To the Graduate Council:

I am submitting herewith a thesis written by Ralph C. Terry entitled "A Microbiological Profile of Commercially Prepared Salads." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Technology.

W. W. Overcast, Major Professor

We have read this thesis
and recommend its acceptance:

Accepted for the council:

Vice Chancellor
Graduate Studies and Research

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A MICROBIOLOGICAL PROFILE OF COMMERCIALLY PREPARED SALADS

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee

Ralph C. Terry

August 1975

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ABSTRACT

Samples of commercially prepared chicken salad, ham salad, potato salad, cole slaw, pimento cheese spread and several other types of salads from producers were analyzed microbiologically upon receipt and five days after the expiration date for aerobic plate count, psychrophilic plate count, coliform count and yeast and mold count. Plating was carried out by homogenizing ten grams of each salad with ten milliliters of 2 percent sodium citrate to a pipettable consistency in a Virtis homogenizer and pouring plates. Aerobic plate count ranges of less than 100 to greater than 30 million organisms per gram of fresh salad and from less than 100 to greater than 1.5 billion organisms per gram of salad after the expiration date were found.

The psychrophilic plate count range was from less than 100 to 31 million organisms per gram of fresh salad and from less than 100 to greater than 970 million organisms per gram of salad after the expiration date. Coliform plate count ranges were from less than one to 1,990 coliforms per gram of salad after the expiration date. Yeast and mold plate count ranges were from less than one to greater than 300,000 organisms per gram of fresh salad and from less than one to greater than 300,000 organisms per gram of salad after the expiration date.

Bacteria from aerobic plate counts, psychrophilic plate counts and coliform counts were isolated according to differences in colony type and location in the agar. The bacteria were characterized for

identification. Identified bacteria included eight species of *Bacillus*, six species of *Lactobacillus*, three species of *Leuconostoc*, five species of *Streptococcus*, three species of *Micrococcus*, one species of *Escherichia*, one species of *Enterobacter* and one species of *Citrobacter*. Members of the genera of *Pseudomonas*, *Xanthomonas* and *Actinomyces* were also found.

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INTRODUCTION

As the American society grows in number and complexity, an increasing demand for faster and simpler methods are required for feeding the population. The housewife of today wishes to spend less time in the kitchen and more time for other purposes. Therefore, convenient food products have been made available on the grocer's shelf.

With the introduction of each new food to the market, there comes a new set of problems in preparation, transport, and storage. To assure a wholesome product for today's families, the making, storing, transporting and preparing of each product must be evaluated with extreme care.

At present, the market for new products expands and declines with great rapidity. Such rapid growth and decline applies much pressure toward getting a new product into the marketplace as soon as possible. For this reason some foods have been placed on the market too quickly and must be removed due to unforeseen problems. Such problems have become the basis of research and food evaluation by universities and sections of government agencies as the Food and Drug Administration (F. D. A.) and the United States Department of Agriculture (U. S. D. A.)

During the 1974 meeting of the Food Safety Task Force for the Southern Region Agricultural Experiment Stations and the U. S. D. A., several problems unforeseen by food producers were discussed. A

decision was made that research was required on processed products which require handling during and after processing (6). The commercially prepared salads are products which require much handling.

The commercial salads industry consists mostly of small producers. Production of the salads or spreads is for a small localized market. A good example is the delicatessen, which is probably the smallest commercial producer of salads or spreads. Each delicatessen has its own assortment of salad types. At the present time, little information is available about the physical, chemical, and microbiological characteristics of these salads. Almost every producer has a different formulation for their salads. With so many different formulas, there could be different physical, chemical, and microbiological characteristics. Characteristics of the salads could be further diversified by the experience of the manufacturer, by consumer preferences, and by employee experience in making salads or spreads. The microbiological characters of salads are especially sensitive to the experience of the manufacturer and consumer preferences because the organisms present may depend upon who makes the salads and when, where and how the salad is made.

Microbiological data on salads are desirable due to the diversity of types and formulations available to the consumer. The object of this research was to determine the resident microflora of some available salads. Research of this nature reveals the basic microbiological makeup for studies to extend shelf-life of the salads and to provide a better product for consumers and a more profitable product for the manufacturer.

CHAPTER I

LITERATURE REVIEW

The market for commercially prepared salads is increasing annually, especially for the more common ones such as chicken salad, ham salad, potato salad, egg salad, tuna salad, cole slaw and pimento cheese spread. One manufacturer in Medina, Ohio (3) increased his sales tenfold between 1968 and 1969 and was expanding to a total office and manufacturing area of 10,000 square feet. More than 500 stores and institutions within a 150-mile radius were buying more than 20,000 pounds of potato salad and more than 10,000 pounds of cole slaw weekly.

Ingredients determine the three categories of commercial salads as salads, cole slaws and pimento cheese spreads. Proportions of ingredients are not readily available to researchers, but contents on packages and cookbook recipes give the contents most common to all salads.

Salads, per se, contain the nomenclature ingredient, plus mayonnaise or salad dressing, eggs, celery, and sweet or sour pickles. The salad may be dressed up by addition of ingredients desired by the manufacturer. Many variations are available in cookbooks (4, 5, 25, 26, 29).

Cole slaws are prepared from cabbage, salad dressing, mayonnaise, or vinegar, plus condiments and some include other vegetables (4).

Pimento cheese spreads are restricted in their content to cheese, (the cheddar variety is used commercially) pimentos and salad dressing or mayonnaise (32).

Available literature on microbiological analysis of salad is scarce; and most of that found (12, 19, 21, 23, 34) concentrates upon contamination by or survival of pathogens. A small number can be classified as dealing with the extension of shelf life (16, 17). Spoilage of salads is usually attributed to lactic acid bacterial souring or yeast fermentation and mold growth.

