

PSYCHROPHILIC MICROBIOLOGICAL DETERIORATIVE CHANGES IN
FRESH BEEF, PORK AND POULTRY AND THEIR PRODUCTS
HELD AT REFRIGERATOR TEMPERATURES

by

SATISH CHANDRA NIVAS

B. V. Sc. & A. H., University of Bihar, India, 1960

A MASTER'S REPORT

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Animal Husbandry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1963

Approved by:


Major Professor

11
1963
A 734
Cop 2

TABLE OF CONTENTS

INTRODUCTION..... 1

PSYCHROPHILES..... 1

 Bacteria..... 4

 Molds..... 6

 Yeasts..... 8

PSYCHROPHILES CAUSING DETERIORATION IN MEAT AT LOW TEMPERATURES..... 9

 Bacteria..... 11

 Bacteria in Relation to Meat and Poultry Pies..... 16

 Bacteria and Ground Meat..... 20

 Yeasts and Molds..... 28

 Yeasts and Molds in Relation to Ground Meat..... 30

PSYCHROPHILES AND BEEF..... 33

 General..... 33

 Bacterial Spoilage..... 40

 Spoilage by Yeasts and Molds..... 46

PSYCHROPHILES AND PORK..... 48

 General..... 48

 Bacterial Spoilage..... 49

PSYCHROPHILES AND POULTRY..... 52

 General..... 52

 Bacterial Spoilage..... 55

 Spoilage by Yeasts and Molds..... 59

ACKNOWLEDGMENTS..... 61

LITERATURE CITED..... 62

INTRODUCTION

With the advancement of science, and newer techniques developed in cold storage of meat and poultry, increased attention is being given to the proper microbiological control of these products. Proper sanitary measures during slaughter of animals, and adequate cleanliness in the handling and processing of their products, is of first and foremost importance in maintaining minimum growth of psychrophilic microorganisms.

Meat is an ideal medium for growth of bacteria, yeasts and molds, as it furnishes the right moisture content and adequate ^{ents} ~~nutrition~~ in the form of proteins, fats, carbohydrates, minerals and vitamins. Thus it is of great importance in providing the right measures for the maintenance of a sound product held at refrigerator temperatures.

The purpose of this study was to review the recent literature of psychrophilic microorganisms causing deteriorative changes in beef, pork and poultry and their products held at low temperatures. Most of the literature cited is from articles and reviews from current periodicals. Some articles have been selected from journals and bulletins of earlier years pertaining to this study.

PSYCHROPHILES

Microorganisms are usually divided into three groups based on temperature requirements; i. e., thermophiles, mesophiles, and psychrophiles. Thermophiles are organisms which thrive at high temperatures; mesophiles are organisms which thrive at

moderate temperatures; and psychrophiles are organisms which have the ability to multiply at refrigerator temperatures.

Table 1 summarizes some of these relationships as based on our current knowledge. (68)

Table 1. Growth temperature relationships of microorganisms.

	Growth Temperature ($^{\circ}\text{C}$)*		
	: Minimum	: Optimum	: Maximum
Psychrophiles	- 5 to + 5	20 to 30	35 to 45
Mesophiles	10 to 15	35 to 45	40 to 50
Thermophiles	35 to 40	55 to 60	65 to 75

*These figures represent temperature limits that appear to be most typical of the group. They are different from many textbook definitions (particularly for the psychrophiles). Many common species have growth ranges (minimum to maximum) that are much wider or narrower than indicated by the summary in this table (e. g., some mesophiles can grow from 5°C to 50°C whereas some pathogens can tolerate temperatures only a few degrees from the body temperature of the host).

Stokes (60) quotes a paper by the Dutch investigator, J. Forster in 1887 who was the first to describe psychrophilic bacteria in the Centralblatt für Bakteriologie and Parasitenkunde as saying:

Our bacteria exhibit at certain temperatures a very special property which to my knowledge, at least with pure cultures, has not been previously observed.... they grow almost as well in the ice box, as at the usual room temperature and even when tubes of streaked nutrient gelatin are placed in a container packed with finely crushed ice in the ice box; that is at 0°C .

Ingraham and Stokes (30) in 1959 defined psychrophiles (a term derived from the Greek words "psychros" meaning cold, and "philos" meaning loving, i. e., cold loving) as cold tolerant

rather than cold loving. They further defined psychrophiles as those bacteria that grew well at 0°C, within two weeks. In other words (30) psychrophiles were bacteria that formed colonies on solid media, easily visible to the naked eye within two weeks at 0°C; or more quantitatively, psychrophiles were bacteria that had a generation time of 48 hours or less at 0°C.

Eddy (12) in 1960 considered that the word psychrotrophic (to increase, thrive) suggested by Mossel (48) in 1960 be used for bacteria able to grow at +5°C and below, whatever their optimum temperature.

Berry and Magoon (8) in 1934 contended that a truly cold-loving or "psychrophilic" group of microorganisms appeared unwarranted by available evidence. While organisms recorded as growing at 0°C or below with propriety could be regarded as forming a cold-tolerant group, their temperature requirements for best growth definitely placed them in the mesophile class.

Mattick (41) in 1951 observed that in spite of their well known adaptability in many ways, it was apparently not easy to induce most mesophilic bacteria to grow at temperatures even moderately low. The rather few truly psychrophilic species, however, succeeded remarkably well. He suggested that it was equally possible for some mesophiles to survive low temperatures, without growing, for very long periods.

Ingram (32) in 1951 said that microbes would grow in cool conditions, though slowly. He goes on to say that in the frozen state, their development was arrested, so it was possible to keep

foods free from microbial spoilage indefinitely, by freezing them; this treatment, however, could sometimes be undesirable because of consequent harmful changes in the food itself.

In 1892, Foster showed that psychrophilic bacteria were widely distributed in nature. He found them in fresh and salt water, on the surface and in the intestines of fresh and salt-water fish, in milk and meat, in garden soil and street dirt, and in canal and meadow ditch water. (30)

Straka and Stokes (62) in 1960 reported the presence of psychrophilic bacteria in soil, skua gull and seal feces, horse feces, in pony debris, and even in glacier ice from Antarctica. They also found one yeast-like organism which was isolated by cultivation at 0°C. The bacteria grew well at 0°C and could develop slowly at -7°C.

Bacteria

According to Ingraham and Stokes (30) in 1959, psychrophilic bacteria were usually gram-negative non-sporeforming rods that had the ability to grow at 0°C. The usual strains belonged to the genus Pseudomonas, Achromobacter, Flavobacterium, Micrococcus and perhaps Alcaligenes. The genera Lactobacillus, Aerobacter and others could contain psychrophilic species. (20)

Mattick (41) in 1951 believed that bacteria died when frozen mainly because they were crushed by extracellular ice crystals. He suggested that there was a critical temperature for survival of bacteria in the frozen state, about -2°C. He

further attributed the death of frozen organisms to denaturation and then the flocculation of some cellular proteins. Quoting Weitserity and Osterud, he said that the intensity of freezing had no influence on immediate death since intracellular ice did not form in bacteria, because a more lethal effect would be expected at temperatures at which ice would be formed in the cells.

Straka and Stokes (61) in 1959 thought that metabolic injury to bacteria at sub-zero temperatures, was manifested by an increase in nutritional requirements. The injured cells could no longer grow on a simple glucose-salt-agar medium, but could develop on a rich complex medium, trypticase soy agar. Dead cells did not grow on trypticase soy agar.

Recovery of injured cells on trypticase soy agar was due to the activity of the trypticase component, an enzymatic digest of casein. Peptides could have been the active substances in the digested casein and this might be required by injured cells for resynthesis of essential proteins denatured by the sub-zero temperatures. (61)

Haines (25) in 1933 made viable counts to determine the rate of growth of three species of bacteria isolated chiefly from meat in nutrient broth saturated with air and air containing 10% and 20% carbon dioxide respectively at 20, 4, and 0°C. He found that carbon dioxide had little action on Proteus, but increased the lag period and lengthened the generation time of Pseudomonas and Achromobacter by about one half at 20°C. On the last two, the effect was more marked at a lower temperature, the generation

Table 2. Effect of plating medium on recovery of Pseudomonas fluorescens after exposed to cold. (61)

Plating Agar	:Percent of Initial Population : Recovered*	
	: Expt. 1	: Expt. 2
Trypticase Soy	42	51
Glucose-Mineral salts (minimal)	19	28
Nutrient	14	
Phytone 2%	17	
Trypticase		
2%	43	52
1%		44
0.5%		32
0.25%		29

*After freezing in 0.5 percent beef extract and storage at -18°C for one day.

time being more than doubled at 0°C by 10% carbon dioxide. He said that 10% carbon dioxide was as effective as 20% in inhibiting the growth of Achromobacter at 0°C but it was not so efficient at 4°C .

Molds

According to Tschistjakow and Botscharowa (70) in 1938, the minimum temperature of the growth of molds lay considerably below 0°C ; low temperatures affected not only different mold species, but even different strains of the same species.

Experimentally they found that some varieties of Penicillium

glaucum, Mucor sp., strains of Botrytis cinerea, Cladosporium herbarum, Chaetostylium fresnii, Monilia nigra, Thamnidium elegans, and some species of Fusarium proved to be the most enduring at low temperatures. Also they noticed that at -18°C and -12°C , no growth of molds could be observed during a period of about 18 months.

Jensen (33) described species of molds which grew in cold storage as being of the genus Rhizopus, Mucor, Thamnidium, Botrytis, Aspergillus niger, Fusaria and Cladosporium herbarum.

Moran, Smith and Tomkins (46), in addition found Sporotrichum carnis, Penicillium expansum and P. anormalium to grow on cold storage meat.

Haines (26) in 1934 reported that several fungi could grow down to about -7°C . He further stated that the lower limit of growth of microorganisms was determined by two factors; firstly the temperature, and secondly the amount of water frozen out of the medium. According to him, when the medium froze, yeasts and molds tended to dominate and bacteria to be inhibited. Yeasts and molds could also tolerate high osmotic pressure of the medium.

Berry and Magoon (8) in 1934 stated that spore germination of certain molds had been observed at temperatures as low as -7.7°C . Any microbial growth below -10°C seemed unlikely.

From available evidence, it was stated (46) that a high concentration of carbon dioxide of the order of 50% or more, was required for complete inhibition of growth. But experimentally,

they found that quite low concentrations of carbon dioxide was markedly inhibitory to the growth of these molds, on lean meat, connective tissue, and on artificial media.

Yeasts

Yeasts, mostly asporogenous ones, are psychrophilic. Under aerobic conditions, yeasts might grow on the surface of meats to cause sliminess, lipolysis, off odors and tastes; and white, cream, pink, or brown discolorations, due to pigments in the yeasts. (20)

According to Lund and Halvorson (39) in 1951, rapid freezing caused greater injury to yeasts than slow freezing. They contended that the degree of injury during freezing and thawing depended upon the composition of the suspending medium. Colloids and sugars contributed some protective action, while acids, both mineral and organic, increased the injury caused by freezing and thawing.

