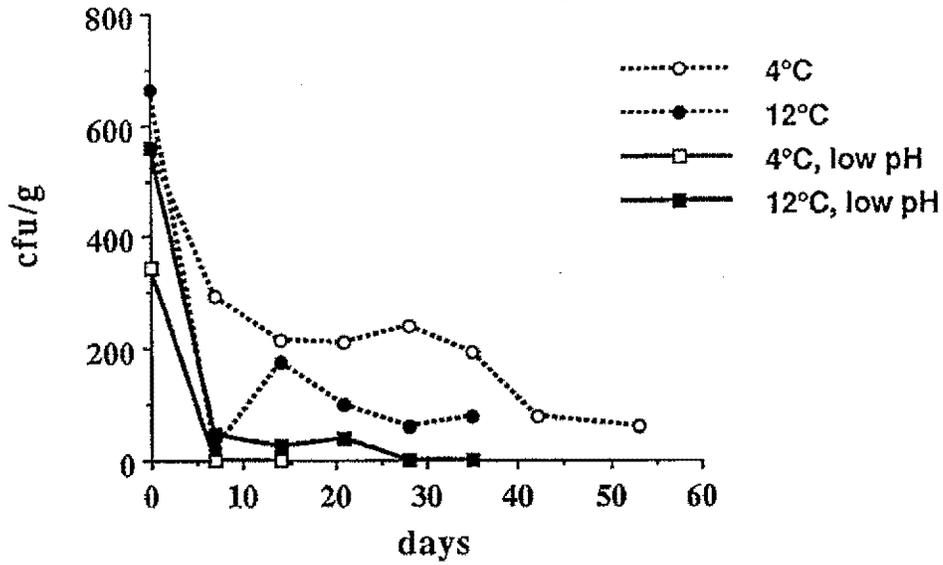
Chicken salad; Potassium sorbate

Listeria monocytogenes



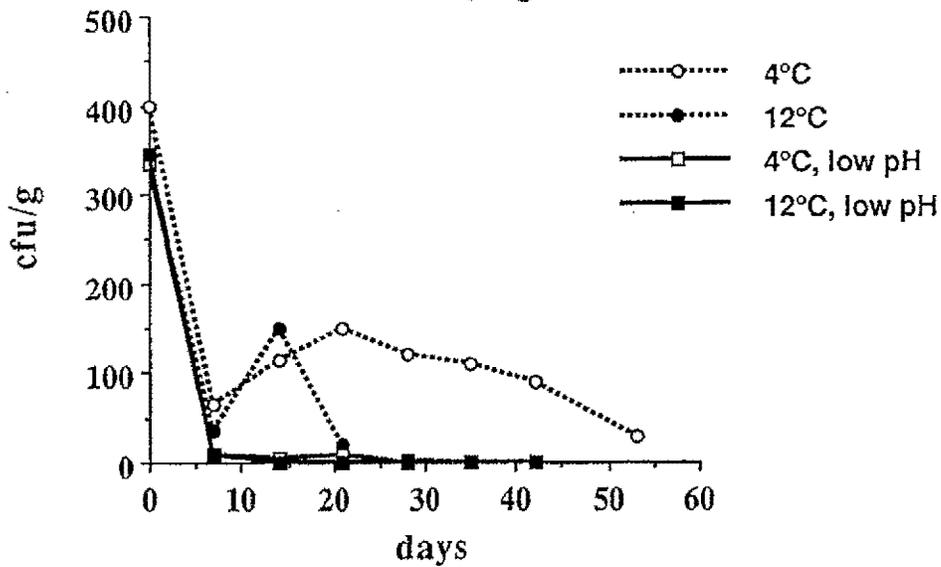
Chicken salad; EDTA

Listeria monocytogenes



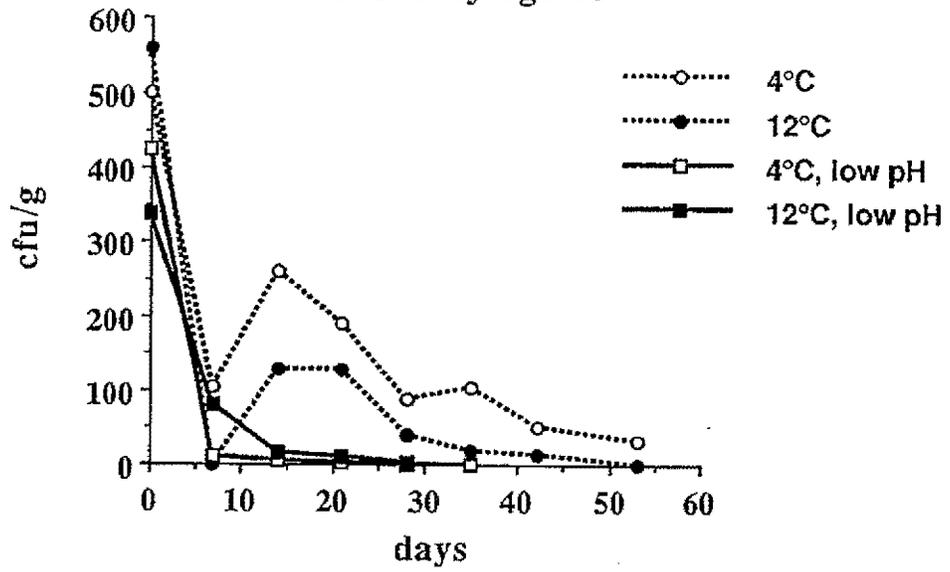
Chicken salad; ALTA 2341

Listeria monocytogenes



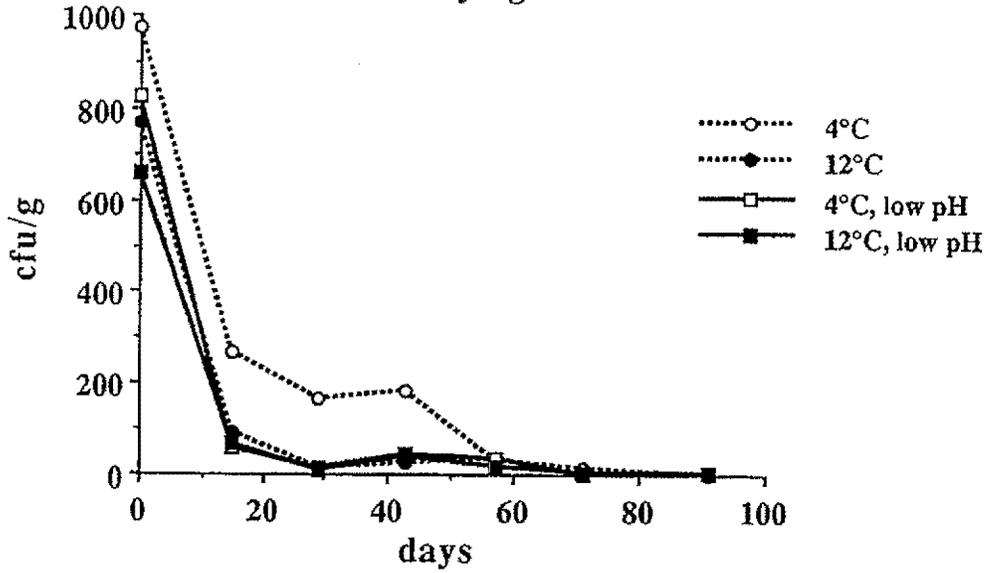
Chicken salad: Lysozyme

Listeria monocytogenes



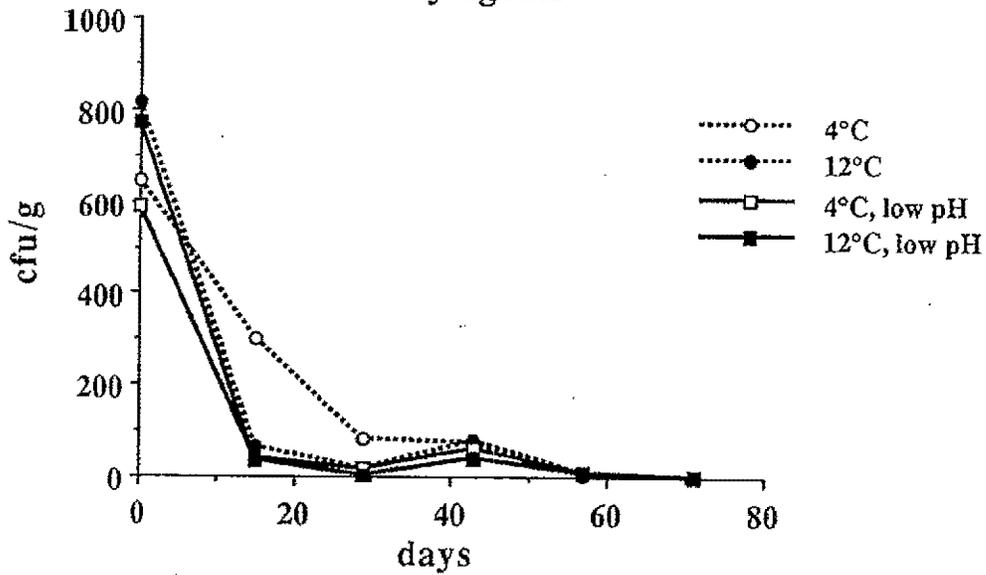
Pimento cheese spread: Control

Listeria monocytogenes



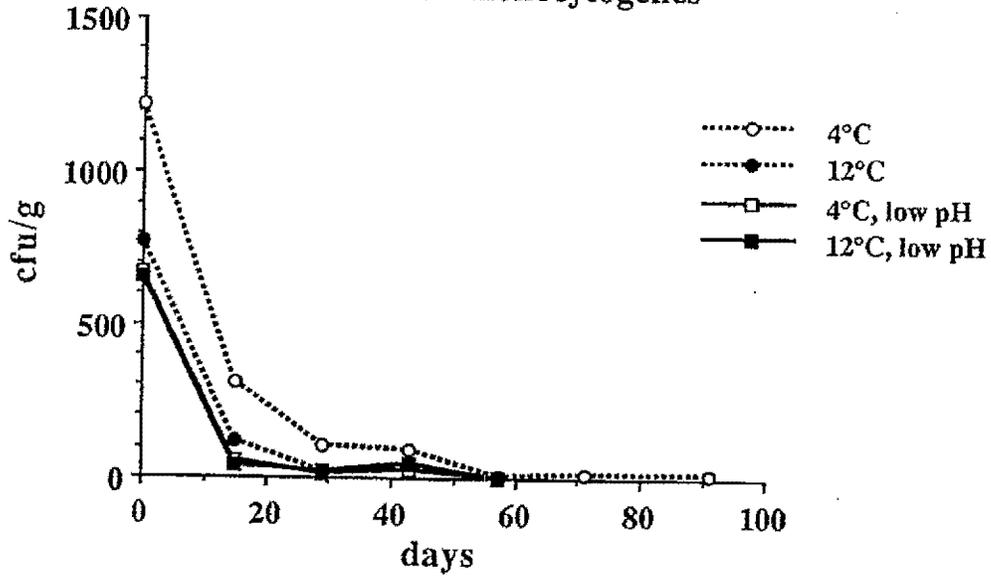