Hill et al., (19) dealt directly with the outbreak of food poisoning caused by eating of tuna salad. Streptococcus pyogenes was determined to be the cause of illness of cadets at the United States Air Force Academy. The tuna salad had been made from eggs contaminated with Streptococcus pyogenes. A kitchen worker who had peeled the eggs was a carrier of an asymptomatic infection and had contaminated the eggs.

The repopulation of the digestive tract of drug treated persons became a concern of Shooter et al. (34). They discovered that foods, several salads among them, served in hospitals and canteens were contaminated with enough bacteria to establish intestinal colonies in healthy persons. Ill persons recently taken off antibiotic therapy were of the greatest concern because of the high numbers of Escherichia coli, Pseudomonas aeruginosa and Klebsiella species detected. Hospital health problems were foreseen due to higher susceptibility of debilitated or drug treated people to lower numbers of organisms than healthy individuals.

Salads from restaurants and cafeterias were found to contain relatively high bacterial and yeast counts by Jopke and Riley (23). The results indicated that salads made in restaurants and cafeterias

were manufactured under unsanitary conditions. Aerobic plate counts showed as many as 1.6×10^6 bacteria per gram of salad. Yeast content was as high as 1.7×10^6 per gram of salad. Fewer coliforms and pathogens were seen with coliform levels up to 3.5×10^5 per gram of salad. Staphlococcus aureus high counts were only 380 organisms per gram of salad. No Salmonella, Shigella, or Clostridium were found and no spoilage or spoilage organisms were noted.

Salads from retail outlets proved to be little different than the restaurant or cafeteria salads. In Raleigh, North Carolina, Christiansen and King (12) found that aerobic plate counts reached a high of 2.9×10^7 per gram, with yeast and mold counts of 3.4×10^6 per gram. Coliforms were found at 2.5×10^4 levels in some of the salads. The numbers of pathogens were highest in Staphlococcus aureus with 2.0×10^5 per gram of salad; but neither Salmonella nor Clostridium were found in high numbers.

Christiansen and King (12) were convinced that the contamination levels of both coliforms and pathogens were higher than the levels detectable. They postulated that low pH and low temperature either killed pathogens or prevented their growth. Because of the pH and temperature controls, it was stated that the only source for pathogenic toxins was contaminated ingredients. Only lactic acid bacteria and yeasts and molds were deemed capable of growth, even with incubation of salads at higher temperatures more favorable to pathogen growth.

Holtzapffel and Mossel (21) approached the pathogen growth differently by inoculating meat and fish salads with known pathogens.

Staphlococcus aureus, Salmonella typhimurium, Salmonella panama, Clostridium perfringens and Bacillus cereus were inoculated into salads purchased from commercial producers and incubated at 20° C and 9° C to compare survival of pathogens. Standards were maintained at 3° C. Scheduled sampling showed that while the aerobic plate counts, lactic acid bacteria counts and yeast and mold counts increased at both 20° C and 9° C, the pathogens decreased. Clostridium perfringens and Bacillus cereus showed the slowest decrease due to their spore formation.

Spoilage of the salads tested (21) was brought about by souring due to lactic acid bacteria or growth of yeasts and molds. Identification of spoilage organisms was not carried out, but 140 lactic acid bacteria were isolated. The lactic acid bacteria isolated consisted of 89 percent rods and 11 percent cocci. Low storage temperature was a deterent to lactic acid bacterial spoilage, but even at 3° C the yeasts and molds grew slowly.

Sources of contamination of salads were included in a study of delicatessen foods by Hankin and Ullman (16). High counts in the aerobic plate counts, oxidase counts and coliform counts were utilized to detect the sources of contamination of salads during manufacture. High aerobic plate counts were caused by ingredients with high bacterial counts before being incorporated into the salads. Dirty equipment was pointed out as causing an increase in the oxidase count and high coliforms were caused by poor handling of the salads during and after manufacture.

The keeping quality of potato salad and cole slaw was examined by Hankin and Stephens (17) by correlation of the aerobic plate count, coliform count, oxidase count, yeast and mold count, pH and total acidity to organoleptic scores of acceptability for sale. Of all of these tests, only two were statistically related to panel scores of quality. The pH of potato salad was negatively correlated to its quality at the 0.01 percent level of probability. For cole slaw, the oxidase count was negatively correlated to quality at the 0.05 percent level of probability.

CHAPTER II

MATERIALS AND METHODS

Nine brands of salads, including chicken salad, cole slaw, ham salad, potato salad, pimento cheese spread and several other types of salads were collected from the producers or local retail outlets. Samples were packed in ice, in non-local and brought to the laboratory within 24 hours. Upon receipt, samples were refrigerated at 3 to 6° C until the first analysis within 48 hours of receipt. Following the first sampling, salads were again refrigerated at 3 to 6° C until the final sampling. Final sampling took place five days following the expiration date of large producers and fifteen days after receipt of delicatessen salads.

Media used were produced by Baltimore Biological Laboratories, Cockeysville, Maryland. Aerobic plate count (APC) and psychrophilic plate count (PPC) were carried out in Standard Methods Agar. Aerobic plate count plates were incubated at 32° C for 48 hours (7). Psychrophilic plate counts were incubated at 3 to 6° C for ten days (7). Coliform counts were performed in Violet Red Bile Agar (7) incubated at 32° C for 24 hours. Mycophil Agar, acidified with 10 percent lactic acid to pH 4.0, was incubated at 21° C for five days for yeast and mold counts.

A ten-gram sample of salad was weighed into a sanitized, stainless steel Virtis homogenizer jar. Ten milliliters of sterile 2 percent sodium citrate (28) was added as diluent and emulsifier.