The yeasts commonly found at low temperatures were Saccharomyces cerevisiae (32); Rodotorula, Geotrichoides and Candida. (15)

Table 3. The effect of acidity in increasing the mortality of Saccharomyces cerevisiae cells held frozen in sucrose solutions. (32)

Solution % sucrose	Weeks for 99% kill when stored at			
	-10°C pH 5*	-10°C pH 3.7	-20°C pH 5*	-20°C pH 3.7
0	8	15	>28	--
1	>>28	2	>>28	15
5	>28	2	>>28	6
10	>28	3	>>28	6
15	28	6	>28	15
20	20	15	>28	20
30	>>28	8	>>28	--
50	>>28	17	>>28	--

*Results not significantly different at pH 6.5.

PSYCHROPHILES CAUSING DETERIORATION IN MEAT AT LOW TEMPERATURES

Psychrophilic deterioration in meat at low temperatures usually occurs due to handling practices at the time of slaughter and subsequent processing before it enters the cooler. Frazier (20) says that the physiological condition of the animal just prior to slaughter also had an influence on the subsequent growth. Factors like fever, fatigue, excitement, and incomplete bleeding encouraged the spread of microorganisms. The rate of cooling of the carcass was another factor since rapid cooling reduced the rate of invasion of the tissues by microorganisms. He (20) listed the following to be the factors that influenced

Table 4. Growth of microorganisms on inoculated frozen meats. (33)

Op Temp.	Time in days				Flora
	: Bacterial growth	: Mold growth	: Yeast growth	: Actino- mycetes growth	
31	7	14	6	5	14 Thamnidium, Aspergillus, musty Pseudomonas, Achromobacter, Micrococcus.
30	7	14	6	6	24 Same as above & Rhizopus, 20% Pseudomonas, 10% Flavobacterium, 70% Achromobacter.
27 to 29	13	30	25	25	50 Same as above; 50% Achromobacter (not musty); 50% Pseudomonas (not musty).
26 to 28	25	25	25	--	43 Rhizopus, Thamnidium, Aspergillus, Torula, Actinomyces sp., 100% Achromobacter.
22 to 24	135	65	60	none	155 Rhizopus, Penicillium, Aspergillus, Sporotrichum, Cladosporium, Thamnidium, white yeasts, Achromobacter.
19 to 21	167	65	105	none	135 Same as above.
18 to 20	none	144	none	none	190 Unidentified white molds.
15 to 17	none	144	none	none	170 Pinpoint white molds.
12 to 14	none	144	none	none	260 Unidentified white molds.
10 to 12	none	none	none	none	OK 500
6 to 8	none	none	none	none	OK 500
0	none	none	none	none	OK 500
-5	none	none	none	none	OK 500

the growth of microorganisms and hence the kind of spoilage:

1) the kind and amount of contamination with microorganisms and the spread of these organisms in the meat;

2) the physical properties of the meat--the grinding of meat greatly increased the surface and encouraged microbial growth because of the exposed area and also because it released moisture and distributed bacteria throughout the meat;

3) the chemical properties of the meat--moisture content (meat could be so dry as to permit no growth, a little moist to allow mold growth, more moist to encourage yeasts, and very moist to favor bacterial growth); presence or absence of fermentable carbohydrate and the high protein content; pH of raw meat (a higher pH favored microbial growth);

4) availability of oxygen--true putrefaction was favored by anaerobic conditions;

5) temperature--at chilling temperatures, psychrophiles were favored and proteolysis was likely, but true putrefaction was rare.

Bacteria

Moran (44) in 1935 commonly found bacteria on meat stored at about 5°C or below to belong to two groups, namely, Achromobacter and Pseudomonas. These organisms were found to have an optimum temperature for growth at about 20°C and would grow at temperatures as low as about -3°C.

He (44) found that in case of meat, storage could be carried out at a temperature of -7°C to -10°C, because at these temperatures attack by bacteria was impossible or extremely slow. It was found to be difficult to determine precisely the exact temperature at which growth ceased, but for all practical purposes it was approximately -3°C for bacteria. The result was

that many frozen meats were edible and wholesome after storage for months or even years.

Haines (24) in 1931 described meat as chilled if it was stored in the range of temperature 0°C to about -2.5°C ; frozen meat was stored at lower temperatures, the maximum being about -5°C .

He (24) found that storage at -5°C led to a steady fall in the number of microorganisms, followed by a period when the numbers were in a minimum. Haines (24) observed that a temperature of -5°C was not sufficiently low to completely inhibit all microbial growth. It was probably safe to say that no growth would take place at -10°C .

He suggested that if the conditions during slaughter and dressing were satisfactory and the humidity during storage was subcritical, meats such as beef, mutton and pork could be stored for as long as 60 days at 0°C without appreciable deterioration. On the other hand, if these conditions were not realized, spoilage could occur due to the action of bacteria, the common organism found being of the genus Pseudomonas.

Haines (23) in 1933 found that with the exception of certain number of organisms of the Pseudomonas group, and a few Proteus, almost all of the bacteria growing on lean meat stored in the range of 4°C to 0°C belonged to the Achromobacter group. These were small gram-negative rods of which some strains were motile, most strains liquefied gelatin and almost all strains produced acid in dextrose.

Table 5. Rate of growth of Achromobacter on lean meat at 0°C.
Organism per sq. cm. of surface. (23)

Time in days :	Sample number				
days :	1	2	3	4	5
0	4×10^4	1.73×10^4	2.7×10^3	$2.7 \times 10^{2*}$	93
4	8.75×10^5	3.5×10^4	5.6×10^3	3×10^3	2×10^2
8	5.6×10^7	6.5×10^6	9.8×10^5	2.6×10^4	5.9×10^4
11	1.65×10^9	7×10^7	---	---	5.95×10^5
15	2×10^{10}	2×10^9	1.13×10^{10}	1.21×10^7	4.5×10^7
Time of appearance of slime	8 days	10 days	11 days	16 days	18 days

*Stored at relative humidity 70%.

Slime apparently became noticeable in every case when the bacterial count was of the order of approximately $10^{7.5}$ organisms per sq. cm. Meat being put into storage with an initial load of 40,000 organisms per sq. cm. was practically inedible after eight days' storage, while meat giving an initial count of 43 could be kept for eighteen days.

Eddy and Mitchell (13) in 1959 isolated twenty-eight strains of coli-aerogenes bacteria, one Aeromonas which grew well at +1.5°C and some which grew well at -1.5°C. The optimum growth temperature for most of these strains was found to be nearer 37°C than 30°C. Nine strains (including the Aeromonas) fermented lactose; the remainder fermented lactose slowly or not at all.

The authors suggested that the fact that the optimum temperature of most of the strains was 37°C indicated that these

organisms were not typical psychrophiles but could more accurately be described as cold-tolerant mesophiles.

Frazier (20) in 1958 found that when meat was held at temperatures near 0°C (32°F), bacteria were limited to psychrophiles. These included many of the types that produced sliminess, discoloration, and spots of growth on the surface, and many that could cause souring such as Pseudomonas, Lactobacillus, Leuconostoc, Streptococcus and Flavobacterium species.

Changes in fats occurred as the oxidation of unsaturated fats in meats took place chemically in air and could be catalyzed by light and copper. Lipolytic bacteria could cause some lipolysis and also accelerate the oxidation of fats. Most animal fats developed "oxidative rancidity" when oxidized, with off-odors due to aldehydes and acids. Rancidity of fats might be caused by lipolytic species of Pseudomonas and Achromobacter. (20)

Jensen (33) observed that proteases of animal or microbial origins acted to produce rancidity in fatty tissues by destruction of the natural antioxidants of the fat-bearing tissues. He also observed that tissue fat was more vulnerable to microbial attack than oils and fats.

Various surface colors were observed due to pigmented bacteria. The "red spot" could be caused by Serratia marcescens. Pseudomonas syncyanea could impart a blue color on the surface. Yellow discolorations were caused by bacteria with yellow pigments, usually species of Micrococcus or Flavobacterium. Chromobacterium lividum gave greenish-blue to brownish-black spots on

stored beef. (20)

Under anaerobic conditions, Frazier (20) found souring to be the result from (a) action of meat's own enzymes during aging or ripening; (b) anaerobic production of fatty acids or lactic acid by the bacterial action; or (c) proteolysis without putrefaction, caused by facultative or anaerobic bacteria and sometimes called "stinking sour fermentation."

Putrefaction could be caused or be assisted in its production chiefly by bacteria of the genera Pseudomonas and Achromobacter. Also, some species of Proteus were putrefactive. (20)

In forty samples of putrefying meat, Weinzirl and Fellow (75) in 1924 found the following species: Sarcina lutea, Proteus vulgaris, Bacillus mesentericus, Bacillus subtilis, Bacillus vulgatus, E. coli, Pseudomonas fluorescens and Micrococcus liquefaciens.

They (75) suggested that the bacterial count was only a partial indicator of spoilage. It indicated the sanitary conditions, especially temperature under which the meat was kept, and since these conditions determined whether or not putrefaction took place, this was thus indirectly regarded as an indicator of putrefaction.

Finally they (75) concluded by saying that aerobic and facultative bacteria assisted in the spoilage of meat, but the putrefactive odor was mainly due to anaerobes.

Bacteria in Relation to Meat and Poultry Pies

Straka and Stokes (63) in 1956 stated some factors which caused microbial contamination in chicken pies. The contamination could occur during the production of chicken pies. Contamination of the chicken carcasses could occur after they were cooked and then allowed to cool for a short period; secondly, contamination of the meat could occur because of being picked from the bones by hand. In some plants twenty or more pairs of hands may work over the carcass as it moved along the operating line. Thirdly, the picked meat could be diced in special machines, some of which might be difficult to clean and sanitize. In some plants the diced chicken might be stored for considerable periods of time, without refrigeration. Eventually the diced chicken might be picked by hand by one worker, weighed and then transferred by hand by another worker to the unbaked pie.

Litsky, Fagerson and Fellers (37) in 1957 on microbiologically testing beef and poultry pies, found that over 70% of the pies had total counts under 25,000 per gram and 84% of the total had total counts lower than 100,000 per gram. They (37) said that by virtue of the composition and pH, precooked frozen meat pies (beef, chicken) offered fertile media for growth of many types of microorganisms. In addition, there were many possibilities of contamination during the manufacture of these products. Because of initial contamination the fact that the product had been frozen and was then heated prior to serving, was no assurance that it was safe.

Kereluk and Gunderson (35) in 1959 examined 188 frozen meat pies (frozen chicken meat pies, frozen turkey meat pies, frozen beef pies and frozen tuna meat pies) commercially produced and procured at the retail level. They found 14 of the total number of 188 frozen meat pies to have bacterial counts over 100,000 per gram; or, 93% had total counts under 100,000 per gram. About 83% of the frozen meat pies had total counts under 50,000 per gram and 75% had total counts under 25,000 per gram. They (35,37) thought that a bacteriological standard of 100,000 per gram was reasonable for frozen meat pies.

Kereluk, Peterson and Gunderson (34) in 1961 isolated two strains of Pseudomonas fluorescens from chicken pies. Strain I was isolated from a defrosted chicken meat pie, whereas Strain II, from routine bacteriological examination of a normal chicken meat pie. They observed that Pseudomonas Strain I had marked "psychrophilic characteristics" because no growth took place at 37°C and the organism reached the maximum stationary stage at 0°C and 5°C at the end of 5 days whereas it was shorter at 10°C and 20°C. Quoting literature they said that the typical psychrophile was a food spoilage organism, but not a food poisoning one.