Pimento cheese spread: Acetic acid

Listeria monocytogenes



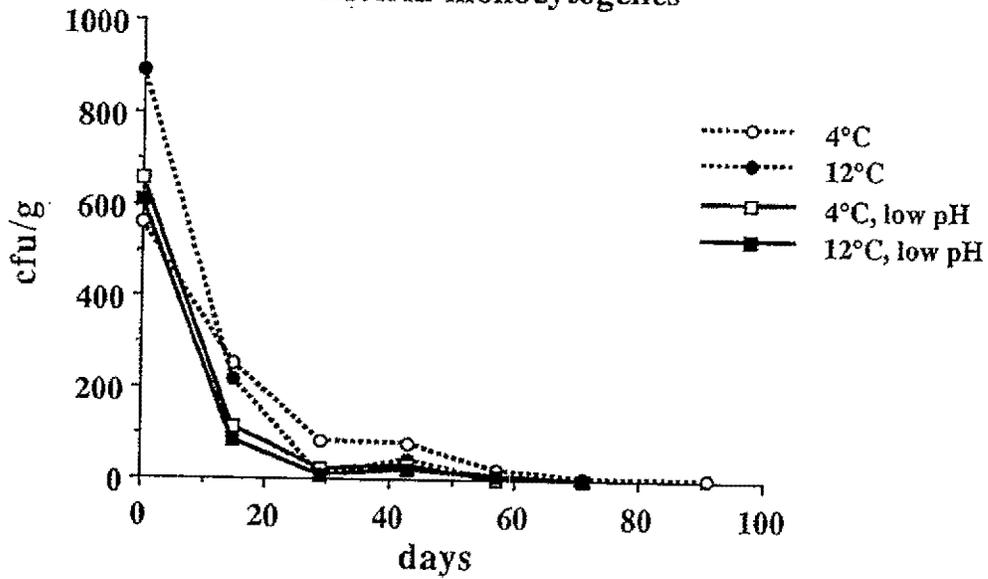
Pimento cheese spread: Sodium benzoate

Listeria monocytogenes



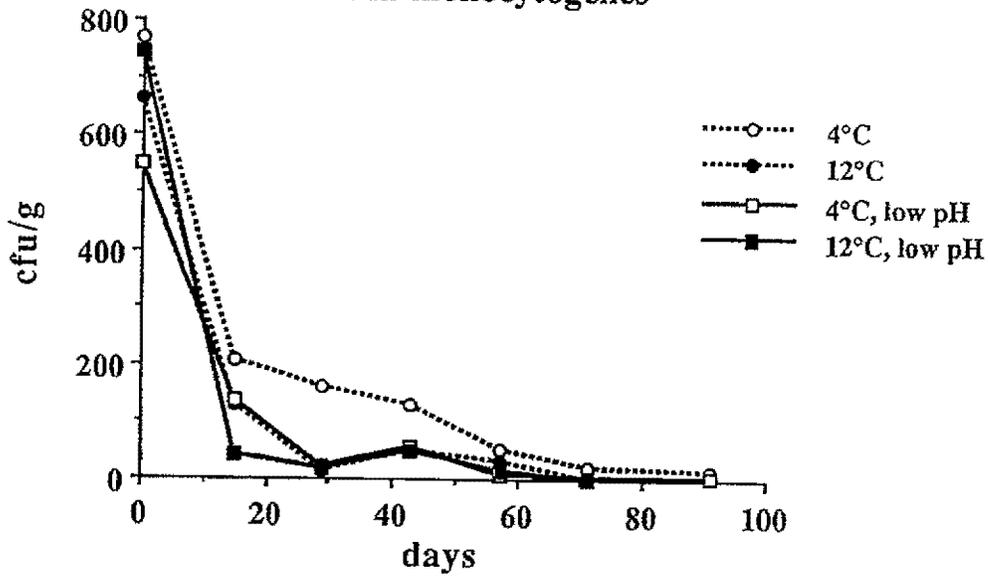
Pimento cheese spread: Potassium sorbate

Listeria monocytogenes



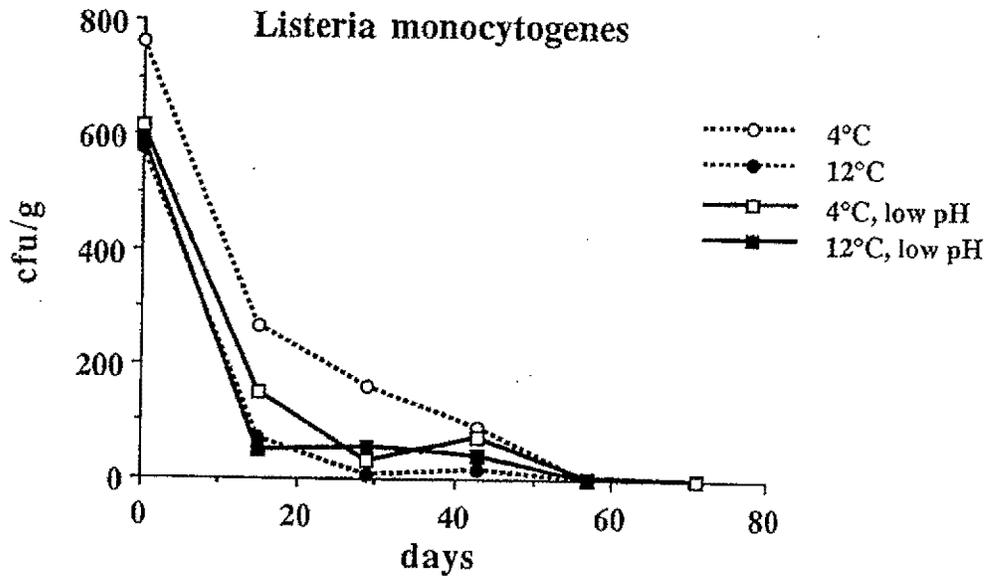
Pimento cheese spread: Sodium lactate

Listeria monocytogenes



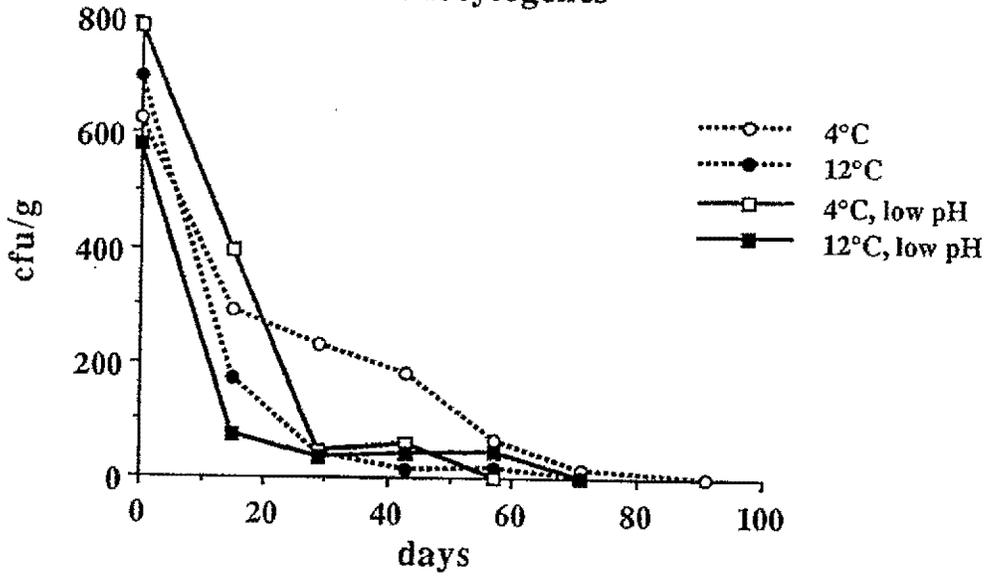
Pimento cheese spread: Mixture of sodium citrate, ascorbate, and diacetate