The salad and diluent mixture was homogenized for one minute at a speed predetermined to allow the homogenate to be pipetted in a standard 2.2 milliliter pipette. Speed depended upon the various salads. Yeast and mold and coliform dilutions of 1 to 1 and 1 to 10 were taken directly from the container and plated as 2.0 milliliters and 0.2 milliliters respectively. Two milliliters of homogenate were placed into a 99 milliliter phosphate diluent (7) for a 1 to 100 dilution. All aerobic plate counts and psychrophilic plate counts were plated at dilutions of 1 to 100, 1 to 1,000 and 1 to 10,000 utilizing the phosphate diluent.

pH values for both original and final samplings were taken by mixing equal volumes of salad with distilled water and measured with a Beckman pH meter.

From each set of aerobic plate count, psychrophilic plate count and coliform plates, at least one colony of each type found was picked for purification and identification. Differences in size, shape, color, location on the plate (surface or subsurface) and appearance (smooth, matte, etc.) were the bases for the chosen colonies. Colonies chosen were purified on Standard Methods Agar incubated at temperatures of 32° C for aerobic plate counts and coliform colonies and 21° C for psychrophilic plate count colonies. All colonies were identified by sequential numbers of A--- for those from the first plating and B--- for those from the final plating following expiration date of the salads.

Purification of colonies was accomplished by addition of a portion of the colony to a 99 milliliter phosphate dilution blank (7),

mixing and pour plating a drop of the dilution using Standard Methods Agar. A colony from the resulting plate, of similar description to the original, was diluted and replated as before. Three transfers were carried out in this manner. A few required more transfers for purification or a dilution blank of 0.1 percent peptone water (13) due to non-survival in a phosphate buffer.

Storage was on a slant of Standard Methods Agar at 3 to 6° C until needed for identification. Isolates were rejuvenated every six months by transferal and regrowth on a similar slant.

The organisms were then identified using the keys presented in Appendix A and Appendix B. Where possible, selective media were used as an aid to identification and rechecked against the key. (For example, Eosin Methylene Blue Agar for identification of Escherichia coli and Citrobacter intermedia). Salmonella-Shigella Agar and Baird-Parker Agar were used for pathogen screening.

CHAPTER III

RESULTS AND DISCUSSION

Tables discussed individually contain the results obtained from all samples of salad upon receipt and at the final plating. Producers were assigned a random identification number when their salads were first received.

The salads obtained from most of the producers had a lower range of all counts than those examined by Christiansen and King (12) and by Holtzapffel and Mossel (21). Like those salads studied previously by the above mentioned researchers, however, the pH and low temperatures of storage for the following study kept growth of microorganisms present in check. Some were even reduced in number.

The salads from producer 57703 (Table I) had less than 1.0×10^5 aerobic plate count bacteria per gram in four of six salads when first received and less than 1.0×10^5 aerobic plate count bacteria per gram in all salads upon the final plating. In all but one of the samples, the potato salad, the detectable bacteria were reduced in number between the first and second plating. In those salads containing coliforms and yeasts and molds at detectable levels, the pattern of decrease in counts was established. The pH values of all salads in this group also dropped at least one-tenth of a pH unit between the first sampling and the final sampling. Since all pH values of the salads were less than pH 5.5 when first samples and the temperature of storage was 3 to 6° C, the indicated counts and pH values support the theories of Christiansen and King (12) and Jopke and Riley (23) that a low pH

TABLE I

MICROBIOLOGICAL PROFILES AND pH OF SALADS FROM PRODUCER 57703
AT THE ORIGINAL AND FINAL EXAMINATIONS

Salad	APC	PPC	Coliform	Yeast and mold	pH
Original examination					
	---------- counts per gram ----------				
Chicken	70,000	11,900	1170	1450	5.05
Cole slaw	21,000	<100	<1	180	4.60
Egg and ham	400	<100	<1	230	4.96
Ham	123,000	1300	1070	80	4.91
Potato	300	<100	<1	1	4.60
Pimento cheese	610,000	<100	<1	3100	5.30
Final examination					
Chicken	6400	1600	180	2	4.70
Cole slaw	2500	<100	<1	<1	4.21
Egg and ham	100	700	<1	<1	4.65
Ham	23,600	32,000	30	20	4.55
Potato	500	900	<1	<1	4.25
Pimento cheese	88,000	7000	<1	40	4.88

APC = Aerobic plate count.
PPC = Psychrophilic plate count.

and low storage temperature are detrimental to bacterial growth in salads. In this set of samples, the yeasts and molds were evidently affected also.

The psychrophilic plate counts in Table I indicated a different trend than the other organism counts. In four of the six salads sampled, the psychrophilic plate counts increased during storage between the first and second sampling. The psychrophilic plate count increases, plus the fact that the pH decreased in the salads may have indicated growth of acid-producing bacteria. However, unknown chemical reactions could have caused the pH decreases. There may have been evidence for the chemical origin of the pH decreases, since the chicken salad and cole slaw had no increase in any counts but still had lowered pH values upon the second sampling. In the chicken salad samples, a decrease in psychrophilic plate count was seen, but the pH value decreased from pH 5.05 to pH 4.72. This was unexplainable with the information available because no growth of any organism was indicated.

One interesting note about the pimento cheese aerobic plate count in Table I was that it was the highest found in any of these salad samples at either plating. The high aerobic plate count may have been caused by the use of cheddar cheese as the main ingredient in the product. Cheddar type cheese is a cultured product and naturally contains high numbers of organisms. Conceivably, a large number of the organisms found in the producer's pimento cheese came from the cheese used for its manufacture.

During storage of the samples from producer 39948 (Table II), four of the six aerobic plate counts and four of the six psychrophilic plate counts increased, which indicated growth of bacteria capable of growth at both 2 to 6° C and 32° C. Three of the five salads containing detectable yeasts and molds also increased in yeast and mold population between the first and second plating. This yeast and mold growth agreed with the theories of Christiansen and King (12) and with Holtzapffel and Mossel (21) that yeasts and molds can grow in salads with low pH values while stored at low temperature. Along with the increases in aerobic plate count, psychrophilic plate count and yeast and mold count, the egg salad samples showed an increase in the coliform count as well. Evidently, under existing pH and temperature conditions coliforms were able to grow. In the cole slaw and potato salad samples, which also contained detectable coliforms, the pH was slightly lower than the pH of the egg salad. In these salads coliforms were unable to grow, in fact, their numbers decreased.