Ercole and Ordal (16) in 1957 observed that poultry pies had a higher degree of bacterial contamination than did other types of precooked frozen foods. They concluded that the greatest public health hazard would come from pies that were baked and not consumed immediately and which were improperly refrigerated between baking and consumption.

Fanelli and Ayres (19) in 1959 found that total numbers of organisms in chicken pot pies were reduced by blast freezing. The numbers of organisms growing anaerobically were not reduced by freezing.

Mucker and David (29) in 1957 observed that out of 113 strains isolated from the frozen chicken pies, 38 were Micrococci, 21 were spore-bearing rods, 30 gram-negative rods and 24 were the large biscuit-shaped types. They could not identify these biscuit-shaped strains, though it was commonly encountered in many frozen products. They thought that these might be variants of known species of Micrococci or they might even constitute a new genus. Little difference in total count was noted as between the light and dark meats, but the broth in many instances showed a considerable lower count. They observed that alternate freezing and thawing did not increase the total flora unless the conditions (temperature and time) of the thawed phase initiated growth. No increase in count occurred when thawing chicken pies were held at 2°C (35.6°F), 7°C (44.6°F) and 18°C (64.8°F) for 48 hours.

Frozen chicken pies were stored at 41°F for periods up to 14 days. (56) Fig. 1 presents data on the numbers of bacteria per gram of chicken pie gravy as a function of time of incubation. The first definite off-flavors and aromas started appearing on about the third day of storage at 41°F. The number of bacteria was of the order of 10,000 per gram. By the fifth to sixth days the pies were very definitely organoleptically

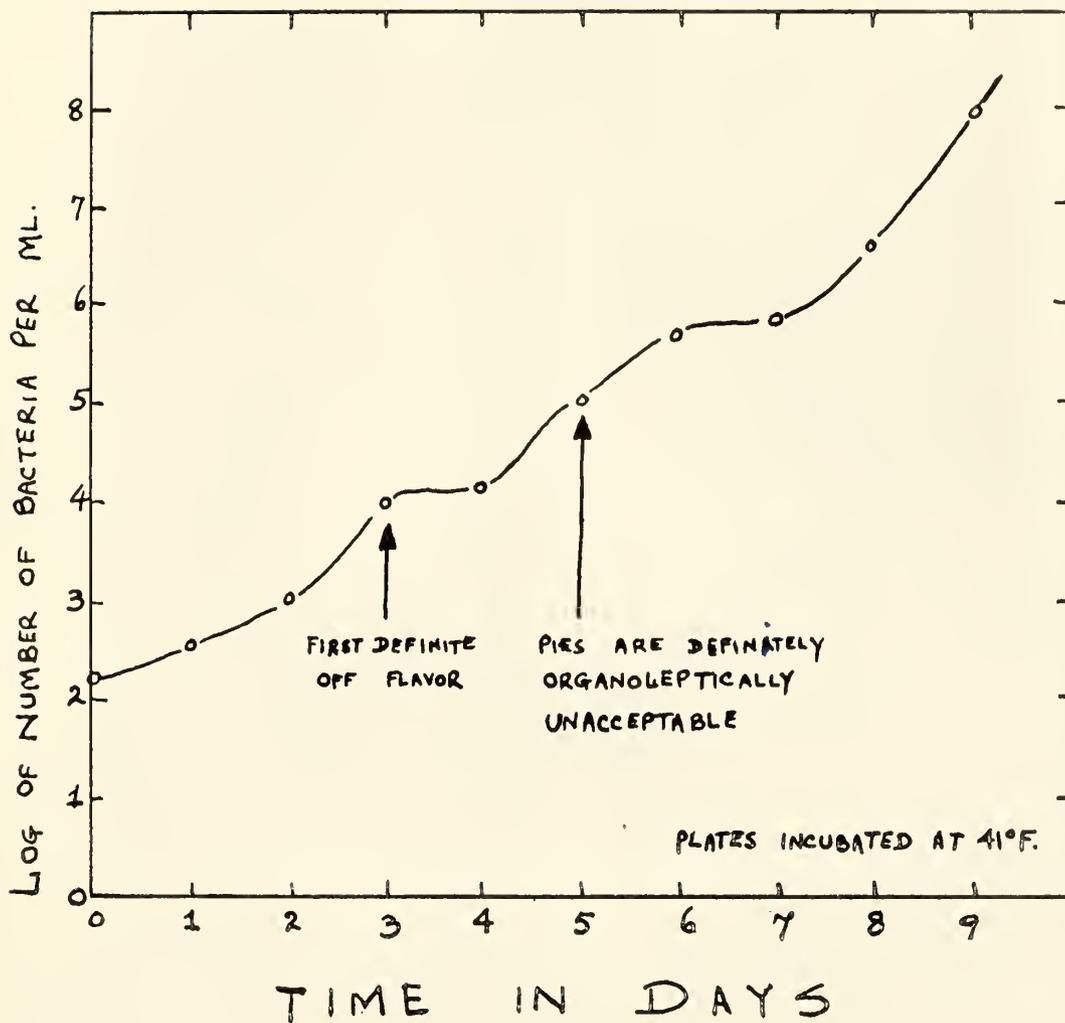


FIGURE 1. (56). BACTERIA COUNTS ON CHICKEN PIES INCUBATED AT 41° F. (5° C).

spoiled. The bacterial population ranged from 100,000 to 1,000,000 per gram. Thus, at the time spoilage became incipient no great number of bacteria were present. Even at spoilage the total number of bacteria per gram of chicken pies was still comparatively low and did not reach the vast numbers ordinarily associated with spoilage and which the extreme organoleptic change would suggest. (56)

The authors (56) observed the proteolytic and saccharolytic activities of both extra and endocellular enzymes from Pseudomonas fluorescens isolated from the chicken pie. Previously noticed defrost effects were observed to be duplicated by the addition of these enzymes to normal chicken pies. It was demonstrated that once the enzymes of psychrophilic bacteria were elaborated and liberated in the growth menstrum, lowering their temperature would slow their action, but would not stop it and definitely would not remedy damage already done.

Bacteria and Ground Meat

Rogers and McCleskey (57) observed that ground or comminuted meats provided a highly favorable environment for the multiplication of bacteria. Fragmentation of tissues with the liberation of cell juices and the intimate mixing of the bacteria normally found on the moist surface with the macerated tissues resulted in a product subject to rapid modification by bacteria.

The bacterial population in ground meat was dependent on three main factors: bacteriological quality of the meats used

for grinding, cleanliness of equipment, and time and temperature of storage.

Table 6. Changes in bacterial flora of ground beef stored at 7°C. (57)

Organism	:Isolated at 37°C*		: Isolated at 7°C*	
	:0 days	14 days	: 0 days	14 days
<i>Pseudomonas</i> and/or <i>Achromobacter</i>	11	64	4	84
<i>Bacillus</i>	8	8	28	6
<i>Flavobacterium</i>	0	6	0	0
<i>Microbacterium</i>	0	7	0	5
<i>Micrococcus</i>	50	14	26	5
<i>Sarcina</i>	6	1	0	0
Yeasts	25	0	42	0
Total	100	100	100	100

*Expressed as percent of total flora.

Micrococcus, Bacillus, and yeasts were the most prevalent organisms at the beginning of the storage period; members of the genus Pseudomonas and/or Achromobacter were most abundant after 14 days storage at 7°C. A mixture of gram-negative, non-spore-forming rods, and various species of the family Micrococcae were predominant in meat when purchased. Organisms that grew most rapidly during storage at 0°C and +2°C were members of the genus Pseudomonas.

Geer, Murray and Smith (22) in 1933 found the following observations on counts of ten samples of fresh unfrozen hamburger steak as bought in the markets and ten samples of Birdseye

Frosted Hamburg Steak, bought from retail stores:

Table 7. Ten samples of unfrozen hamburger steak.

Market	Bacteria per gram
1.	24,300,000*
2.	43,700,000
3.	6,000,000
4.	6,700,000
5.	18,300,000
6.	16,200,000
7.	8,500,000*
8.	10,100,000*
9.	27,100,000
10.	20,100,000

Average of ten samples---
18,100,000.

Table 8. Ten samples of frosted hamburger steak.

Dealer	Bacteria per gram
1.	1,000,000
2.	2,600,000
3.	2,400,000
4.	1,500,000
5.	2,400,000
6.	3,200,000
7.	2,100,000
8.	2,300,000
9.	1,900,000
10.	700,000

Average of ten samples---
2,000,000.

*Freshly ground at
time of purchase.

Kirsch et al. (36) in 1952 observed that the most evident sign of spoilage of the hamburger was an undesirable odor. This could be described as a stale, sour smell such as that usually associated with meat no longer fresh. This sour odor was detectable between the eighth and twelfth day after storage. Fading of the color at the surface of the meat where it was exposed to air usually was noticeable at four to six days. The bacteria usually found were members of the genus Pseudomonas and/or Achromobacter. Lactobacillus species as well as Proteus species

were also observed. Surprisingly the authors (36) observed the absence of Flavobacterium species, since these organisms could grow at temperatures near 0°C and were reported in many studies of refrigerated food, for example poultry and beef. The frequent occurrence of Lactobacilli in the hamburger sample was unexpected.

Sulzbacher (64) in 1952 observed that there was no scientific basis for the commonly held belief that frozen meat became more perishable after thawing than fresh meat.

Sulzbacher and McLean (66) in 1951 found 74% of the isolates from fresh pork sausage to belong to the genera Pseudomonas, Microbacterium, Alcaligenes, Achromobacter, Bacterium and Bacillus. Seventy percent of the Pseudomonas cultures studied were found as lipase-forming organisms in new sausage.

Table 9. Habitat distribution of bacteria isolated. (66)

Genus	:No. of :isolates:	: Percent : of total:	:New :Sausage:	: Old : Sausage:	: :Spices:	:Equip- :ment
<u>Pseudomonas</u>	34	10.8	+++	-	-	+
<u>Microbacterium</u>	47	14.9	++	++++	-	-
<u>Alcaligenes</u>	19	6.0	+	++	-	-
<u>Achromobacter</u>	40	12.9	++	++	-	+
<u>Bacterium</u>	65	20.6	+++	++	-	+
<u>Bacillus</u>	28	8.9	+	+	++	+
Total	233	74.1				

Table 9 shows the relative prevalence in either freshly prepared sausage, older samples stored in the refrigerator, spices or plant equipment. They (66) observed that species of Microbacterium made up a rather large proportion of the flora of fresh pork sausage which had been stored at home refrigerator temperatures (5-8°C), and this could have been the cause for the acid flavor of these samples.

McLean and Sulzbacher (42) in 1953 observed a new bacterium from fresh pork sausage which was non-heat resistant and they named it Microbacterium thermosphactum, Spec. Nov. This bacterium was a member of the genus Microbacterium but differed from it in its source and heat resistance.