Listeria monocytogenes



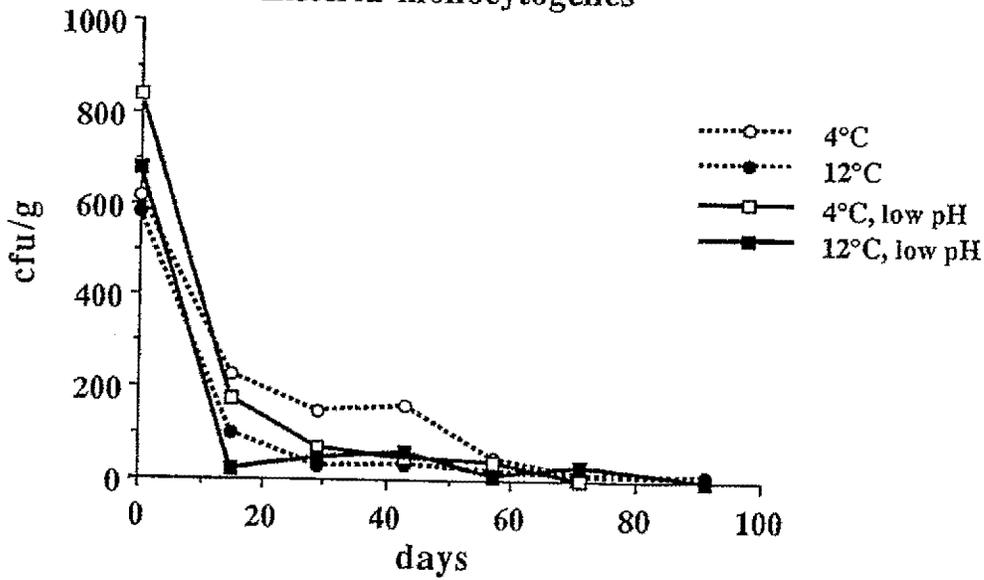
Pimento cheese spread: EDTA

Listeria monocytogenes



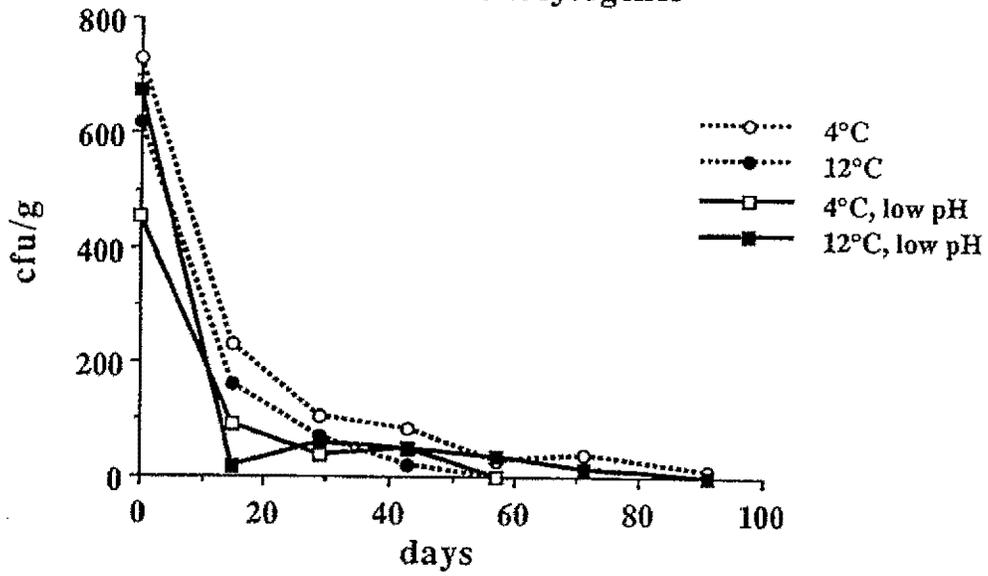
Pimento cheese spread: ALTA 2341

Listeria monocytogenes



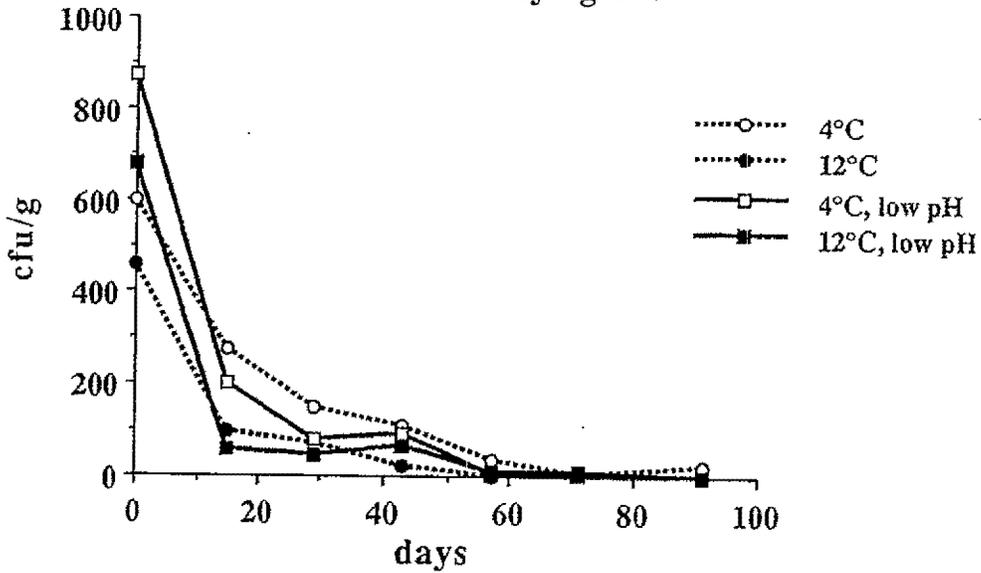
Pimento cheese spread: Lysozyme

Listeria monocytogenes



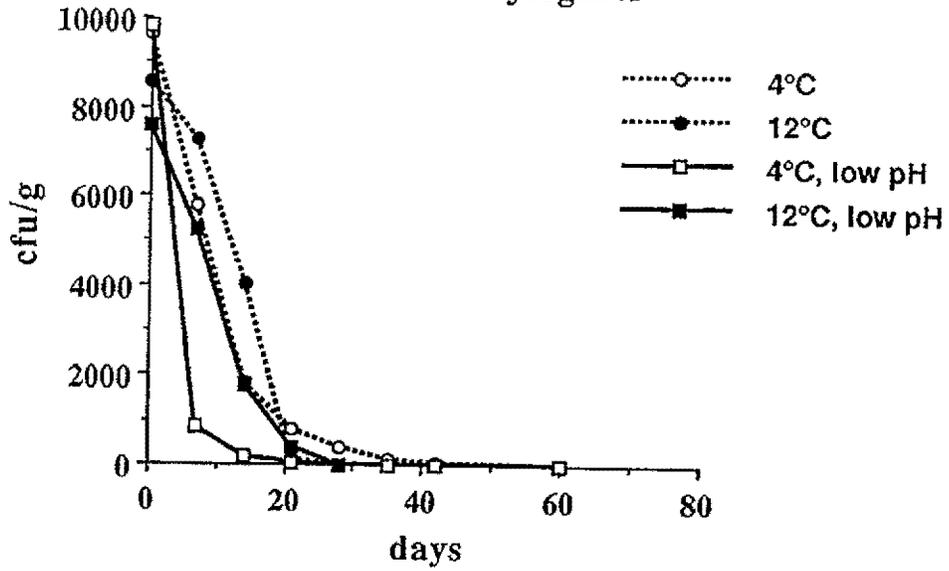
Pimento cheese spread: ALTA 2001

Listeria monocytogenes



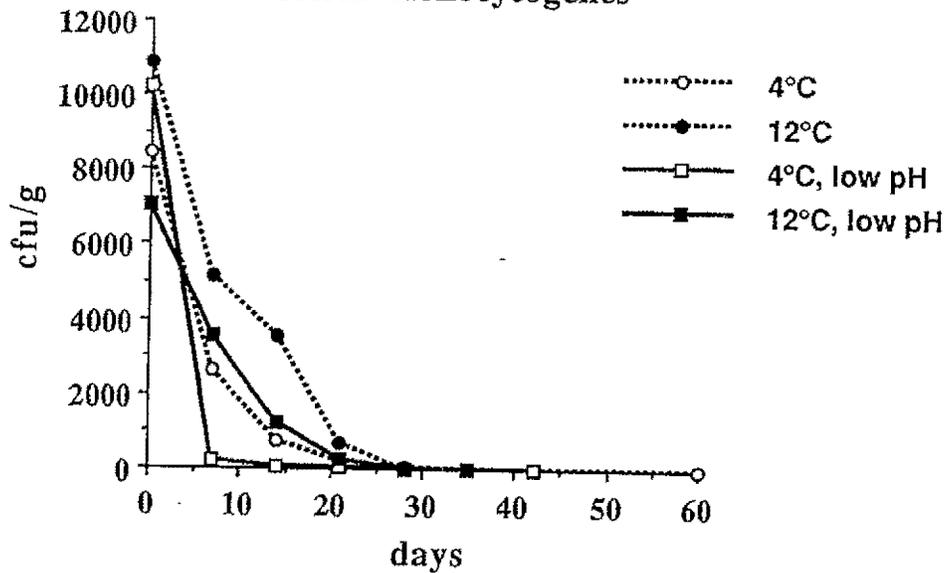
Potato salad: Control

Listeria monocytogenes