High aerobic plate counts characterized half of the salad samples from producer 26615 (Table III) when they were first received. The chicken salad, ham salad and tuna salad samples had more than 3.0 x 10^7 bacteria per gram. These were the highest counts of prepared salad found in this study. The aerobic plate counts of the salads in this set of samples generally decreased between the first and second samplings. This decrease, plus the low pH values of the salads tends to support the theories of Christiansen and King (12) and of Holtzapffel and Mossel (21) that low pH and low temperature of storage kills some bacteria in salads. Increases in psychrophilic plate counts of potato

TABLE II

MICROBIOLOGICAL PROFILES AND pH OF SALADS FROM PRODUCER 39948
AT THE ORIGINAL AND FINAL EXAMINATIONS

Salad	APC	PPC	Coliform	Yeast and mold	pH
	counts per gram				
Original examination					
Chicken	100	100	<1	170	4.85
Cole slaw	700,000	24,100	1030	7000	4.80
Ham	6900	300	<1	1450	4.55
Egg	1800	20,000	<1	710	5.45
Potato	180,000	72,000	900	19,000	4.95
Pimento cheese	2,390,000	25,300,000	<1	<1	5.60
Final examination					
Chicken	135,000	3000	<1	3000	4.80
Cole slaw	630,000	720,000	<1	240,000	4.60
Ham	1500	100	<1	<1	5.40
Egg	6,900,000	6,000,000	20	9000	5.42
Potato	39,800,000	29,800,000	70	1700	5.04
Pimento cheese	7,500,000	5,900,000	<1	<1	4.55

APC = Aerobic plate count.
PPC = Psychrophilic plate count.

TABLE III

MICROBIOLOGICAL PROFILES AND pH OF SALADS FROM PRODUCER 26615
AT THE ORIGINAL AND FINAL EXAMINATIONS

Salad	APC	PPC	Coliform	Yeast and mold	pH
		counts per gram			
Original examination					
Chicken	>30,000,000	17,200,000	<1	>300,000	4.70
Cole slaw	900	<100	<1	<1	3.90
Ham	>30,000,000	30,600,000	<1	>300,000	4.95
Potato	9200	10,700	<1	16,200	5.06
Pimento cheese	7300	1400	<1	860	5.27
Tuna	>30,000,000	6,000,000	<1	<1	4.72
Final examination					
Chicken	1,250,000	1,500,000	10	>300,000	4.22
Cole slaw	300	<100	<1	<1	3.51
Ham	18,700,000	12,400,000	<1	<1	4.59
Potato	21,600	57,000	<1	18,800	4.69
Pimento cheese	4200	4000	<1	3300	4.96
Tuna	9,600,000	7,280,000	<1	<1	4.30

APC = Aerobic plate count.
PPC = Psychrophilic plate count.

salad, pimento cheese and tuna salad samples and decreases in pH values of these same salads may have indicated the growth of acid-producing bacteria. A chemical reaction could have accounted for the same pH reduction in those salads where the pH value decreased. If a chemical reaction took place, it is doubtful that the growing bacteria were acid-producing bacteria.

In this group of salads, there was another indication of coliform growth. The chicken salad had no detectable coliforms in the first sample taken; but there were ten coliform organisms found in the second sampling. Growth of coliforms should not have been able to take place at such a low pH (pH 4.70 with a decrease to pH 4.22) and low temperature (3 to 6° C). The chicken salad sample of producer 57703 (Table I) had a higher pH value (pH 5.05 with a decrease to pH 4.72). This sample was stored at the same temperature (3 to 6° C) with a larger number of coliforms. Those coliforms were unable to grow. Most of them died off.

The psychrophilic plate counts and pH values are the only characteristics to indicate a directional trend in the salads received from producer 94719 (Table IV). The increase in psychrophilic plate counts in three of the five salads (cole slaw, potato salad and pimento cheese) was indicative of growth. The fact that pH values decreased in these same salads may have indicated growth of acid-producing bacteria or a chemical reduction of pH. Chemical reduction of pH is supported by the lowered pH of chicken salad. The salads of this group showed erratic results in aerobic plate counts and yeast and mold counts, with some increases and some decreases in counts. Two salads, cole

TABLE IV

MICROBIOLOGICAL PROFILES AND pH OF SALADS FROM PRODUCER 94719
AT THE ORIGINAL AND FINAL EXAMINATIONS

Salad	APC	PPC	Coliform	Yeast and mold	pH
		counts per gram			
Original examination					
Chicken	1300	<100	42	35	5.14
Cole slaw	400	<100	<1	3	4.48
Ham	100	<100	<1	30	4.91
Potato	1700	<100	<1	11	5.06
Pimento cheese	490,000	100	<1	>300,000	5.14
Final examination					
Chicken	100	<100	<1	3	4.80
Cole slaw	400	400	<1	38	4.15
Ham	100	100	<1	17	5.00
Potato	6900	3200	<1	4360	4.51
Pimento cheese	550,000	2100	<1	18	4.95

APC = Aerobic plate count.
PPC = Psychropholic plate count.

slaw and ham salad, did not show either an increase or decrease in aerobic plate count. Chicken salad was the only salad with detectable coliforms, which evidently died before the final plating. Lack of other coliform counts for comparison eliminated the chance to observe a tendency of increase or decrease.

The salads from producer 00023 (Table V) had variable increases and decreases in yeast and mold counts and in pH values. Some increases, some decreases and some stable results were seen. However, the growth of bacteria capable of growth at 32° C and at 3 to 6° C was indicated by increases in the aerobic plate count between the first and second plating, except in the cases of chicken salad and cole slaw. Detectable coliforms showed a decrease between the first and second plating in three of the salads. The decrease of coliforms was evidently caused by the low pH and low temperature of storage.