Butler (10) in 1950 treated eight equal portions of meat with salt, pepper and sage as follows: A--no seasoning; B--sage only; C--pepper only; D--sage and pepper; E--salt only; F--salt and sage; G--salt and pepper; H--salt, pepper and sage. After seasoning, the meat was ground in a meat grinder. The grinding was done twice. Quick freezing was accomplished by eighteen hours storage in the sharp freezer at -20°F. The following observations were noted:

1. Commercial salt increased the oxidation of frozen ground pork;
2. Salt in the presence of pepper was not as active in promoting rancidity as salt alone, but the inhibitory action of pepper on salt was limited;
3. Completely seasoned sausage containing 2% salt, 0.375%

sage, 0.25% black pepper remained stable longer than unseasoned ground pork;

4. Salt in the presence of sage had some pro-oxidant activity, but much less than when acting alone or in combination with pepper;
5. Black pepper had rather weak anti-oxidant properties;
6. Sage had decided anti-oxidant properties;
7. No synergistic effect was noted as a result of the combination of sage and pepper;
8. Light produced by an incandescent bulb of 100 watts seemed to have a pro-oxidant effect.

Hall, Harrison and Mackintosh (28) in 1962 observed that flavor deteriorated rapidly in frozen stored sausage with only sodium chloride added. Sausage containing sage with sodium chloride maintained normal flavor comparable with that of unseasoned sausage.

Miller (43) in 1955 observed on inoculating into fresh unseasoned ground pork (50% fat) a food poisoning strain of Micrococcus pyogenes var. aureus at the rate of approximately 325,000 cells per gram, a decrease to less than 150,000 per gram at 0°F to -8°F, and thereafter, a further gradual decrease to about 50,000 per gram in ten months. At eighteen months approximately 40,000 food poisoning micrococci per gram still remained in the frozen meat. He found the natural flora of the ground meat to comprise essentially of psychrophilic, gram-negative rod types (carcass stored ten days at 34°F before cutting). These

non-pathogenic bacteria, which caused deterioration of meat at low temperatures, declined from 1.4 million to less than 0.6 million per gram in one week at 0°F to -8°F. After one week the decrease in numbers of psychrophiles was gradual.

The author (43) concluded from the above statement that food poisoning micrococci died at a slower rate than psychrophilic, gram-negative rod bacteria in frozen, unseasoned, ground pork.

Sausages prepared from cured comminuted meats were subject to several types of spoilage, the majority of which were manifested by a visual change in color of the product. Most, but not all of these discoloration problems were known to be of microbial origin. These surface discolorations were first manifested by the small greenish spots on the damp surface indicative of spoilage which tended to spread under favorable conditions. Accompanying this discoloration was a slight greasy or slimy appearance of the product. This type of discoloration on cured sausage usually resulted from surface contamination after the sausage had been smoked or cooked. All sausage-greening cultures were salt-tolerant and capable of growing at low temperatures. This sausage discoloration resulted from the production of hydrogen peroxide by the microorganisms.

The bacteria usually associated in this case were either heterofermentative Lactobacilli or members of the genus Leuconostoc (52, 53).

Heterofermentative Lactobacilli isolated from various types of greening of cured meat products had widely different degrees

of heat resistance even though they were identical in their physiological, serological and nutritional characteristics. (54)

Niven (51) classified cases of bacterial discolorations of sausages into three categories, namely:

1. Surface discoloration, resulting from contamination of the sausage surface after heat processing, and followed by holding conditions that allowed extensive growth of these contaminating bacteria. This surface discoloration may or may not be accompanied by a certain degree of sliming or "greasy" appearance.

The bacteria usually associated with this type of spoilage were Lactobacillus and Leuconostoc. These greening Lactobacillus and Leuconostoc cultures had certain distinctive characteristics--(a) they grew quite well at low temperatures; (b) they were relatively tolerant to salt; (c) they appeared to produce hydrogen peroxide when grown in the presence of air, and (d) these were usually of the gas-producing variety.

2. Green cores appeared on the cut surface a few hours after the sausage was sliced, caused by faulty heat processing, and poor refrigeration of the finished product. The bacteria usually found in cases of "green cores" were species of Leuconostoc and Lactobacillus.
3. Green rings, resulting from an unusually high bacterial count of the sausage mix before heat processing. It seemed hard to suggest what types of bacteria were

associated with green rings, since the microorganisms in such sausages as examined in the laboratory appeared to be dead.

Allen and Foster (1) in 1960 found that vacuum packed sliced cold meats during storage at refrigerator temperatures developed spoilage as exemplified by slime formation and development of atypical flavors. They also found lactic acid bacteria in great numbers to be associated with spoilage.

Yeasts and Molds

Yeasts and molds of importance in meat spoilage were found to be psychrophilic in nature. They were obligately aerobic and tolerant to both acid and dry environments. Many of them could utilize nitrite and nitrate as a source of nitrogen. Yeasts and molds usually predominated on meat products that were salted, dried or fermented. Some species could grow below 0°C., but on fresh meats at low temperatures, their growth rate was somewhat slower than that of the Pseudomonads. (68)

Under aerobic conditions, yeasts could grow on the surface of meats to cause sliminess, lipolysis, off-odors and off-flavors, and white, cream, pink or brown discolorations, due to pigments in the yeasts. (20)

The aerobic growth of molds could cause: (9, 20, 33, 78)

1. Stickiness;
2. Whiskers--a white fuzzy growth appeared on meat held at temperatures near freezing. This could be caused by

- such molds as Thamnidium, Mucor, Rhizopus and others;
3. Black spot--usually caused by Cladosporium herbarum, but other molds with dark pigments could be responsible e. g., Mucor mucedo, Penicillium glaucum, Rhizopus. (Brown spots on beef could be caused by a Saccharomyces);
 4. White spot--usually caused by Sporotrichum carnis, and Geotrichum species;
 5. Green patches--usually due to the green spores of species of Penicillium such as P. expansum, P. asperulum, and P. oxalicum;
 6. Decomposition of fats--due to lipases and thus bringing the hydrolysis of fats. Sometimes molds aided the oxidation of fats;
 7. Off-odors and off-flavors.

Jensen (33) stated that mold growth was, among other factors, dependent on moisture content. Thus air circulation was necessary to maintain an adequately low relative humidity so that no molding and sliming resulted.

Macara (40) observed the minimum relative humidity at which growth occurred to be slightly below 75% corresponding to a water content of 10% in dried meat containing 40% fat. The molds which developed in the dried meat were species of Penicillium and Aspergillus.

Evans and Niven (18) described the steps whereby slime development was detected. The term "greasy" was often used to refer to the incipient stages of slime development. As this

advanced, the term "slippery" was sometimes employed, which adequately described the physical nature of the product surface. By drawing a knife blade across the surface of the product, a soft creamy material was obtained on the blade which consisted almost entirely of cells of the microorganisms which grew extensively to produce the spoilage condition. This simple test was an easy diagnosis for the early stages of sliming.

Jensen (33) encountered black yeasts on meats on rare occasions. These, asporogenous yeasts or Torula were not vigorous fermenters.

Yeasts and Molds in Relation to Ground Meat

Yeasts among other bacteria were found to be the most prevalent organisms at the beginning of the storage period in ground or comminuted meat. (57) Weiners often were found to be covered with a pasty, yeast-like slime resulting from the growth of microorganisms. The yeast isolated from fresh sausage were observed to be cultures of Debaryomyces closely resembling D. guilliermondie. D. membranefaciens was also observed. (49)

Jensen (33) quoted Kuhl's finding of a white yeast as a cause of slimes on sausage. He observed that practically every saprophytic mesophile and psychrophile could grow on the moisture film of the casing and thus form a slime. He stated that the physical conditions, not the kinds of microbes, were important in this problem of preventing growths on sausage.

Frazier (20) observed molds of the genus Penicillium and Mucor to grow on hamburger meat.

Table 10 (concl.)

Genera	: Talayrach: Masse: Manvoisin: Bidault: Brooks	: Brooks & Moran: Empey & Ayres
represented	: 1901 : 1912: 1918 : 1921 : & Kidd : Mansford: et al: Scott	: 1954
:	:	: 1921 : 1923 : 1932: 1939 a, b:

YLAISTS

Rhodotorula (+)^c

+

Mardomyces

+

Saccharomyces

+

^aMost mycologists use the name Torula to refer to certain of the Dematiaceae (Skinner et al. 1947) and, in particular, the genus Cladosporium. Brooks and Hansford (1923) did not consider the organism that they had isolated to be a species of Cladosporium.

^bAlso includes Dematiacium and Hormodendrum.

^cDescribed a pink yeast.

PSYCHROPHILES AND BEEF

General

Scott (58) in 1936 discussed the influence of water content of substrate on the growth of microorganisms on ox muscle at -1°C . They determined this on slices of ox muscle of which the water contents were in equilibrium with the aqueous vapor tension of their storage temperatures. As shown in Table 11, the critical water content of muscles, expressed as percentages of the dry weight were found to be between 85 and 90 for Achromobacter, between 140 and 180 for Pseudomonas and between 45 and 55 for asporogenous yeasts belonging to the genera Candida, Geotrichoides and Mycotorula. The biceps femoris muscle of the ox was used throughout, since it was generally free from bands of connective tissue and fat. Achromobacter seemed to be one of

Table 11. (58)

Organism	:Critical range :percentage r. h.	: Critical range water, : percentage dry weight
Achromobacter No. 7 & 483	96 to 96.5	85 to 90
Pseudomonas No. 1 & 451	98 to 98.5	140 to 180
Candida sp.	91 to 92	50 to 54
Geotrichoides sp.	90 to 91	47 to 50
Mycotorula sp.	89 to 90	45 to 47

the most important organisms causing spoilage in beef. Considerable restriction in the rate of growth of Achromobacter occurred when the water content of the surface tissues were maintained below 90% during storage in the meat works.

Moran (45) in 1930 observed that at -3°C (26.6°F), 70% of the muscle was present as ice; at -5°C (23°F), 82%; and at -10°C (14°F), 94% separated out as ice. Hence, drying or desiccation of the substrate accounted largely for failure of microorganisms to grow on frozen substrates. With decreasing temperatures more ice separated out, and the cell water remaining behind became more concentrated.

Jensen (33) in 1954 observed that slow freezing promoted larger ice crystals in the tissues with resultant fiber rupture and excessive drip during thawing. Rapid or sharp freezing promoted more desirable features, such as smaller ice crystals with less fiber rupture and much less drip upon thawing. He concluded by saying that "obviously the more drip, the better pabulum for microorganisms." But Jensen again goes on to say that sharp freezing or slow freezing of meat did not destroy the bacteria commonly encountered on beef carcasses. When frozen meats were held at -4.4°C (24°F), there was greater destruction of vegetative bacterial cells than at lower temperatures, -30°C (-22°F). This was especially noticeable after three weeks' storage.

Scott (59) in 1937 observed the rates of growth of certain microorganisms at different temperatures so as to predict the relative influence of spoilage exerted by the various organisms

at each storage temperature.

Further, it would be feasible to predict the possible extent of the changes in population which various organisms might undergo during the initial cooling of sides of beef in the meat works when the meat surfaces were frequently at temperatures very favorable to microbial proliferation. (59)

Table 12 (59) shows typical maximum population for three of the organisms studied.

The initial contamination of beef occurring in the packing plant is of fundamental importance because it is at this stage that the microorganisms have the chance of growth and multiplication. Whatever subsequent measures are taken, its microbiological quality is open to question if the initial methods of slaughtering, handling and processing have been faulty. Other factors such as excitement, over exercise or high pH should be taken into account, since they promote greater activity of the microorganisms and thus greater chances of spoilage exist. Temperature is another factor which should always be kept in mind to understand the types of organisms growing on beef carcasses.