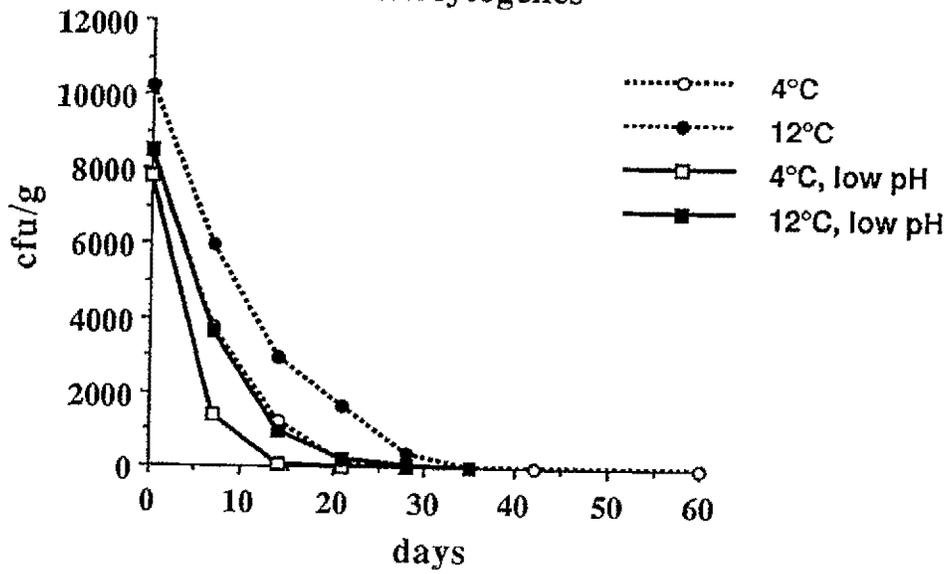
Potato salad: Acetic acid

Listeria monocytogenes



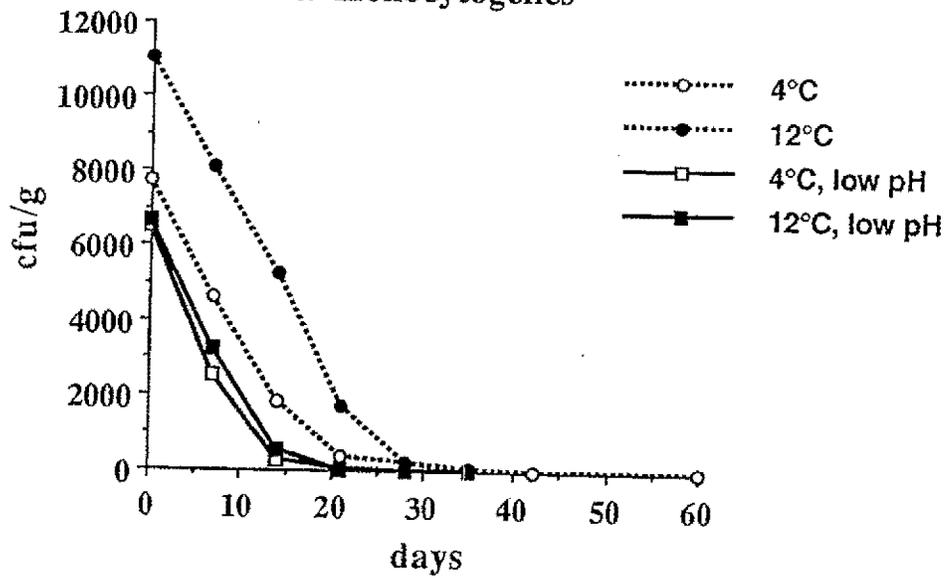
Potato salad: Sodium benzoate

Listeria monocytogenes



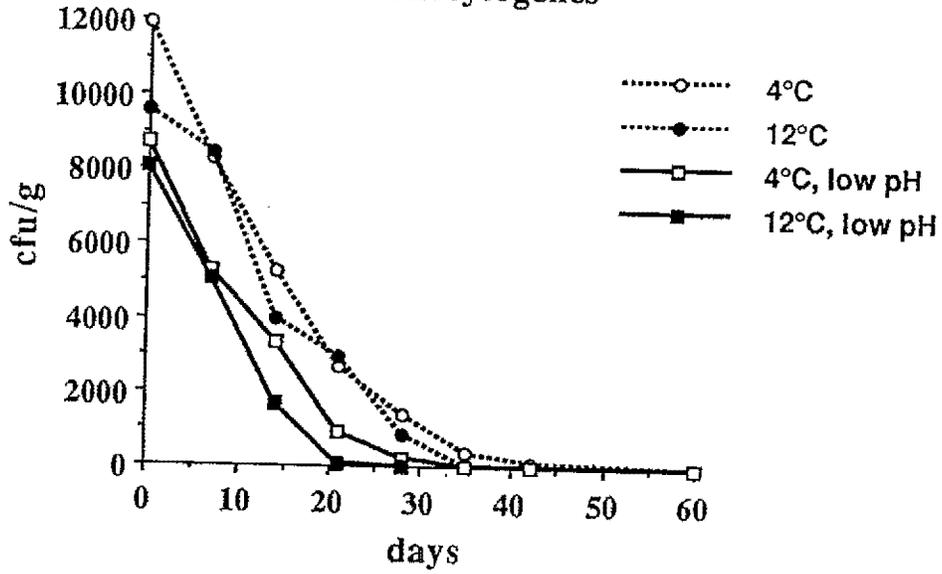
Potato salad: Potassium sorbate

Listeria monocytogenes



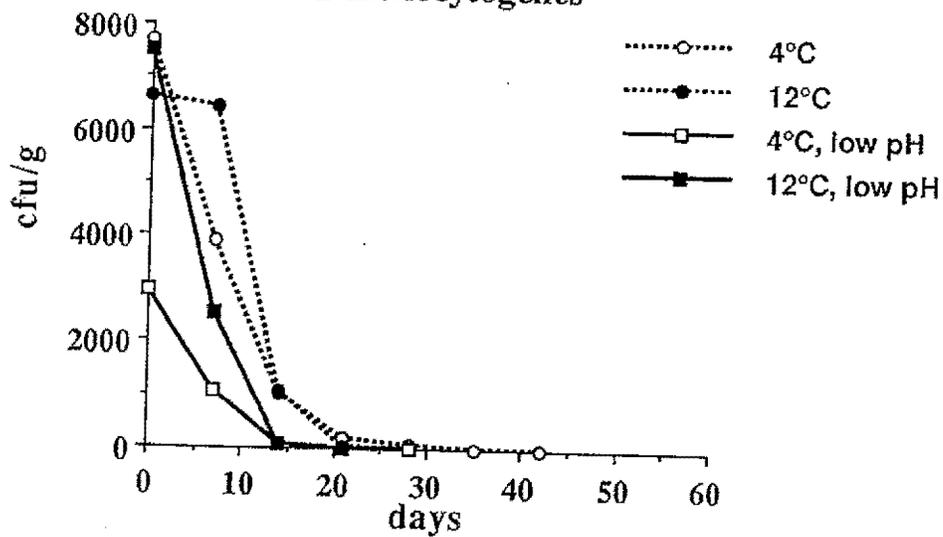
Potato salad: Sodium lactate

Listeria monocytogenes



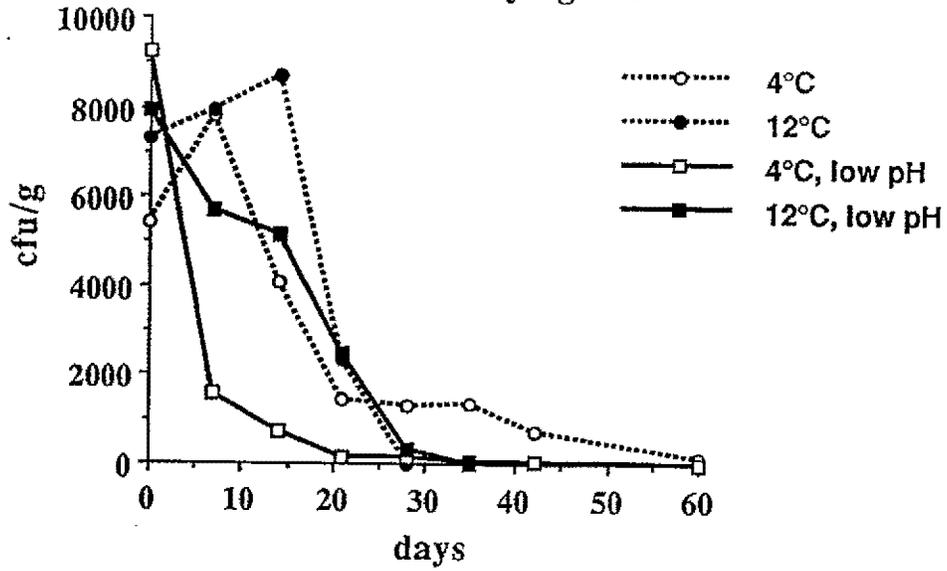
Potato salad: Mixture of sodium citrate, diacetate, and ascorbate