Generally, salads from producer 97532 (Table VI) showed a decrease in coliform counts and in aerobic plate counts. Lower counts in chicken salad, cole slaw, potato salad and ham salad samples indicated that pH and low temperatures of storage may have caused death of some organisms. Yeast and mold results did not produce a trend of growth or death because yeast and mold counts from potato salad and cole slaw increased while those from ham salad and chicken salad decreased. The increased yeast and mold counts occurred at a lower pH value than the decreased counts. Psychrophilic plate counts decreased in three of the five salads. In this group of salads, the low pH and low temperature of storage did not prevent the growth of psychrophilic bacteria. The largest increase in psychrophilic plate counts was seen in ham and

TABLE V

MICROBIOLOGICAL PROFILES AND pH OF SALADS FROM PRODUCER 00023
AT THE ORIGINAL AND FINAL EXAMINATIONS

Salad	APC	PPC	Coliform	Yeast and mold	pH
Original examination					
	---------- counts per gram ----------				
Chicken	<100	<100	<1	2	4.71
Cole slaw	42,000	3400	50	660	4.75
Ham	20,500	<100	<1	1	4.36
Potato	6900	600	680	1660	4.57
Pimento cheese	33,000	51,000	15	>300,000	5.68
Tuna	380,000	214,000	<1	>300,000	5.34
Final examination					
Chicken	100	<100	<1	60	4.59
Cole slaw	7400	3400	<1	2460	4.70
Ham	38,000	<100	<1	9	4.37
Potato	16,000	1300	<1	1500	4.46
Pimento cheese	83,000	20,000	<1	26,400	5.80
Tuna	64,800,000	24,600,000	<1	1	5.10

APC = Aerobic plate count.
PPC = Psychrophilic plate count.

TABLE VI

MICROBIOLOGICAL PROFILES AND pH OF SALADS FROM PRODUCER 97532
AT THE ORIGINAL AND FINAL EXAMINATIONS

Salad	APC	PPC	Coliform	Yeast and mold	pH
	---------- counts per gram ----------				
Original examination					
Chicken	29,000	10,200	46	1890	4.80
Cole slaw	7100	5400	580	1220	4.55
Ham	12,800	24,600	23	>300,000	5.01
Potato	4100	11,000	<1	1	4.44
Pimento cheese	8,040,000	5,000,000	<1	<1	5.24
Final examination					
Chicken	20,500	56,000	20	500	4.82
Cole slaw	3700	4000	28	2600	4.55
Ham	9000	22,200	<1	6700	5.04
Potato	2900	900	<1	2800	4.46
Pimento cheese	15,100,000	14,800,000	<1	<1	5.23

APC = Aerobic plate count.
PPC = Psychrophilic plate count.

cheese salad and in the pimento cheese. A pH increase was also seen. The organisms which grew were not acid-producing bacteria. Pseudomonas or other unknown psychrophiles were the most likely to grow.

The dry salads of producer 70010 (Table VII) were the only salads of this type studied. These salads were not plated before storage. In the results obtained after storage, very high aerobic plate counts (in excess of 7.5×10^8), psychrophilic plate counts (more than 9.0×10^8) and yeast and mold counts (more than 1.0×10^5) were observed. These results were anticipated because the ingredients were chopped or sliced during preparation, which freed the plant fluids to provide a good growth medium for bacteria. The lack of a pH lowering agent, such as mayonnaise or salad dressing used in the preparation of the other salads of this group, allowed the growth of bacteria capable of growth at low temperatures. A slimy, dextran-like material was noticeable on the salads when they were plated, which suggested the growth of Pseudomonas or Leuconostoc.

The salad samples from producers 28449 (Table VIII) and 20589 (Table IX) were similar in their characteristics. Both contained less than 1.0×10^5 organisms per gram in most of their aerobic plate counts and less than forty coliforms in three of four samples where coliforms were found. The chicken salad of producer 28449 (Table VIII) contained more than 7.5×10^3 coliforms per gram in fresh salad but had a low aerobic plate count of 4.5×10^4 organisms per gram. These coliforms could have been present on the chicken used to manufacture the salad, due to lack of hygiene on the part of personnel who prepared the salad or due to dirty equipment or ingredients. Coliforms are especially

TABLE VII

MICROBIOLOGICAL PROFILES AND pH OF SALADS FROM PRODUCER 70010
AT THE ORIGINAL AND FINAL EXAMINATIONS

Salad	APC	PPC	Coliform	Yeast and mold	pH
Original examination					
	counts per gram				
Chicken	1000	100	60	800	4.34
Cole slaw (hot)[a]	5400	<100	<1	10	4.10
Egg and olive	17,900	3000	<1	70	4.50
Ham	2200	<100	<1	<1	4.25
Ham and cheese	1,330,000	400	62	116	4.40
Potato	33,000	<100	<1	2	4.06
Pimento cheese	141,000	3500	<1	>300,000	4.69
Dry tossed[b]	----[c]	----	--	----	----
Dry cole slaw[b]	----	----	--	----	----

TABLE VII (continued)

Salad	APC	PPC	Coliform	Yeast and mold	pH
Final examination					
Chicken	800	300	<1	32	4.30
Cole slaw (hot)	8200	<100	<1	<1	4.20
Egg and olive	11,000	<100	<1	3	4.60
Ham	1400	<100	<1	8	4.50
Ham and cheese	1,100,000	337,000	<1	86	4.70
Potato	18,600	200	<1	1390	4.10
Pimento cheese	142,000	16,100	<1	16	4.80
Dry tossed	1,620,000,000	936,000,000	<1	135,000	----
Dry cole slaw	756,000,000	972,000,000	<1	270,000	----

APC = Aerobic plate count.
PPC = Psychrophilic plate count.
[a]This cole slaw contained hot pepper while others examined did not.
[b]These salads did not contain mayonnaise or salad dressing.
[c]Data not obtained.