Empey and Scott (15) in 1939 observed that the sources of microbial contamination acquired in the packing plant were:

1. Soil;
2. Hide and hair;
3. Aqueous sources such as samples of water used in the dressing of beef and the cleansing of slaughter floors;
4. Air-borne;
5. Utensils and equipment;
6. Contents of G. I. tract.

Table 12. Maximum populations on muscle at various temperatures.

Organism	Temp. °C						
	-1	2	4	10	15	20	25
Achromobacter No. 7	1.1×10^9	1.8×10^9	1.4×10^9	3.4×10^9	2.6×10^9	1.1×10^9	1.5×10^9
Pseudomonas No. 451	6.0×10^8	--	8.8×10^8	2.1×10^9	--	2.0×10^9	2.4×10^9
Geotrichoides Y 9	3.2×10^6	--	8.1×10^6	10×10^6	10×10^6	7.6×10^6	--

Summarizing their data they (15) observed:

Spoilage of chilled beef held at temperatures close to -1°C was due to the proliferation of the beef surface of various bacteria, yeasts and molds. The initial contamination acquired by beef surfaces during dressing included more than 99 percent bacteria amongst the organisms viable at ordinary temperatures (20°C). These populations usually contained less than 1 percent of organisms viable at -1°C . The percentage of yeasts and molds in -1°C populations were greater than the respective percentages in the 20°C populations. Of the organisms viable at -1°C , four principal bacterial genera were represented--Achromobacter, 90 percent; Micrococcus, 7 percent; Flavobacterium, 3 percent; and Pseudomonas, less than 1 percent. Yeasts were represented by the following genera: Mycotorula, Candida, Geotrichoides, Blastodendron, and Rhodotorula. Of the mold genera, the most common was Penicillium, followed in order by Mucor, Cladosporium, Alternaria, Sporotrichum, and Thamnidium. The chief source of the superficial beef microflora was found to be the hide and hair of the slaughtered animals. The hide microflora is, to an extent, dependent on the microflora of soils from the pastures on which the cattle graze.

The microflora of beef surfaces is similar to the microflora of hides, with the exception that the incidence of yeasts on beef is significantly greater. This difference, so far, remains unexplained. All microbial populations from beef and other sources show a variability such that the logarithms of the populations are distributed in an approximately normal manner. Statistical treatment of these logarithmic values shows that the percentage incidence of organisms viable at -1°C decreases during summer, and it also shows a decrease with decreasing geographical latitude. Both the seasonal and geographical variations are shown to be due to a negative regression of low temperature type organisms against temperature. The results are similar for beef, hide, and soil.

Vickery (71) in 1936 discussed the lipolytic activity of several typical strains of the bacterial genera Achromobacter and Pseudomonas, and also of asporogenous yeasts, all of which were capable of comparatively vigorous growth on beef adipose tissue stored at a temperature of -1°C . All strains of yeasts and Pseudomonas tested were found to be responsible for appreciable

lipolysis, but apparently only one strain of Achromobacter had this power and that only to a slight degree. The author observed that the maximum population in the fat-emulsion medium were reached in 7 to 10 days at 20°C, 18 to 26 days at 4°C and 30 to 45 days at -1°C; the duration of storage at the respective temperatures were, therefore, always adjusted to be in excess of these times.

Table 13. (72)

Organism	:No. of strains : :tested	: No. of strains causing : appreciable lipolysis
Asporogenous yeasts	4	4
Achromobacter	9	4
Pseudomonas	8	4

Bacteria carried out two types of enzymatic attack on fats; namely, hydrolysis by a lipase, and oxidation of the fatty acids by oxidases. Some of the products of these reactions gave rise to the odors and flavors that were usually recognized as rancidity. Microorganisms elaborating only lipase did not complicate the problem of rancidity as did the dual-acting microbes. The lipolytic organisms usually found to give trouble on meat products were of the genus Pseudomonas and Achromobacter and other gram-negative organisms. These same organisms were also quite active in the oxidative degradation of the fatty acids. (20, 33, 68)

Ayres (3) in 1955 quoted Lea (1931) and Haines (1933 b) as

saying that certain of the unpleasant tastes and odors in fat of stored beef were caused by microorganisms growing either in fatty tissue or in adjacent muscles. Lea found that the fat of beef carcasses stored in still air at 0°C (32°F) was good after 25 days but somewhat tainted at 42 days. Later Lea (1938) stated that tainted fat might contain several million bacteria per gram.

Jensen (33) encountered cultures of oxidizing bacteria which imparted tallowy flavors to meat mixtures containing animal fats.

By the help of certain tests, he (33) showed that microorganisms could induce oxidative rancidity; hydrolysis with high free fatty acid formation (lipase formers); tallowiness (oxidizers) in beef fat, mutton fat, and beef lard; and, in addition, wherever oxidizing types of microbes exerted their influence, flavor changes took place. Some changes were "flavor reversions" such as one finds in deodorized oils and fats, and some were "flavor adjuvants" in which condition the flavor was reinforced and also altered in character.

Research workers at the American Meat Institute Foundation (68) have stated:

The free fatty acids liberated by hydrolytic cleavage of fats are quite inhibitory to a wide range of microorganisms. Thus, it has been observed that the total microbial population of a rancid meat product may decrease as rancidity develops. The peroxides produced during oxidation of unsaturated fatty acids also are quite toxic to many microorganisms and may give a similar effect.

Bacterial Spoilage

Ayres (3) compiled the genera of viable bacteria on refrigerated beef as indicated on Table 14. The most common bacteria viable on refrigerated beef appear to be Pseudomonas, Micrococcus, Achromobacter, Flavobacterium and Bacillus.

Haines and Smith (27) gave examples of the effect of time and temperature on the spoilage of meat. In one experiment they showed the increase in count in fresh beef at 32°F to be from about two thousand to seven billion per sq. cm. (0.15 sq. in.) in 15 days.

The amount of initial contamination was found to have a direct effect on the time in which slime appeared (6, 27). Such slime was a criterion of definite bacterial spoilage and non-salability. If the initial count was between 100 and 1000 per sq. cm., slime appeared in 15 days at 32°F; if it was between 10,000 and 100,000, slime appeared in about half the time or 8 days.

The authors (27) showed the striking influence of temperature on growth in an experiment with Achromobacter, one of the slime-producing bacteria. In an artificial medium, the time required to reach a count of 30,000,000 per sq. cm. decreased from 8 days at 32°F to 1 day at 68°F.

Ayres (6) in 1960 observed that the critical value for slime production was 6×10^7 organisms or a log value of 7.8. Referring to Fig. 2 the author (6) noted that even though the meats tested had very low counts initially, they developed

Table 14. Genera of bacteria viable on refrigerated beef.

Genera represented	: : 1931 : 1933 a, b	: : 1933 : 1933 a, b	: : 1933 : 1933 a, b	: : 1939 a, b	: : 1940 : 1940	: : 1944 : 1944	: : 1952 : 1952	: : Kirsch : et al. : 1954	: : Ayres : 1954
Pseudomonas	+	+	+	+	+	+	+	+	+
Azotobacter type	+								
Chromobacterium			+			+			
Micrococcus	+	+	+	+	+	+	+	+	+
Gaffkya				+					
Sarcina				+				+	
Diplococcus				+				+	
Streptococcus	+		+						+
Lactobacillus								+	
Achromobacter	+		+					+	+
Flavobacterium			+					+	+
Serratia								+	
Proteus	+							+	
Bacterium								+	
Bacillus								+	+

Table 15. Rate of growth of bacteria on lean meat at 32°F. (27)

Time (Days)	Number of bacteria per sq. cm. (0.15 sq. in.)
0	2,200
4	7,500
8	1,330,000
11	250,000,000
15	6,850,000,000

off-odor by the second or third day when held at 25°C, by the third day when held at 20°C, by the fifth day when stored at 10 or 15°C, by the tenth day when held at 5°C, and by the twentieth day when stored at 0°C. Growth curves of organisms growing aerobically or anaerobically were similar. In either case, a short lag in growth rate was noted at the beginning of the storage period. At the end of the sixth day of storage at 10°C, the number of organisms had reached 50 million per sq. cm. and by the seventh day the meat had pronounced off-odor. Slime appeared before the eighth day of storage. At 20°C, spoilage was evident before the fourth day. On samples stored at 5°C, slime usually appeared by the thirteenth day. Meat stored at 0°C developed off-odor and slime at twenty and twenty-two days respectively. Gas production was not noted in the package at this temperature until about eight days after the formation of slime. When gas was formed, either ammonia or hydrogen sulfide could be detected.

After one day of storage, the microbial population on meat

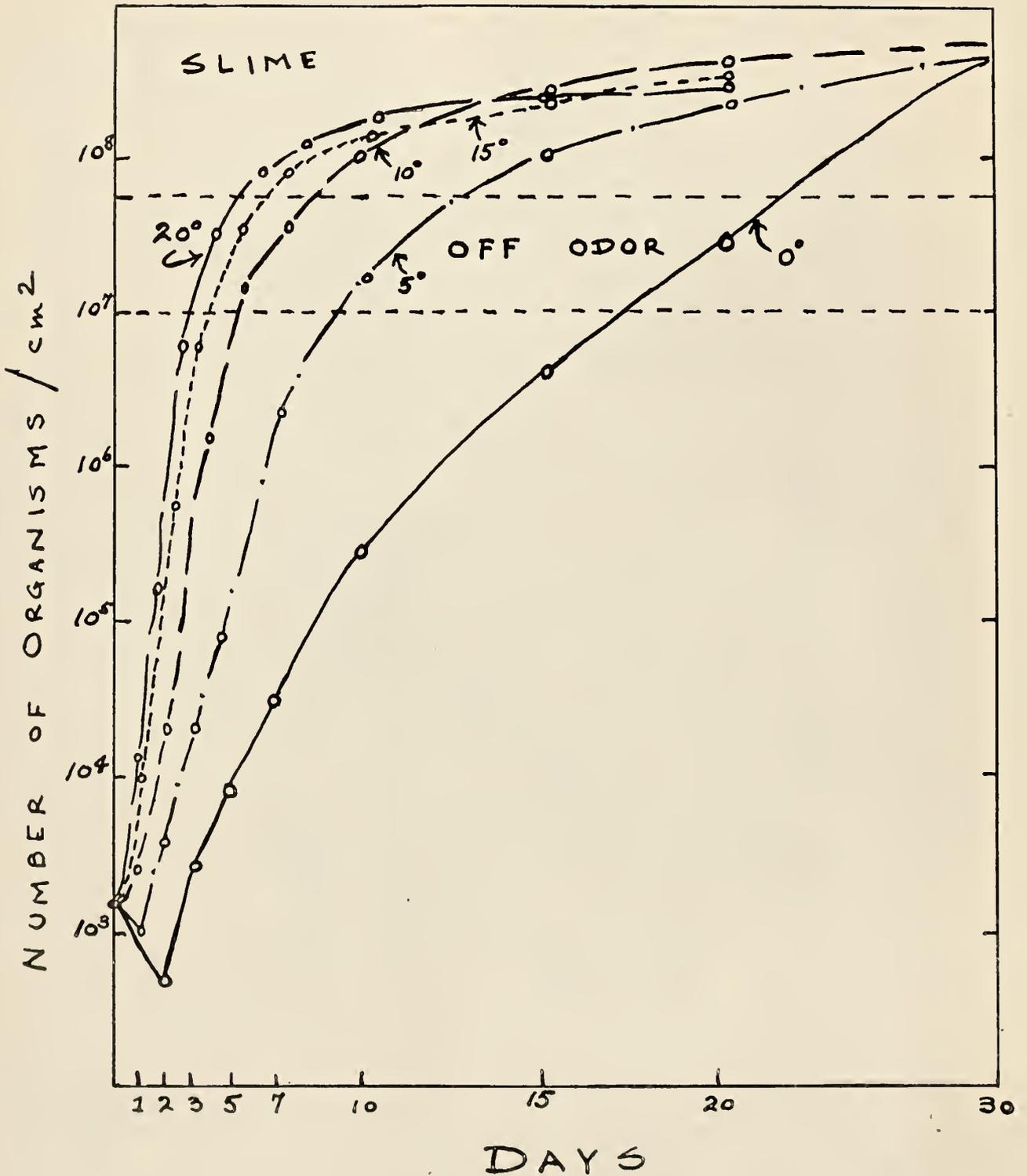


FIGURE 2. (6). RATES OF GROWTH OF ORGANISMS ON BEEF STORED AT 0°, 5°, 10°, 15°, AND 20° C.