Listeria monocytogenes



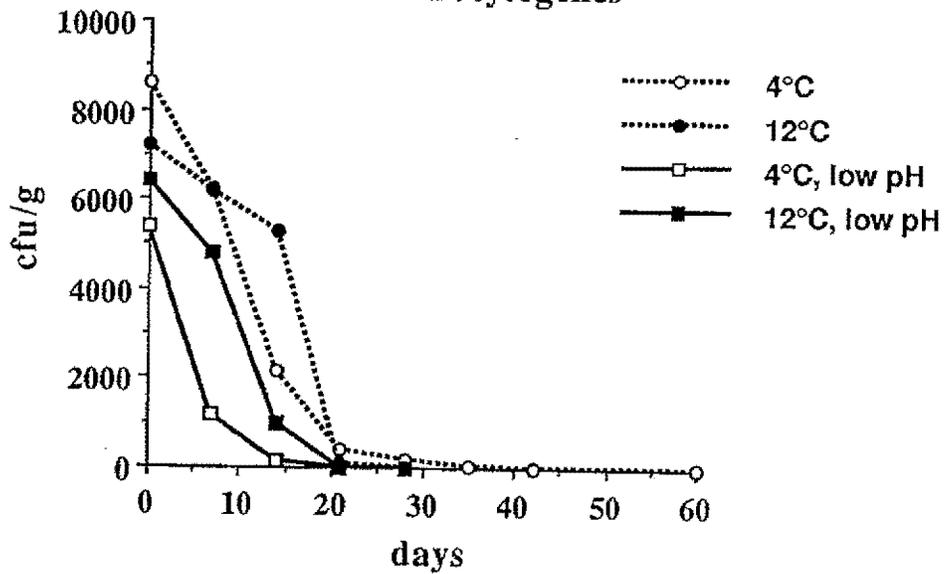
Potato salad: EDTA

Listeria monocytogenes



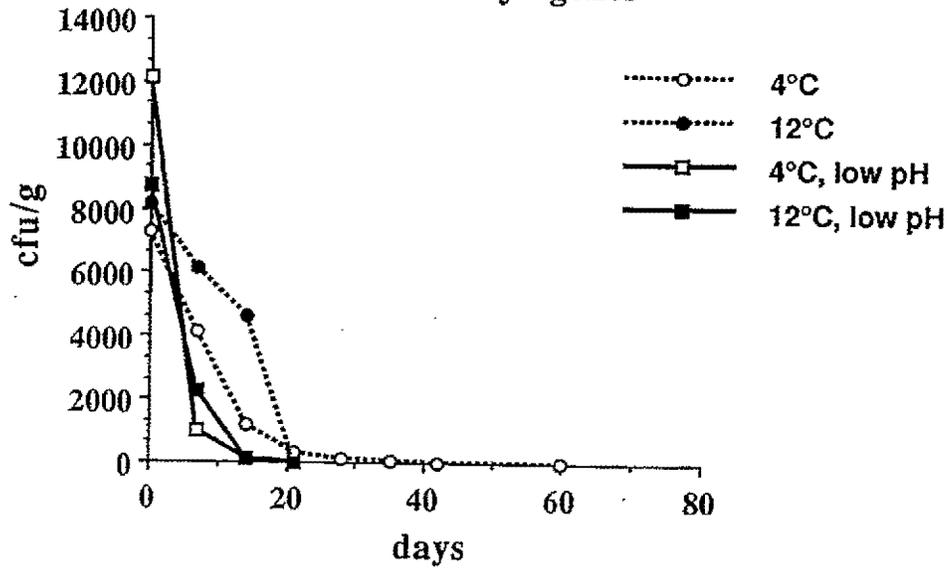
Potato salad: ALTA 2341

Listeria monocytogenes



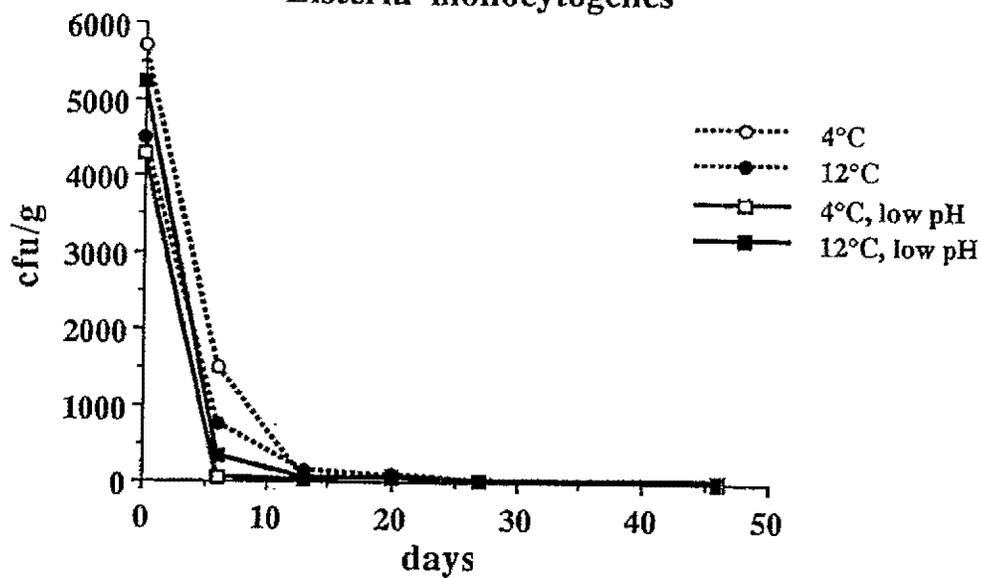
Potato salad: Lysozyme

Listeria monocytogenes



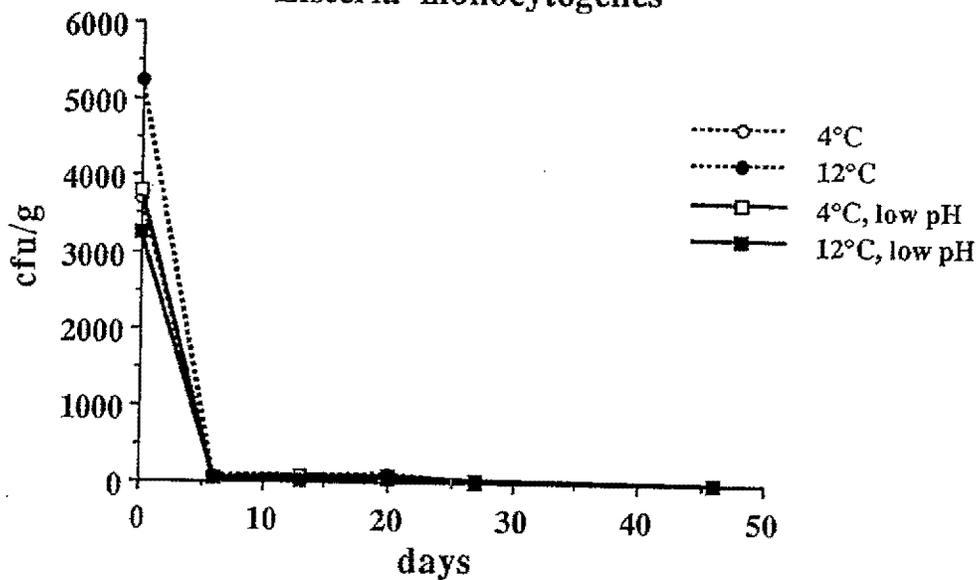
Pasta salad: Control

Listeria monocytogenes



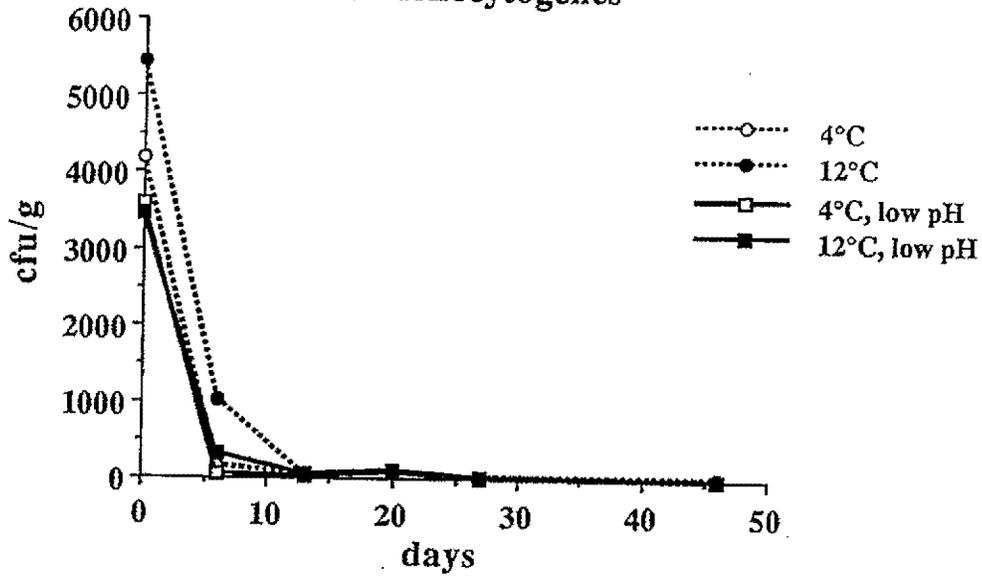