TABLE VIII

MICROBIOLOGICAL PROFILES AND pH OF SALADS FROM PRODUCER 28449
AT THE ORIGINAL AND FINAL EXAMINATIONS

Salad	APC	PPC	Coliform	Yeast and mold	pH
Original examination					
	---------- counts per gram ----------				
Chicken	45,000	95,000	7740	7800	4.94
Cole slaw	6000	1000	<1	7	4.29
Ham	100	100	<1	<1	4.43
Ham and cheese	239,000	206,000	<1	25,000	4.88
Potato	3400	100	<1	80	4.27
Pimento cheese	3000	1200	40	2000	5.17
Final examination					
Chicken	64,000	76,000	1990	----[a]	4.91
Cole slaw	1700	600	<1	----	4.24
Ham	200	100	<1	----	4.43
Ham and cheese	338,000	2,100,000	<1	----	4.85
Potato	8000	6300	<1	----	4.25
Pimento cheese	11,400	6400	14	----	5.05

APC = Aerobic plate count.
PPC = Psychrophilic plate count.
[a]Data not obtained.

TABLE IX

MICROBIOLOGICAL PROFILES AND pH OF SALADS FROM PRODUCER 20589
AT THE ORIGINAL AND FINAL EXAMINATIONS

Salad	APC	PPC	Coliform	Yeast and mold	pH
		counts per gram			
Original examination					
Chicken	2600	<100	<1	<1	4.58
Cole slaw	10,200	7000	<1	----[a]	4.20
Ham	4100	1700	10	----	4.36
Potato	175,000	30,700	<1	10	4.46
Pimento cheese	5,570,000	<100	<1	4700	5.16
Tuna	600	<100	10	<1	4.55
Final examination					
Chicken	1400	<100	<1	----	4.54
Cole slaw	3000	<100	<1	----	4.30
Ham	5600	1600	<1	----	4.36
Potato	5,480,000	17,400,000	<1	----	----
Pimento cheese	7,090,000	100	<1	----	5.10
Tuna	1100	<100	<1	----	4.54

APC = Aerobic plate count.
PPC = Psychrophilic plate count.
[a]Data not obtained.

found in these places and could easily have been introduced into the salad in these ways. This coliform count was the single highest found in all of the salads tested. Even among those salad samples with millions of aerobic plate count organisms other than coliforms (Table III), none had this high a coliform count.

In both sets of samples (Tables VIII and IX) the coliforms were reduced in number during storage, while the majority of the aerobic plate counts and some of the psychrophilic plate counts increased in number. The growing organisms in these salads were probably capable of growth at 32° C and at 3 to 6° C, but did not produce acid, as the pH of most of the samples showed little or no increase between samplings.

The date from these last two sets of samples (Tables VIII and IX) contradicted the theory of Holtzappfel and Mossel (21) that only lactic acid-producing bacteria and yeasts and molds were capable of growth in salads stored at low temperatures and having a low pH. Little or no acid was produced by the bacteria present, and therefore, they cannot be considered as acid-producing bacteria.

The salad samples studied by Holtzapffel and Mossel (21) were spoiled by either lactic acid bacteria or yeasts and molds, but they did not identify the bacteria present in the salads. The primary purpose of this study was to find and identify the bacteria present in the salads. Bacteria isolated and identified are listed in Table X.

Holtzapffel and Mossel (21) noted that of the 140 lactic acid bacteria they isolated, 89 percent were rods and 11 percent

TABLE X

BACTERIA AND QUANTITY OF EACH SPECIES
ISOLATED AND IDENTIFIED FROM ALL SALADS

Bacteria	Quantity
Bacillus	146
Bacillus brevis	1
Bacillus megaterium	1
Bacillus coagulans	4
Bacillus firmus	5
Bacillus cereus	9
Bacillus licheniformis	29
Bacillus subtilis	45
Bacillus pumilis	52
Lactobacillus	75[a]
Lactobacillus lactis	2
Lactobacillus delbrueckii	2
Lactobacillus brevis	4
Lactobacillus casei	10
Lactobacillus leichmannii	12
Lactobacillus plantarum	12
Leuconostoc	50
Leuconostoc dextranicum	5
Leuconostoc mesenteroides	21
Leuconostoc paramesenteroides	24
Streptococcus	15
Streptococcus lactis	1
Streptococcus uberis	2
Streptococcus cremoris	3
Streptococcus faecalis	4
Streptococcus durans	5
Staphlococcus	12
Staphlococcus epidermidis	10
Staphlococcus aureus	2
Micrococcus	41
Micrococcus roseus	1
Micrococcus varians	17
Micrococcus luteus	23

TABLE X (continued)

Bacteria	Quantity
Enterobacteriaceae	12
Escherichia coli	4
Enterobacter aerogenes	4
Citrobacter intermedia	4
Pseudomonas	20
Xanthomonas	4
Actinomyces	1

[a]33 *Lactobacillus* were not identified to the species level.

were cocci. With the same number of lactic acid bacteria, 140, a much higher ratio of cocci were found in this study. The 65 lactic acid cocci were mostly Leuconostoc, 50, with several Streptococcus, 15. Some 75 organisms were identified as Lactobacillus, but only 42 were identified to the species level.

The Leuconostoc identified consisted of 42 percent Leuconostoc mesenteroides, 48 percent Leuconostoc paramesenteroides, and ten percent Leuconostoc dextranicum. Only five of the known species of Streptococcus were found in the salads. Streptococcus lactis, Streptococcus uberis, Streptococcus cremoris, Streptococcus faecalis and Streptococcus durans were the streptococci identified. The bulk of the Lactobacillus were Lactobacillus leichmannii, Lactobacillus plantarum and Lactobacillus casei, with some Lactobacillus lactis, Lactobacillus delbrueckii and Lactobacillus brevis.