stored at 5°C was almost invariably less than it was initially and at 0°C, the number of microorganisms progressively decreased during the first two days of storage. Apparently, the nutrients were inadequate for many of the organisms residing on the surfaces of meat or the bacteria were unable to survive at these low temperatures. (6)

Microorganisms isolated from refrigerated beef were classified and it was found that at 10°C or lower, the bacteria responsible for the production of slime were almost without exception, Pseudomonads. At 15°C or above there was approximately an equal incidence of organisms of the genus Micrococcus and Pseudomonas. Other bacteria found in sliced meat were Achromobacter, Flavobacterium, Microbacterium, Alcaligenes, Aeromonas, Bacillus, Clostridium, and Streptococcus. (6)

Haines (23) in 1933 considered that the cut flesh was subject to an increase in numbers of microorganisms even while it was stored at refrigerator temperatures. Ingram (31) in 1949 pointed out that the low temperature group of bacteria was greatly affected by humidity, tending to form a slime more readily on cut surfaces than on skin, connective tissue or fat. Microorganisms appeared in damp pockets, such as folds between the fore leg and breast of a carcass and their spread was greatly promoted by the condensation which occurred when cold carcass was exposed to warm damp air.

Empey and Vickery (14) in 1933 observed that 95% of the initial flora of beef capable of growth at -1°C (30.2°F)

consisted of members of the genus Achromobacter, the remainder were species of Pseudomonas and Micrococcus. During storage the relative numbers of Achromobacter and Pseudomonas increased while those of Micrococcus decreased.

More recent reports, Ayres (5) and Kirsch et al., (36), have indicated that species of Pseudomonas have a relatively greater importance in causing off-odor and slime than that assigned them by the earlier workers.

Wolin, Evans and Niven (77) in 1957 also indicated that when fresh beef was allowed to remain at 0 to 2°C in a moist environment, it spoiled within a few days due to bacterial growth. The onset of spoilage was detected by the presence of surface slime, discoloration of the meat pigment and the production of a musty odor. The predominant organism was generally classified as Achromobacter species but current methods of classification identified many of these as Pseudomonas species.

Spoilage by Yeasts and Molds

Jensen (33) observed that yeasts and molds could grow on frozen meat at a lower temperature than bacteria. Very few bacteria grew below -3°C, but yeasts and molds could grow down to -8°C (17.6°F). A. M. I. F. studies (68) showed that the most vulnerable characteristic was their need for oxygen. Thus, vacuum packaging or other methods of excluding oxygen were very effective in preventing yeast and mold growth.

Cladosporium herbarum grew on meat at -6°C (21.2°F) and

many Thamnidia grew on frozen meat at -5°C (23°F).

Proper air-circulation seemed necessary in meat coolers, refrigeration rooms and storage rooms. Circulating air, dried the surface of the meat so that if the relative humidity was "right", no molding or sliming could result. (33)

Ayres (6) noted that species of Penicillium were more commonly isolated from beef than were any other molds. Cladosporium, Thamnidium, Mucor and Rhizopus were next in order. Aspergillus was encountered occasionally on meat held at temperatures of 10°C . Also, two separate isolations of Monilia and single isolations of Alternaria and Sporotrichum were made. For the most part the activity of molds was restricted to the outer surfaces where aerobic conditions were obtained. On samples of meat that had been refrigerated for several days, Penicillium and Cladosporium were found growing on the connective tissue or on the native layer of fat covering the muscular tissue. Either of these organisms produced spots ranging in color from yellow to black although discolorations attributed to Cladosporium ordinarily were darker in color than those identified as Penicillium.

In yeast growth, slime could be detected at 99% relative humidity when the population was between 2×10^6 and 10^7 depending on the size of the individual yeast cells. (58)

Strains of organisms belonging to the asporogenous genera of yeasts--Torulopsis, Candida, Rhodotorula, Mycotorula, Geotrichoides and Blastodendrion were observed on refrigerated

beef. (6, 15) Trichosporon scottii has also been observed. (47)

PSYCHROPHILES AND PORK

General

Generally the factors influencing the growth of microorganisms in pork tissues is the same as for meats and beef. Exercise should be emphasized since over-exercise before slaughter of the pig leads to the glycogen being used up and so, very little production of lactic acid. A higher pH thus results. The muscle has a darker appearance and it has a closed structure. Further there is a higher water-holding capacity. The tissues of the swine are more susceptible to microbiological spoilage since, among other factors, the resistance of the animal is lowered, when the animal is over-exercised before slaughter.

Jensen (33) stated that ham fats were subjected to the enzymes of many species of salt-tolerant and other kinds of microbes. Bacon fat held at -17.8°C (0°F) could become rancid after a period ranging from four months to one year.

Ham souring, as recorded by Jensen (33) and Frazier (20) was caused by species of the genera Clostridia usually C. putrefaciens. Other bacteria involved were Achromobacter, Bacillus, Pseudomonas, Lactobacillus, Proteus, Serratia, Bacterium, Micrococcus and others. General sanitation and rapid chilling of carcasses following slaughter were essential to the prevention of ham souring.

There were six types of ham sours, namely: "shank sours,"

"body sours" or "loin sours," "aitchbone sours," "stifle-joint sours," "body-bone sours," and "butt sours." Jensen (12) quoting Tanner states, "the term 'sour' is used in the packing industry to indicate a condition of putrefaction, and not formation of acidity as might be expected." There are various theories as to the causes of souring, among them being: (1) faulty exsanguination of the hog; (2) improper chilling; (3) contamination through salt used in pickling solution; (4) employing "second" or used pickle from vats previously showing sours; (5) mutilations such as bruises, cuts, and tears during processing of hams; and (6) failure of salt and curing ingredients to penetrate into bone marrow.

Sours appear to be prevented by bleeding hogs properly. Prompt curing with artery pumping has considerably reduced incidence of souring as well as the use of curing pickles that are low in bacterial count. Adequate refrigeration is also an important factor. (33)

"Taints," or undesirable odors and tastes, that appear in meat as the result of the growth of bacteria on the surface often were evident before other signs of spoilage. The term "bone taint" of meats referred to either souring or putrefaction next to the bones, especially in hams. Usually it meant putrefaction.

Bacterial Spoilage

Mossel and Ingram (47) in 1955 listed factors which influenced spoilage as follows: (1) the initial infection of the

substrate; (2) factors depending on the properties of the substrate ("intrinsic factors"); (3) the conditions of storage ("extrinsic factors"); (4) the properties of the dominant microorganisms, for whose influences the term "implicit" factors was coined.

Tanaka, Nozaki and Yoshida (67) in 1951 found the following species of bacteria on clean pork in storage:

A. Bacteriaceae

1. Flavobacterium anteniforme
2. F. sulfurcum
3. Achromobacter pestifer
4. A. liquidum
5. A. solitarium

B. Coccaceae

1. Micrococcus subflavescens
2. M. ureae
3. M. nitrificans
4. M. percitrens
5. M. flavescens

Besides these, Alcaligenes ammoniagenes, Proteus and Escherichia, were detected. Each species had its own stages of multiplication. Flavobacterium and Alcaligenes were detected in the fresh stage. Micrococcus was seen in the stages of rigor mortis and ripening of the meat. Achromobacter multiplied in the putrefied stage, and prevailed over other bacteria.

Judging from the apparent observations, the preservable

period of the pork was as follows:

Table 16. (67)

Temp.	Period	No. of bacteria	Sol. N %	Amino N.	N of NH ₃ Mg. %
2-6°C	12 days	10 ⁷	1.10	0.68	0.30
8-12°C	6 days	10 ⁷	1.00	0.65	0.26

When the multiplication of bacteria was controlled, amino nitrogen increased by autolysis, but soluble nitrogen was nearly constant. The multiplication of bacteria preceded the change of the chemical components.

Sulzbacher (65) in 1950 made studies on frozen pork packaged in various ways and stored at 0°F (-18°C) and 25°F (-4°C) for twelve weeks and showed that: (1) aerobic counts made at 99°F (37°C) decreased during the storage period for all samples, regardless of storage temperature and protective methods; (2) counts of total aerobic and lipase-forming organisms made at 68°F (20°C) increased during the storage period. This increase was particularly great for protected samples at 25°F (-4°C) but was also evident for samples stored at 0°F (-18°C).

Twenty-eight Pseudomonas and four Achromobacter strains were isolated from frozen pork. All grew rapidly in the range of 25°F (-4°C) to 21°F (-6°C).

Callow (11) in 1932 observed that: (1) small pieces of pork could be kept in perfect condition for over two months at 0°C in desiccators filled with commercial carbon dioxide. The

author found that after seventy days, the meat tasted better than fresh pork, because it was more tender. There was practically no loss in weight; (2) pork stored in air at 0°C was completely spoiled after seventeen days. The use of carbon dioxide thus increased the storage life of pork more than four times; (3) an atmosphere of nitrogen at 0°C prevented the aerobic spoilage of pork but encouraged the growth of anaerobic organisms. Nitrogen was therefore useless for the storage of uncured meat; and (4) carbon dioxide had an inhibitory action on the growth of microorganisms obtained from pork which had suffered from aerobic and anaerobic spoilage. Culture medium heavily inoculated with these organisms showed no signs of growth at -1°C in carbon dioxide even after fifty days, whereas in nitrogen and in air, there were signs of growth after four days.