Pasta salad: Acetic acid

Listeria monocytogenes



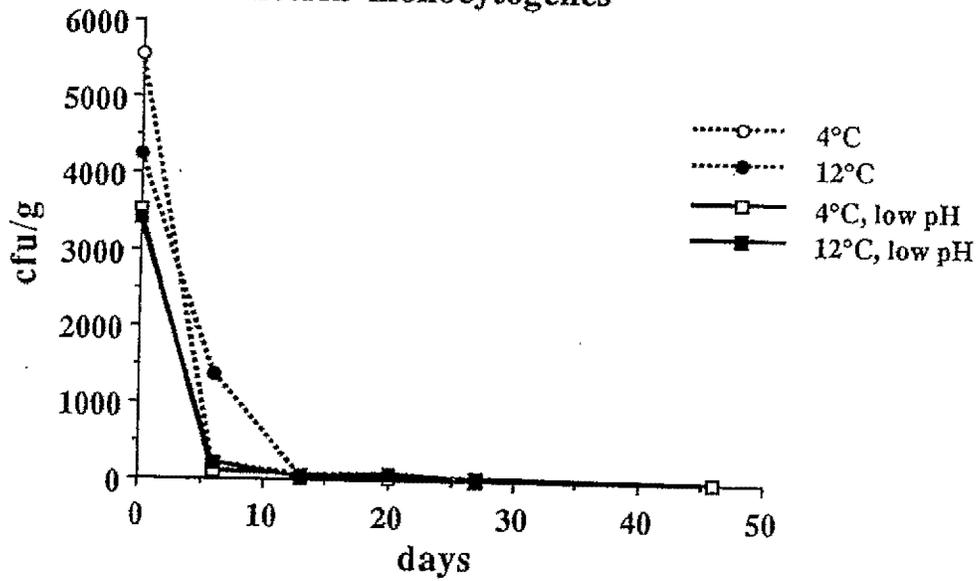
Pasta salad: Sodium benzoate

Listeria monocytogenes



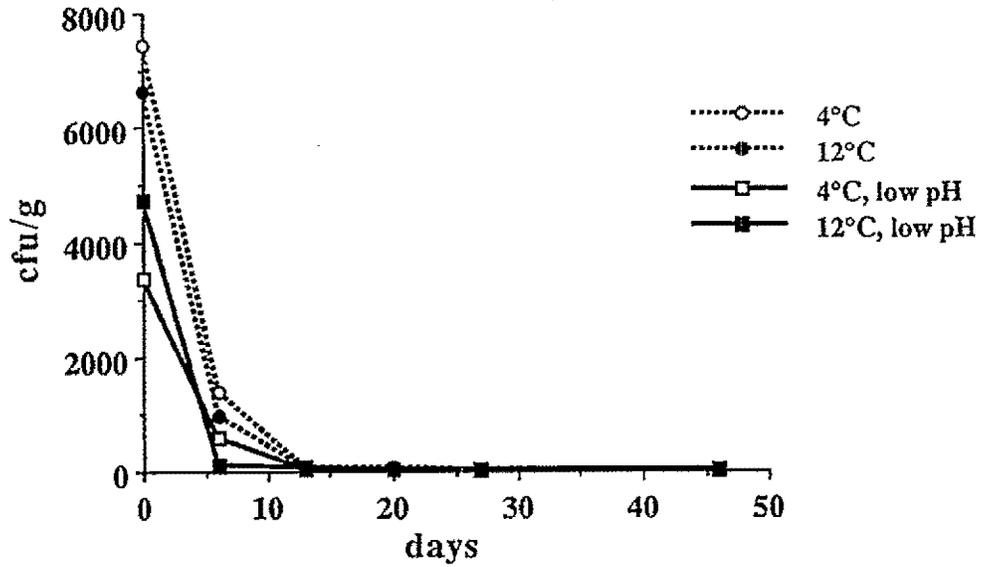
Pasta salad: Potassium sorbate

Listeria monocytogenes



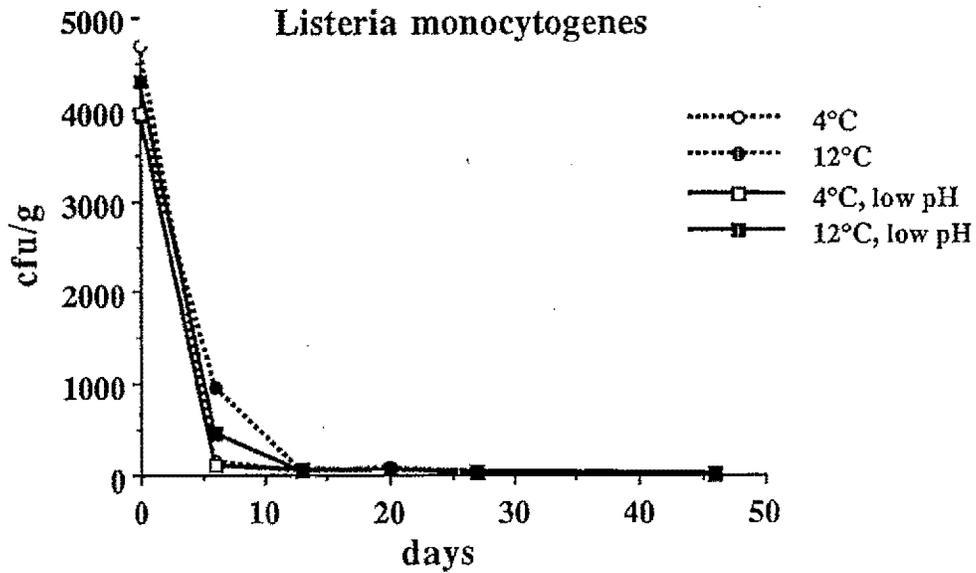
Pasta salad: Sodium lactate

Listeria monocytogenes



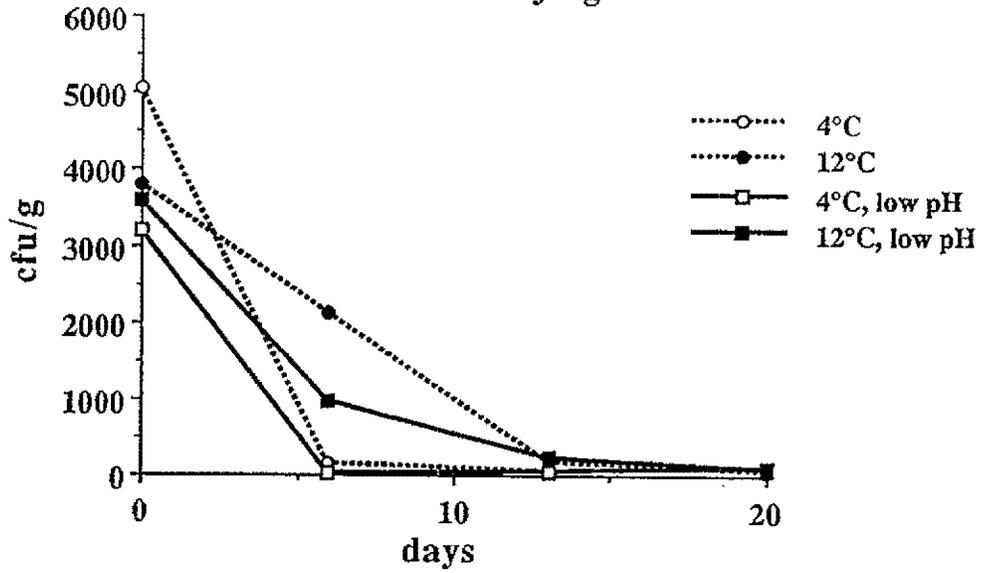
Pasta salad: Mixture of sodium citrate, ascorbate, and diacetate.