Bacillus was the only other genus of bacteria found in as high a frequency as the lactic acid bacteria. This was an anticipated result, as most vegetables are in close contact with the soil. Bacillus are considered soil organisms. The salads which have a high vegetable content would contain these organisms or their spores. The majority of Bacillus were Bacillus pumilus, Bacillus subtilis, and Bacillus licheniformis with a few Bacillus cereus, Bacillus firmus, Bacillus coagulans, Bacillus megaterium and one Bacillus brevis.

According to Bergey's Manual of Determinative Bacteriology (10), there are only three species of Micrococcus. All three were represented in the salad samples. Micrococcus luteus was most prevalent with 56 percent of the 41 organisms isolated. The remainder were

Micrococcus varians and one Micrococcus roseus, with a 42 percent and 2 percent, respectively, of the total.

Staphlococci constituted only twelve of the isolates, and only two were Staphlococcus aureus. The remaining ten were identified as Staphlococcus epidermidis.

Most of the remaining genera isolated in this study were either in the family of Enterobacteriaceae or Pseudomonadaceae. A few Citrobacter, Enterobacter and Escherichia were found; but the majority of the organisms were either Pseudomonas or Xanthomonas. One actinomycete was found.

CHAPTER IV

SUMMARY

Throughout this study, there were no patterns established which included all the salads. Bacterial counts and yeast and mold counts increased in some salads, decreased in others and remained the same in still others. Further studies would be necessary to determine the causes of the variabilities.

The pH of the salads probably shows the most noticeable variability and could have been affected by bacterial or yeast and mold growth as well as by natural chemical or buffering capacities of the contents of the salads. If microbial growth caused variations in the pH in the salads, then with some of the salads, the growth of microorganisms would not have been detectable. If pH changes were due to chemical reactions, they were not studied in this experiment. In either case, more in-depth studies of salads will be required to uncover the causes of the pH changes.

The bacilli and some of the lactic acid bacteria were most often isolated in this study. The lactic acid bacteria would be the most likely to spoil the salads if the theories of Holtzapffel and Mossel (21) and Christiansen and King (12) are accepted. The other bacteria found were a lesser percentage of the isolates.

The salads of this study were probably manufactured under better sanitary conditions than those previously reported by Holtzapffel and Mossel (21) and Christiansen and King (12). The salads of the aforementioned researchers generally had higher bacterial counts and yeast and mold counts than those in this study.

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APPENDICES

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APPENDIX A

PARTIAL IDENTIFICATION SCHEME FOR GENERA OF BACTERIA

I. Gram positive.

A. Rods

1. Spores present.

1. *Bacillus*

2. Non-sporulating.

2. *Lactobacillus*

B. Cocci

1. Catalase positive.

a. Dextrose fermented.

3. *Staphlococcus*

aa. Dextrose oxidized or no reaction.

4. *Micrococcus*

2. Catalase negative.

a. Carbon dioxide produced anaerobically from glucose.

5. *Leuconostoc*

aa. No carbon dioxide produced anaerobically from glucose.

6. *Streptococcus*

II. Gram negative.

A. Rods

1. Pigments produced on Standard Methods Agar.

a. Non-diffusible yellow or orange pigment.

b. Oxidative or no attack on glucose.

c. Polar flagella.

7. Xanthomonas

cc. Peritrichous flagella.

8. Flavobacterium

bb. Fermentative attack on glucose.

9. Enterobacteriaceae

aa. Diffusible yellow, green-yellow, green, or brown pigments.

b. Oxidative attack on glucose.

c. Motile.

d. Polarly flagellated.

10. Pseudomonas

dd. Peritrichous flagella.

e. Utilizes inorganic nitrogen as sole nitrogen source.

11. Agrobacterium

ee. Does not utilize inorganic nitrogen.

12. Achromobacter

cc. Non-motile.

13. Acinetobacter

bb. No attack on glucose.

c. Motile.

d. Polarly flagellated.

10. Pseudomonas

dd. Peritrichous flagella.

11. Alcaligenes

2. Non-pigmented on Standard Methods Agar.

a. Oxidative or no attack on glucose.

b. Polarly flagellated.

10. Pseudomonas

aa. Fermentative attack on glucose.

b. Motile.

c. Polarly flagellated.

d. Gelatin hydrolyzed.

e. Positive Møller arginine test.

14. Aeromonas

ee. Negative Møller arginine test.

15. Vibrio

dd. Gelatin not hydrolyzed.

16. Plesiomonas

cc. Peritrichous flagella.

9. Enterobacteriaceae

bb. Non-motile.

c. Oxidase positive.

14. Aeromonas

cc. Oxidase negative.

9. Enterobacteriaceae

APPENDIX B

PARTIAL IDENTIFICATION SCHEME FOR SPECIES OF BACTERIA

Bacillus

I. Spores not definitely swollen.

A. Protoplasm of 24-hour cells grown on glucose agar vacuolated, if lightly stained.

1. Acetylmethylcarbinol not produced.

1. Bacillus megaterium

2. Acetylmethylcarbinol produced.

2. Bacillus cereus

B. Protoplasm of 24-hour cells grown on glucose agar not vacuolated, if lightly stained.

1. Growth on glucose agar as good or better than on Standard Methods Agar. Good growth on soybean agar.

a. Grows in Tryptícase Soy Broth, plus seven percent NaCl.

b. Starch hydrolyzed. Nitrites produced from nitrates.

c. Good growth under anaerobic conditions in glucose broth.

3. Bacillus licheniformis

cc. Scant, if any, growth in glucose broth under anaerobic conditions.

4. Bacillus subtilis

bb. Starch not hydrolyzed. Nitrites not produced from nitrates.

5. Bacillus pumilus

aa. No growth in Trypticase Soy Broth, plus seven percent NaCl.

b. Glucose utilized. Weak, if any, hydrolysis of gelatin.

6. Bacillus coagulans

2. Growth on glucose agar definitely not as good as on Standard Methods Agar. Scant, if any, growth on soybean agar.

a. Urease not produced.