PSYCHROPHILES AND POULTRY

General

Frazier (20) in 1958 discussed the sources of contamination in poultry. The method of killing and bleeding the fowl had an important effect on the quality of the product. Modern methods involved the severing of the jugular vein of the bird, suspending by its feet, with resultant blood drainage. The method of picking or plucking had some influence on the keeping quality of the bird. Dry-plucked birds were more resistant to decomposition than semi-scalded or scalded ones because the skin was less likely to be broken, but more pin feathers were left. Most

picking was by means of the semi-scald method in which the fowl was immersed in water at 125 to 135°F (51.7 to 57.2°C) for about 30 seconds, rather than the older scalding method at 150 to 190°F (65.5 to 87.8°C) or over. Experiments showed that the water in the semi-scald method was not an important source of contaminating microorganisms if reasonable precautions were taken to change the water; in fact the process was one of mild pasteurization. The dressing (plucking) procedure normally did not add much contamination. Counts of numbers of bacteria on the skins of dressed fowls over a long period showed less than 250,000 organisms per gram of skin and few in the adjoining flesh. Evisceration of the fowl added contaminating bacteria from the alimentary tract. Undrawn or New York dressed poultry was not subject to contamination from the alimentary canal of the fowl but developed off-flavors (visceral taints) as a result of microbial growth in the tract. The type of feed given the fowls before slaughter could have an influence on the development of visceral taints. Microorganisms found on the skin, feet, and cut surfaces included Pseudomonas, Achromobacter, Flavobacterium, Micrococcus, coliforms, Alcaligenes, Proteus, Bacillus and others, including some yeasts and molds. (20)

The sanitation of the housing of the birds before killing had some influence on the numbers of microorganisms on the skin at dressing, but even under the best conditions enough spoilage organisms contaminated the skin to permit microbial deterioration if conditions of handling and storage were not good.

Contamination of the lining of the body cavity of the bird could be prevented if the fowl was not eviscerated until sold in the retail market, but visceral taints could develop unless the birds were adequately refrigerated. (20)

Some microorganisms could be removed from the skin during the scalding process that preceded picking and some would be killed, but not enough to be significant. During drawing or evisceration of the bird, microorganisms from the intestinal tract contaminated the lining of the body cavity, but thorough washing with good water removed many of the organisms. (20)

Frazier (20) indicated that poultry could be kept in good condition for months if freezing was prompt and rapid and the storage temperature was low enough. Fairly rapid freezing was desirable for this produced a light-appearing bird because fine ice-crystals were found within the fibers. Slow freezing, on the other hand, caused large crystals to accumulate outside the fibers and the flesh to appear darker. Regardless of the rate of freezing, intact birds did not drip upon thawing. Visceral taints developed slowly in frozen undrawn birds unless the storage temperature was low.

Poultry should be frozen fast enough to retain most of the natural bloom or external appearance of a freshly dressed fowl, and the storage temperature should be below 0°F (-17.8°C), and the relative humidity above 95% to reduce drying of the surface. (20)

Ayres, Ogilvy and Stewart (4) in 1950 found that birds

stored at 0°C, had a storage life of sixteen days; at 5°C, birds spoiled at seven days; and at 10°C, off-odor and slime were observed at three days.

Bacterial Spoilage

While the enzymes of the fowl contributed to the deterioration of the dressed bird, bacteria were the chief cause of spoilage, with the intestines a primary source of these organisms. It has been indicated that most of the bacterial growth took place on the surfaces, that is, the skin, the lining of the body cavity, and any cut surfaces, and the decomposition products diffused slowly into the meat. (20)

Walker and Ayres (74) in 1956 observed that the skin of the live bird had a count of about 1500 organisms per sq. cm., whereas the final product had a count of approximately 35,000 per sq. cm. of skin surface. In general, a tendency was observed for the counts to increase during the processing operation.

Counts of 8200 organisms per ml. were encountered in the scald tank water and more than 200,000 per ml. in the chill tank waters. The low counts obtained in the scald tank water could probably be attributed to the low counts found on the live birds and possibly the temperature of the scald tank had some bactericidal effect. The temperature range of the scald tank was 137 to 140°F (58.3 to 60°C).

Assary, Moore and Kramer (17) in 1958 determined the effect of different scald temperatures, chill times, and storage

temperatures on the bacterial contamination and shelf-life of tray-pack fryers.

Total bacterial counts per sq. cm. of skin from fryers scalded at 128°F for 50 seconds, and 138°F for 30 seconds, when slime developed, were approximately 1×10^7 and 1.6×10^7 respectively. Bacteria isolated from both scald groups were similar in type in that they grew quite well at low temperatures and were common to soil, water, or fecal material. (17)

Shelf-life as determined by odor was not significantly different between fryers at 128°F for 50 seconds, and 138°F for 30 seconds. However, when the scald time was 60 seconds, for each of these scald temperatures, shelf-life was significantly higher at the 1% level of probability for the 128°F scalded fryers. (17)

The influence of different chill times on the subsequent shelf-life was determined by comparing fryers chilled in ice slush for 4 and 16 hours, 2 and 18 hours, 2 and 12 hours, and 4 and 24 hours. In every test the average shelf-life was extended by the shorter of the two chill times. (17)

Fromm (21) in 1958 observed that the re-use of chill tank slush ice, as many as five times, did not significantly influence the bacterial numbers in chilled carcasses nor effect the shelf-life of the carcasses or flavor of the cooked product.

The longest shelf-life observed for any combination of treatments was 14.8 days for fryers scalded at 136°F for 45 seconds, chilled in ice slush for 2 hours and held in a 34°F cooler. (17)

Ayres, Ogilvy and Stewart (4) observed the characteristic sweetly rancid odor which could also be described as "dirty dishrag" odor to develop on cut up chickens stored for a sufficient time at high humidities. The authors described the formation of slime which accompanied the off-odor. Minute, translucent, moist colonies appeared in large numbers on the cut surfaces and skin of the bird. At first these colonies gave a superficial appearance of being droplets of moisture, later, however, they became larger and white or creamy in color and often coalesced as a rather uniform sticky or slimy layer. In this final stage, the meat had a pungent ammoniacal odor in addition to the "dirty dishrag" odor. The odor appeared before the slime formation.

The first sign of surface odor was apparent when the bacteria count on the skin had exceeded approximately 2,500,000 per sq. cm. as reported by Lockhead and Landerkin (38), or 10^8 per sq. cm. as shown by Ayres, Ogilvy and Stewart. (4)

The following bacterial genera were represented from eviscerated poultry (17, 38): Pseudomonas, Micrococcus, Achromobacter, Flavobacterium, Alcaligenes, Proteus, Bacillus, Sarcina, Streptococcus, Eberthella, Salmonella, and Aerobacter. Upon microscopic examination, the predominant flora at the time of sliming were found to be motile, gram-negative rods generally occurring in pairs. However, gram-negative cocci or coccobacilli occasionally persisted throughout the entire period of storage. (17)

The gram-negative rods having polar flagella were classed as members of the genus Pseudomonas. The coccus or coccobacillus forms were similar in many respects to Alcaligenes viscosus but were spherical or almost spherical, and non-motile. (17)

Nagel et al. (50) in 1960 also observed the spoilage organisms in chilled eviscerated chicken to be mostly of the Pseudomonas species. Aeromonas and members of the Achromobacter-Alcaligenes group represented only a minor portion of the bacteria present at time of spoilage of properly chilled poultry meat.

Thornby, Ingram and Barnes (69) observed changes in the microbial population of chilled eviscerated chickens after storage at 1°C, by carrying out viable counts on heart infusion agar (Difco) incubated at 1°C, 20°C, and 37°C. Fecal Streptococci were also determined. These results were summarized in Table 17.

Table 17. The relationship between the development of "off-odors" in chickens stored at 1°C, colony counts at 1°C, 20°C, and 37°C, and numbers of fecal Streptococci. (69)

Stage of storage	Colony count/sq. cm.* at			No. of fecal streptococci
	1	20	37	
Initial	3.5×10^4	1.8×10^5	4.4×10^4	5.5×10^3
Slight "off-odor"	7.5×10^7	7.4×10^7	6.2×10^5	1.7×10^4
Strong "off-odor"	7.8×10^8	1.0×10^9	1.8×10^7	5.0×10^4

*Mean of duplicate results from 3 chickens.

As Table 17 showed, the numbers of psychrophilic bacteria increased about a thousand-fold from the initial state to the first definite trace of unusual odor, and about a further ten-fold by the time the carcass had acquired a strong smell and was frankly spoiled.

Barnes and Shrimpton (7) in 1957 observed the greening of unviscerated poultry carcasses stored at 15°C to be due to the production of hydrogen sulfide following multiplication of bacteria in the gut. The main groups present in the gut after death were fecal Streptococci, coli-aerogenes organisms, Lactobacilli and Clostridia; but their relative importance in causing greening was not yet been established. The hydrogen sulfide diffused from the gut into the muscle tissue and then reacted with the haem pigments of blood and muscle to form derivatives which microspectroscopically were indistinguishable from sulf-hemoglobin.

It was also observed that mechanically plucked birds greened more rapidly over the ribs than hand plucked ones, because the shaking distributed bacteria from the caecum along the gut. (7)

Spoilage by Yeasts and Molds

Usually the mold spoilage in chicken meat occurred after it was treated with the tetracyclines. The molds isolated from spoiled chlortetracycline treated chicken fryers were usually of the genus Cladosporium, Mucor, Penicillium, Rhizopus, Torula, and Trichoderma. Those isolated from controls were Alternaria,

Aspergillus, Mucor, and Rhizopus. (4, 55)

Yeasts too had similar features to mold since these grew more often on chlortetracycline treated than on untreated chicken meat. The results indicated that the development of yeasts upon antibiotic-treated poultry meat was a result of suppression of competition between the yeasts and the bacterial flora of the birds so that the bacteria were not present in large enough numbers to limit the growth of yeasts. (2, 55, 73, 76)

The yeasts commonly encountered in chicken meat were of the following genera: Saccharomyces, Rhodotorula, Geotrichum, Torulopsis, Candida, Cryptococcus, and Trichosporon. (2, 4; 55, 73, 76)

Both pigmented and non-pigmented yeasts were recovered from poultry. (73)

ACKNOWLEDGMENTS

The author expresses his sincere appreciation to Professor D. L. Mackintosh, major professor, for his able guidance throughout the preparation of this report; to Dr. W. A. Miller, without whose aid and helpful advice, this report could not have been accomplished; and to Dr. D. H. Kropf for his suggestions and constructive criticism.

LITERATURE CITED

1. Allen, J. R. and L. M. Foster.
Spoilage of vacuum packed sliced processed meats during refrigerated storage. Food Res. 25:19-25, 1960.
2. Ayres, John C.
Effect of sanitation, packaging and antibiotics on the microbial spoilage of commercially processed poultry. Iowa State J. Sci. 34:27-46, 1959.
3. Ayres, John C.
Microbiological implications in the handling, slaughtering and dressing of meat animals. Adv. in Food Res. 6:109-161, 1955.
4. Ayres, John C., W. S. Ogilvy and G. F. Stewart.
Post-mortem changes in stored meats. I. Microorganisms associated with development of slime on eviscerated cut up poultry. Food Technol. 4:199-205, 1950.
5. Ayres, J. C.
Some bacteriological aspects of spoilage of self-service meats. Iowa State J. Sci. 26:31, 1951.
6. Ayres, J. C.
Temperature relationships and some other characteristics of microbial flora developing on refrigerated beef. Food Res. 25:1-18, 1960.
7. Barnes, Ella M., and D. H. Shrimpton.
Causes of greening of uneviscerated poultry carcasses during storage. J. Appl. Bact. 20:273-285, 1957.
8. Berry, J. A., and C. A. Magoon.
Growth of microorganisms at and below 0°C. Phytopathology. 24:280-296, 1934.
9. Brooks, F. T.
Moulds on frozen meats. J. Soc. Chem. Ind. Trans. and Absts. 43:306T, 1924.
10. Butler, O. D., Jr.
The effect of salt, pepper and sage on the stability of ground pork stored at zero degrees Fahrenheit. J. An. Sci. 9:30-36, 1950.
11. Callow, L. H.
Gas storage of pork and bacon. I. Preliminary experiments. J. Soc. Chem. Ind. Trans. 51:116T-119T, 1932.