Listeria monocytogenes



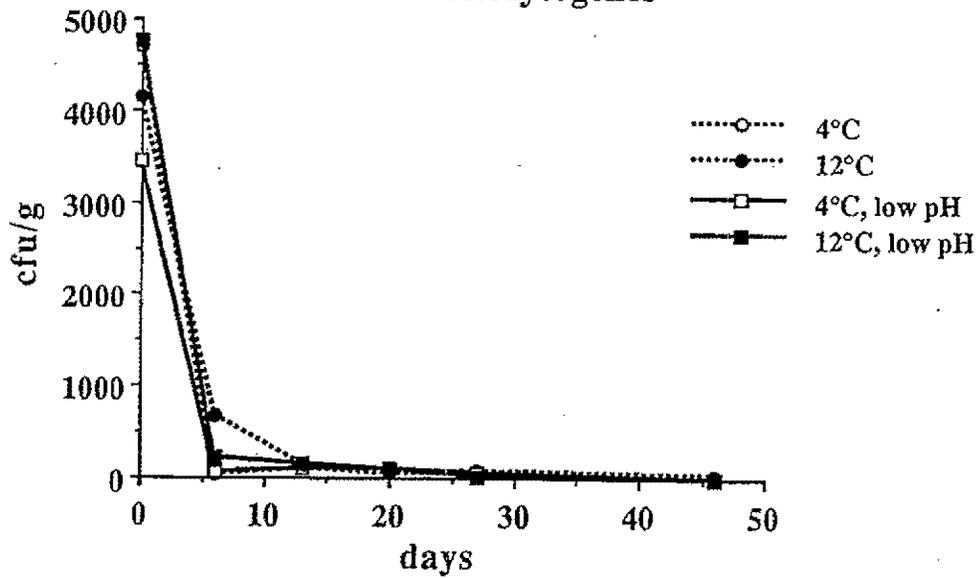
Pasta salad: EDTA

Listeria monocytogenes



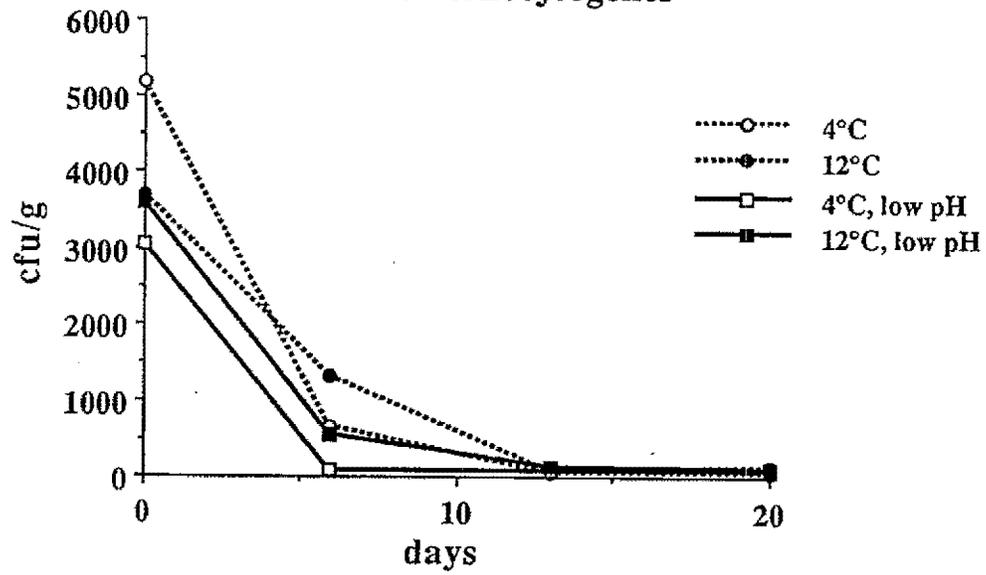
Pasta salad: ALTA 2341

Listeria monocytogenes



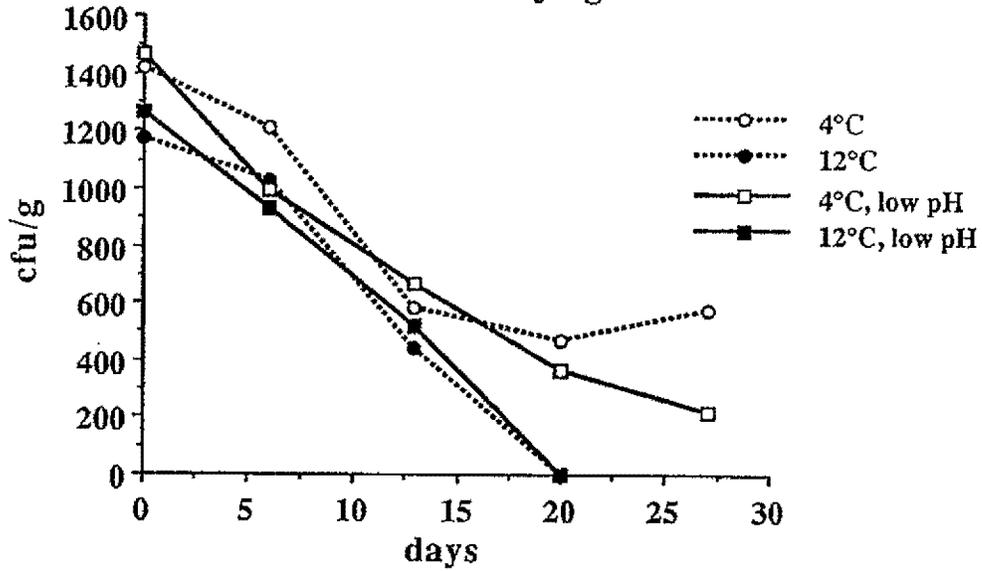
Pasta salad: Lysozyme

Listeria monocytogenes



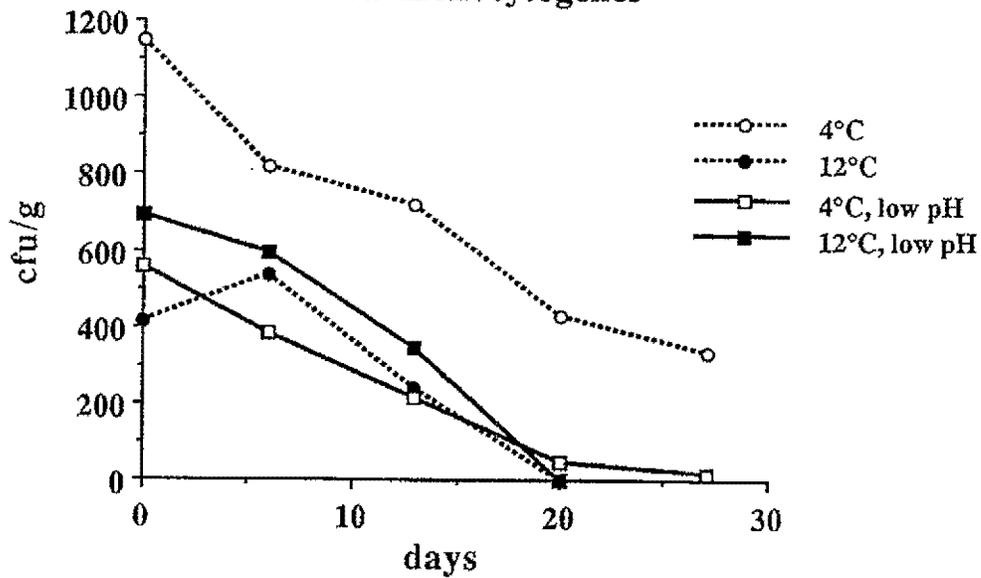
Seafood salad: Control

Listeria monocytogenes

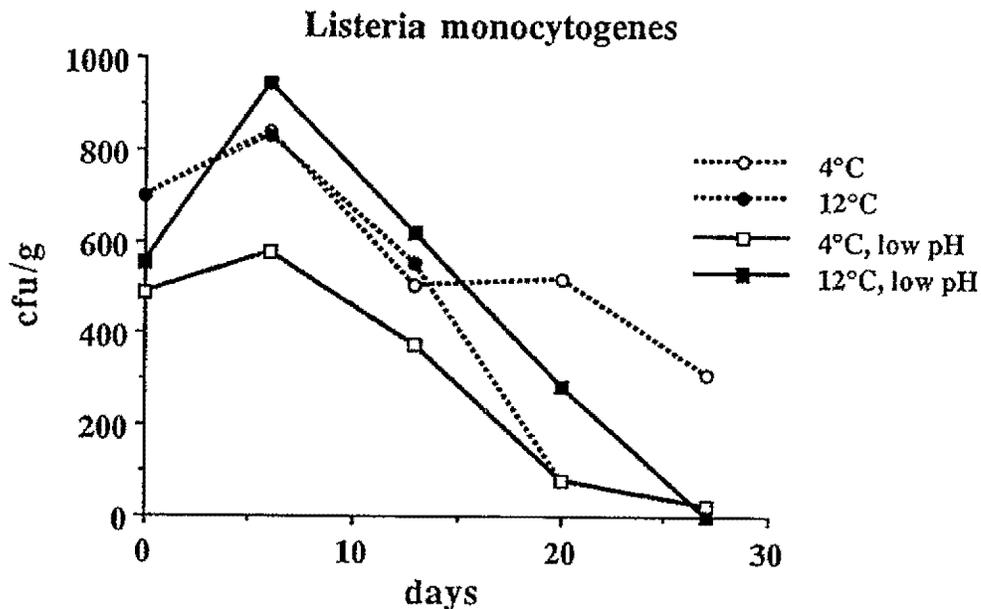


Seafood salad: Acetic acid

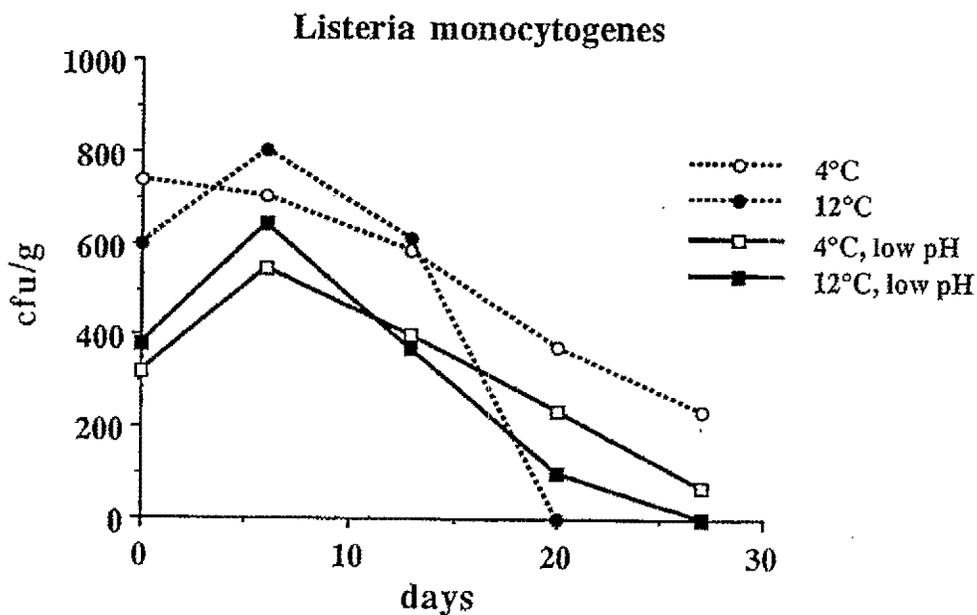
Listeria monocytogenes



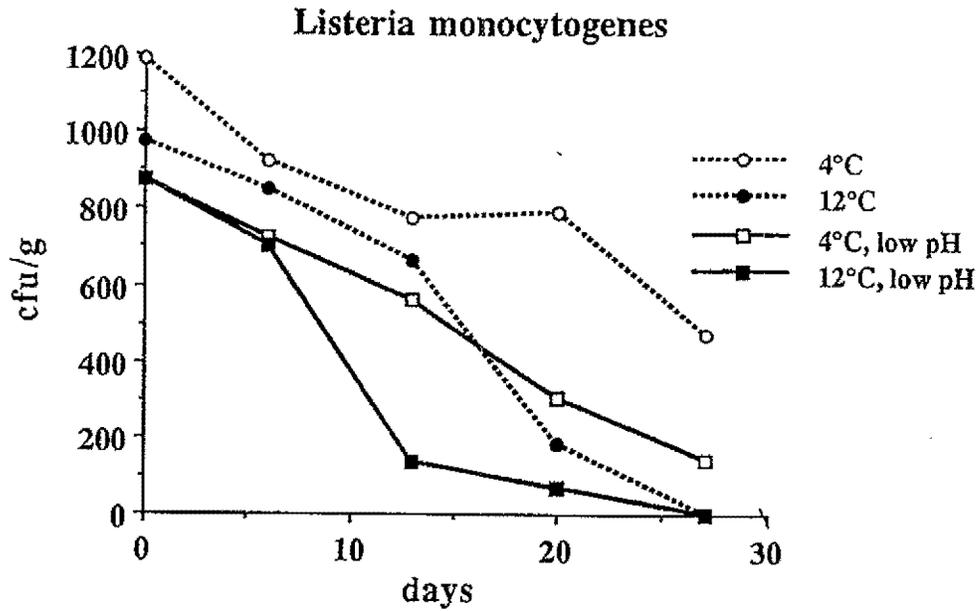
Seafood salad: Sodium benzoate



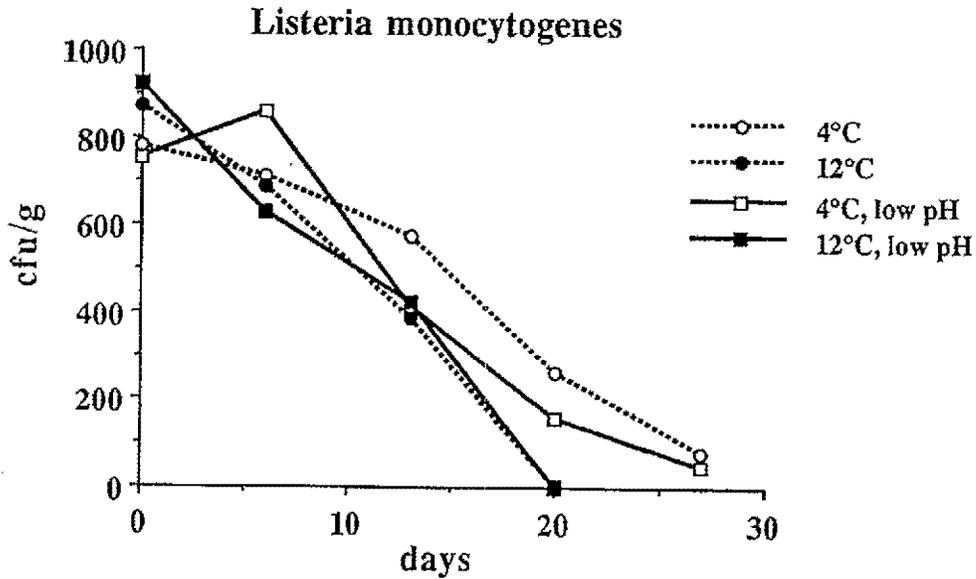