7. Bacillus firmus

aa. Urease produced.

8. Bacillus lentus

II. Spores definitely swollen.

A. Gas not produced from glucose.

1. Saprophytic. Grows on ordinary media.

a. Starch not hydrolyzed.

b. Does not grow in glucose broth under anaerobic conditions.

9. Bacillus brevis

Lactobacillus

I. Homofermentative.

A. Optimum temperature 37° to 60° C or higher.

1. Acid from lactose.

a. Optimum temperature 37° to 45° C.

b. Acid from sucrose.

1. *Lactobacillus lactis*

bb. No acid from sucrose.

c. Acid from cellobiose.

2. *Lactobacillus acidophilus*

cc. No acid from cellobiose.

3. *Lactobacillus helveticus*

aa. Optimum temperature 45° to 62° C.

4. *Lactobacillus bulgaricus*

2. No acid from lactose.

5. *Lactobacillus delbrueckii*

B. Optimum temperature 28° to 32° C.

1. Acid from raffinose.

6. *Lactobacillus plantarum*

a. Acid from mannitol.

7. *Lactobacillus casei*

aa. No acid from mannitol.

8. *Lactobacillus leichmannii*

II. Heterofermentative. CO_2 from glucose.

A. Optimum temperature 28° C to 32° C.

1. Ferments raffinose.

9. *Lactobacillus buchneri*

2. Does not ferment raffinose.

10. Lactobacillus brevis

B. Optimum temperature 35° to 40° C or higher.

11. Lactobacillus fermenti

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Leuconostoc

1. Produces CO_2 from glucose.

   A. Produces acid from sucrose.

      1. Produces acid from trehalose.

         a. Produces dextran.

            b. Produces acid from arabinose.

               1. Leuconostoc mesenteroides

            bb. No acid from arabinose.

               2. Leuconostoc dextranicum

         aa. No dextran produced.

            3. Leuconostoc paramesenteroides

      2. No acid from trehalose.

         4. Leuconostoc lactis

   B. No acid from sucrose.

      5. Leuconostoc cremoris

Streptococcus

I. Growth at 10° C and 45° C. Ammonia produced from arginine.

A. No growth in 6.5 percent broth or in 0.1 percent methylene blue milk.

1. *Streptococcus uberis*

B. Growth in 6.5 percent NaCl broth and in 0.1 percent methylene blue milk.

1. Litmus reduced before coagulation.

2. *Streptococcus faecalis*

2. Litmus reduced after coagulation or not reduced.

3. *Streptococcus durans*

II. Growth at ten degrees C but not at 45° C. Growth in 0.1 percent methylene blue milk.

A. Growth at 40° C. Produces ammonia from arginine.

4. *Streptcococcus lactis*

B. No growth at 40° C. No ammonia produced from arginine.

5. *Streptococcus cremoris*

Staphlococcus

I. Baird-Parker positive.

A. Mannitol positive (acid).

1. *Staphlococcus aureus*

B. Mannitol negative (no acid).

2. *Staphlococcus epidermidis*

Micrococcus

I. Red pigment produced.

1. *Micrococcus roseus*

II. Pigment other than red produced.

A. Yellow pigment produced.

1. Produces acid from glucose.

a. Produces acid from xylose.

2. *Micrococcus varians*

aa. Does not produce acid from xylose.

3. *Micrococcus luteus*

2. Does not produce acid from glucose.

a. Does not produce acid from xylose.

3. *Micrococcus luteus*

B. No pigment produced.

1. Produces acid from glucose.

a. Produces acid from xylose.

2. *Micrococcus varians*

aa. Does not produce acid from xylose.

3. *Micrococcus luteus*

2. Does not produce acid from glucose.

3. *Micrococcus luteus*

Enterobacteriaceae

I. Urease positive.

1. Proteus

II. Urease negative.

A. Simmons Citrate positive.

1. Methyl red positive.

a. Growth in presence of cyanide.

b. Voges-Proskaur positive.

2. Hafnia

bb. Voges-Proskaur negative.

3. Citrobacter

aa. No growth in the presence of cyanide.

b. Acid from lactose.

4. Arizona group

bb. No acid from lactose.

5. Salmonella

2. Methyl red negative.

a. Motile.

b. Acid from lactose.

6. Enterobacter

bb. No acid from lactose.

7. Serratia

aa. Non-motile.

8. Klebsiella

B. Simmons Citrate negative.

1. Motile.

a. Indole positive.

9. Escherichia

aa. Indole negative.

5. Salmonella

2. Non-motile.

10. Shigella

VITA

Ralph C. Terry was born in Camden, Tennessee on October 27, 1950. When he was four years of age, his family moved to Cottage Hills, Illinois where he attended grade school at Cottage Hills Elementary School from 1956 until 1961. In 1961, he began his junior high school education at Wilbur Trimpe Junior High School in Bethalto, Illinois. In 1963, his family moved back to Camden, Tennessee where he completed his secondary education at the Camden Junior High School and Camden Central High School.

Upon receipt of his high school diploma in 1968, he entered the University of Tennessee, Knoxville, in the summer of 1968. He received the Bachelor of Science degree in Liberal Arts from the University of Tennessee in June, of 1973. He worked his way through while attending UT, paying for educational expenses by working at Fort Sanders Presbyterian Hospital, Knoxville during the school year and during summers and also working for ServiceMaster of Knoxville during the summers.

On June 8, 1973 he married the former Patricia Lawson, of Oak Ridge, Tennessee. In June of 1973, he was admitted to the Graduate School of the University of Tennessee, Knoxville and received an assistantship in the Food Technology and Science Department of the University of Tennessee College of Agriculture. He thereupon began work toward the Master of Science Degree in Agriculture.

The degree of Master of Science in Agriculture was conveyed to Mr. Terry in August, of 1975 after completing degree requirements of the College of Agriculture.