Seafood salad: Potassium sorbate



Seafood salad: Sodium lactate

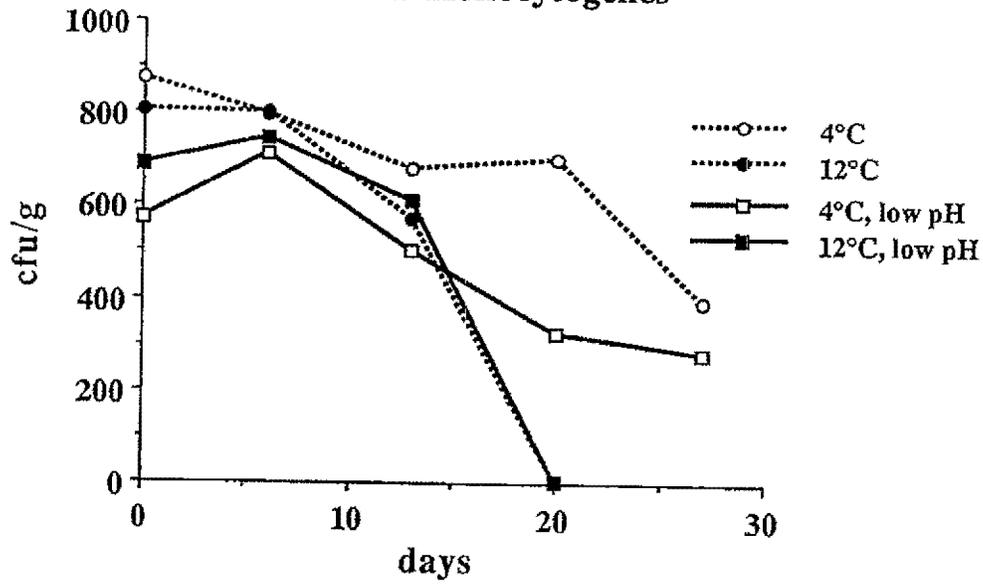


Seafood salad: Mixture of Sodium citrate, diacetate, and ascorbate.



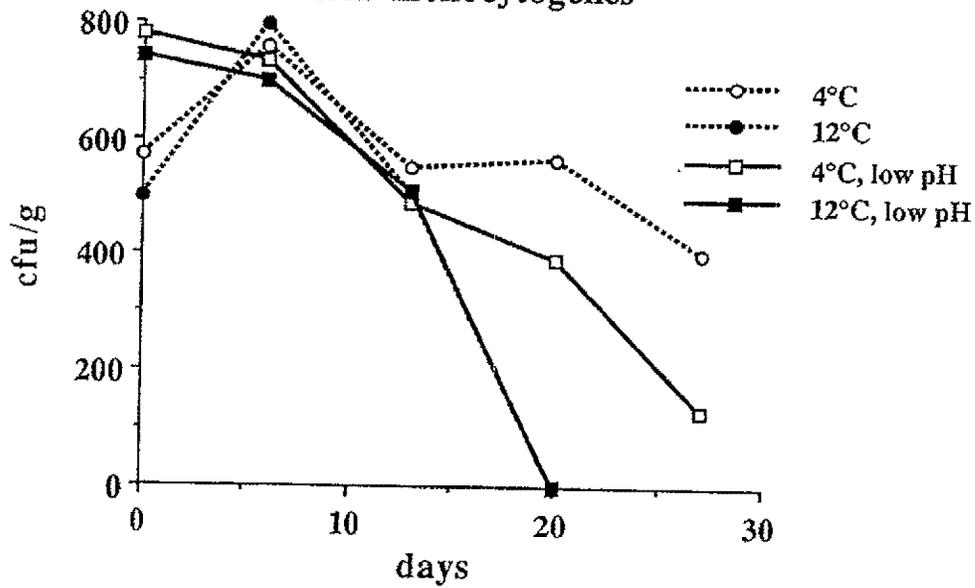
Seafood salad: EDTA

Listeria monocytogenes



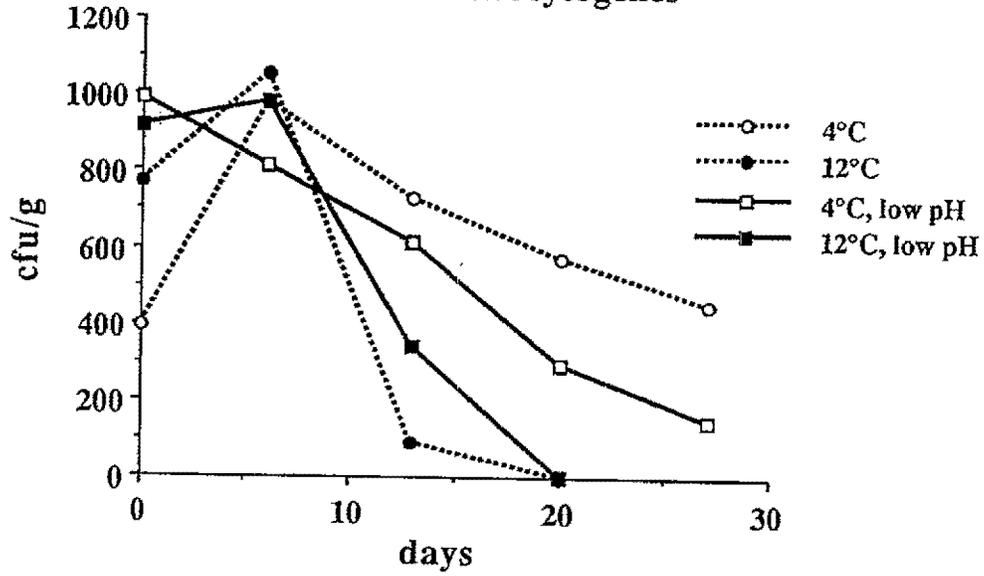
Seafood salad: ALTA 2341

Listeria monocytogenes



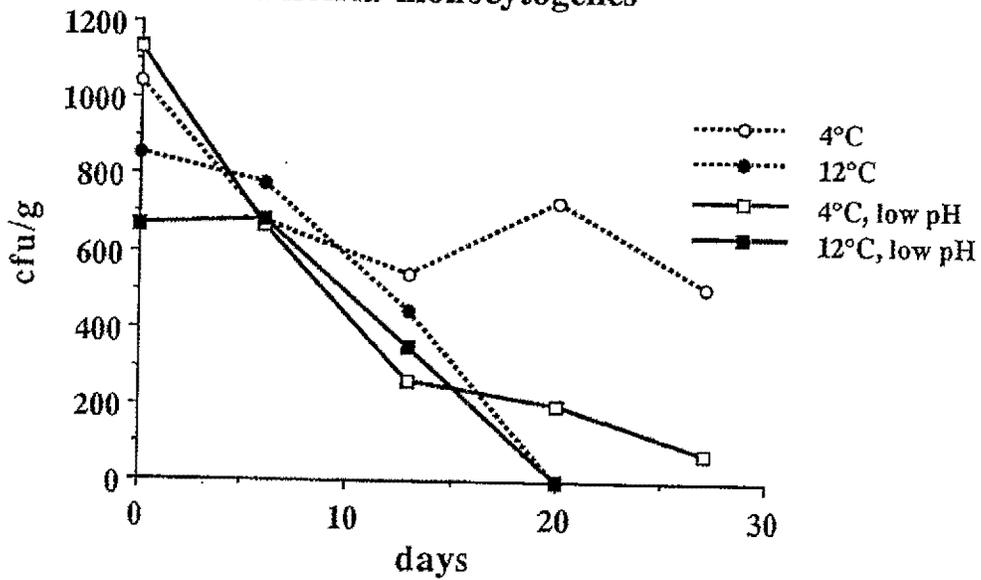
Seafood salad: Lysozyme

Listeria monocytogenes

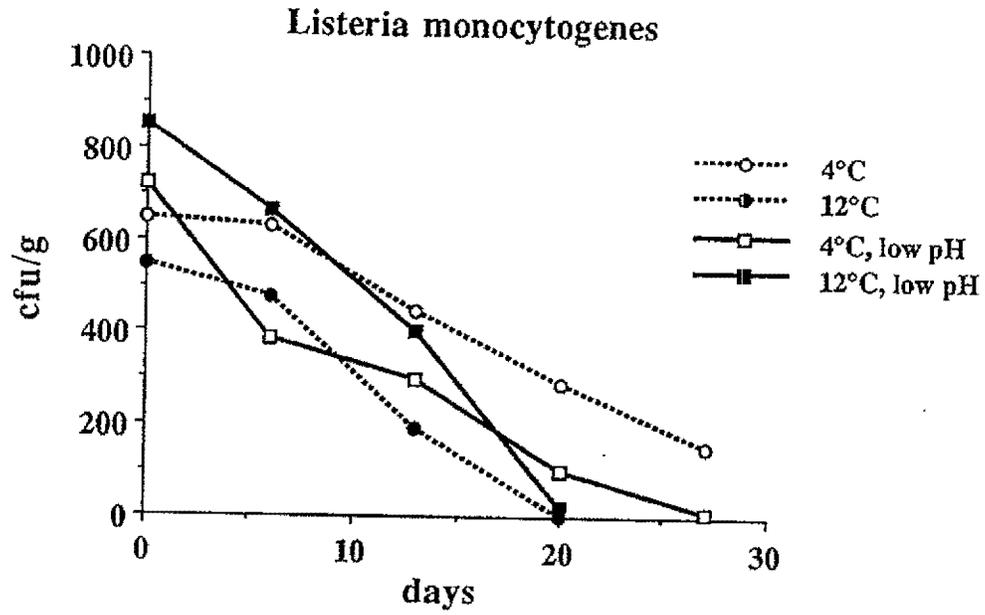


Seafood salad: EDTA + Lysozyme

Listeria monocytogenes



Seafood salad: Acetic acid + Lysozyme



Seafood salad: Sodium lactate + Lysozyme