12. Eddy, B. F.
The use and meaning of the term 'psychrophilic'.
J. Appl. Bact. 23:189-190, 1960.
13. Eddy, B. F., and A. G. Kitchell.
Cold tolerant fermentative gram-negative organisms
from meat and other sources. J. Appl. Bact. 22:57-63,
1959.
14. Empey, W. A., and J. R. Vickery.
The use of carbon dioxide in the storage of chilled
beef. Australia J. Council Sci. Ind. Res. 6:233-243,
1953.
15. Empey, W. A., and W. J. Scott.
Investigations on chilled beef. I. Microbial contamin-
ation acquired in the meatworks. Australia Council
Sci. and Ind. Res. Bull. No. 126:7-71, 1959.
16. Ercole Canole-Farola and L. John Ordal.
A survey of the bacteriological quality of frozen
poultry pies. Food Technol. 11:578-582, 1957.
17. Essary, L. G., W. E. C. Moore and C. Y. Kramer.
Influence of scald temperatures, chill times, and
holding temperatures on the bacterial flora and shelf-
life of freshly chilled, tray-pack poultry. Food
Technol. 12:684-687, 1958.
18. Evans, James B., and C. F. Niven, Jr.
Slime and mold. Amer. Meat Inst. Foundation Bull. No.
24, 1955.
19. Fanelli, M. J., and J. C. Ayres.
Methods of detection and effect of freezing on the
microflora of chicken pies. Food Technol. 13:294-300,
1959.
20. Frazier, W. C.
Food Microbiology. New York: McGraw-Hill Book
Company, Inc., 1958.
21. Fromm, Daniel.
Influence of re-using chill tank slush ice in market
quality of eviscerated broilers. Food Technol.
12:257-259, 1958.
22. Geer, Laurence F., William T. Murray and Ernest Smith.
Bacterial content of frosted hamburger steak. Amer. J.
Public Health. 23:673-676, 1933.

23. Haines, R. B.
The bacterial flora developing on stored lean meat, especially with regard to 'slimy' meat. *J. Hyg.* 33:175-182, 1933.
24. Haines, R. B.
The growth of microorganisms on chilled and frozen meat. *J. Soc. Chem. Ind.* 50:223T-226T, 1931.
25. Haines, R. B.
The influence of carbon dioxide on the rate of multiplication of certain bacteria, as judged by viable counts. *J. Soc. Chem. Ind.* 52:13T-17T, 1933.
26. Haines, R. B.
The minimum temperature of growth of some bacteria. *J. Hyg.* 34:277-281, 1934.
27. Haines, R. B., and L. C. Smith.
The storage of meat in small refrigerators. *Food Investigation Special Report No. 43.*
28. Hall, J. L., Dorothy L. Harrison and J. L. Mackintosh.
Counter-effect of sodium chloride and sage on development of peroxide in frozen stored sausage. *Food Technol.* 16:102-104, 1962.
29. Hucker, C. J., and Elizabeth Russel David.
The effects of alternate freezing and thawing on the total flora of frozen chicken pies. *Food Technol.* 11:354-356, 1957.
30. Ingraham, J. L., and J. L. Stokes.
Psychrophilic bacteria. *Bact. Rev.* 23:97-106, 1959.
31. Ingram, M.
Benjamin Ward Richardson Lecture, Part II. Hygiene and Storage. *J. Roy. Soc. Sanit. Inst.* 69:39, 1949.
32. Ingram, M.
The effect of cold on microorganisms in relation to food. *Proc. Soc. Appl. Bact.* 14:243-260, 1951.
33. Jensen, L. B.
Microbiology of meats, 3rd ed., Illinois: The Garrard Press, Champaign. 1954.
34. Kereluk, A., A. C. Peterson and H. F. Gunderson.
Effect of different temperatures on various bacteria isolated from frozen meat pies. *J. Food Sci.* 26:21-25, 1961.

35. Kereluk, K., and M. F. Gunderson.
Studies on the bacteriological quality of frozen meat pies. I. Bacteriological survey of some commercially frozen meat pies. *Appl. Microbiology*. 7:320-323, 1959.
36. Kirsch, R. H., F. E. Berry, C. L. Baldwin and E. M. Foster.
The bacteriology of refrigerated ground beef. *Food Res.* 17:495-503, 1952.
37. Litsky, Warren, I. V. Pagerson and C. R. Pellers.
A bacteriological survey of commercially frozen beef, poultry and tuna pies. *J. Milk Food Technol.* 20:216-219, 1957.
38. Lockhead, A. C., and G. B. Landerkin.
Bacteriological studies of dressed poultry. I. Preliminary investigations of bacterial action at chill temperatures. *Scientific Agr.* 15:765-770, 1935.
39. Lund, A. J., and H. O. Halvorson.
The effect of low temperatures on enzymes and microorganisms. *Proc. Third Conf. Res. The Coun. Res. Amer. Meat Inst.* 59-62, 1951.
40. Macara, T. J. R.
Dried meat. II. The growth of moulds on dried meat. *Soc. Chem. Ind. J. Trans.* 62:104-106, 1943.
41. Mattick, A. T. R.
The effect of cold on microorganisms: general introduction. *Proc. Soc. Appl. Bact.* 14:211-215, 1951.
42. McLean, Ruth A., and William L. Sulzbacher.
Microbacterium thermosphactum, *Spec. Nov.*; a non-heat resistant bacterium from fresh pork sausage. *J. Bact.* 65:428-433, 1953.
43. Miller, W. A.
Effect of freezing ground pork and subsequent storing above 32°F upon the bacterial flora. *Food Technol.* 9:332-334, 1955.
44. Moran, T.
Post-mortem and refrigeration changes in meat. *J. Soc. Chem. Ind. Trans.* 54:149T-151T, 1935.
45. Moran, T.
The frozen state in mammalian muscle. *Proc. Roy. Soc. London, Ser. B.* 107:182, 1930.

46. Moran, T., E. C. Smith and R. G. Tomkins.
The inhibition of mould growth on bacteria by carbon dioxide. *J. Soc. Chem. Ind.* 15:114T-116T, 1932.
47. Mossel, D. A. A., and M. Ingram.
The physiology of the microbial spoilage of foods. *J. Appl. Bact.* 18:232-268, 1955.
48. Mossel, D. A. A., and H. Zwart.
The rapid tentative recognition of psychrotrophic types among Enterobacteriaceae isolated from foods. *J. Appl. Bact.* 23:185-188, 1960.
49. Mrak, E. M., and Lee Bonar.
A note on yeast obtained from slimy sausage. *Food Res.* 3:615-618, 1938.
50. Nagel, C. W., et al.
Microorganisms associated with spoilage of refrigerated poultry. *Food Technol.* 14:21-23, 1960.
51. Niven, C. F., Jr.
Sausage discolorations of bacterial origin. *Amer. Meat Inst. Foundation Bull. No. 13*, 1951.
52. Niven, C. F., Jr., A. G. Castellani and Virginia Allanson.
A study of the lactic acid bacteria that cause surface discolorations of sausage. *J. Bact.* 58:633-641, 1949.
53. Niven, C. F. Jr., and James B. Evans.
Lactobacillus viridescens Nov. spec., a heterofermentative species that produces a green discoloration of cured meat pigments. *J. Bact.* 73:758-759, 1957.
54. Niven, C. F., Jr., L. G. Buettner and James B. Evans.
Thermal tolerance studies on the heterofermentative *Lactobacilli* that cause greening of cured meat products. *Appl. Microbiology.* 2:26-29, 1954.
55. Njoku-Obi, et al.
A study of the fungal flora of spoiled chlortetracycline treated chicken meat. *Appl. Microbiology.* 6:420-422, 1958.
56. Peterson, A. C., and M. F. Gunderson.
Role of psychrophilic bacteria in frozen food spoilage. *Food Technol.* 14:413-417, 1960.
57. Rogers, Raymond E., and C. B. McCleskey.
Bacteriological quality of ground meat in retail markets. *Food Technol.* 11:318-320, 1957.

58. Scott, W. J.
The growth of microorganisms on ox muscle. I. The influence of water content of substrate on rate of growth at -1°C . Australia Coun. Sci. Ind. Res. J. 9:177-190, 1936.
59. Scott, W. J.
The growth of microorganisms on ox muscle. II. The influence of temperature. Australia Coun. Sci. Ind. Res. J. 10:338-350, 1937.
60. Stokes, J. L.
Growth of microorganisms at low temperatures. Proc. Twelfth Res. Conf. Amer. Meat Inst. Foundation:9-15.
61. Straka, Robert P., and J. L. Stokes.
Metabolic injury to bacteria at low temperatures. J. Bact. 78:185-189, 1959.
62. Straka, Robert P., and J. L. Stokes.
Psychrophilic bacteria from antarctica. J. Bact. 80:622-625, 1960.
63. Straka, Robert P., and J. L. Stokes.
Sound handling practices best antidote for microbiological hazards of precooked frozen food. Quick Frozen Foods. 18:182-186, 1956.
64. Sulzbacher, William L.
Effect of freezing and thawing on the growth rate of bacteria in ground meat. Food Technol. 6:341-343, 1952.
65. Sulzbacher, William L.
Survival of microorganisms in frozen meat. Food Technol. 4:386-390, 1950.
66. Sulzbacher, William L., and Ruth A. McLean.
The bacterial flora of fresh pork sausage. Food Technol. 5:7-8, 1951.
67. Tanaka, H., H. Nozaki and N. Yoshida.
Studies on the pork in storage. III. Bacteriological studies on the pork in storage. Bull. National Inst. Agric. Sci., Yahagi, Chiba, Japan. Series G. 2:45-49, 1951.
68. The Science of Meat and Meat Products.
San Francisco: American Meat Institute Foundation, 1960.

69. Thornby, Margaret J., M. Ingram and Ella M. Barnes.
The effects of antibiotics and irradiation on the
Pseudomonas-Achromobacter flora of chilled poultry.
J. Appl. Bact. 23:487-498, 1960.
70. Tschistjakow, F. M., and Z. Z. Botscharowa.
The influence of low temperature on microorganisms.
II. Influence of low temperature on the development
of moulds. (In Russian with English summary). Micro-
biologiya. VII (IV):498-514, 1938.
71. Vickery, J. R.
The action of microorganisms on fat. I. The hydro-
lysis of beef fat by some bacteria and yeasts toler-
ating low temperatures. Australia Coun. Sci. Ind.
Res. J. 9:107-112, 1936.
72. Vickery, J. R.
The action of microorganisms on fat. II. A note on
the lipolytic activities of further strains of micro-
organisms tolerating low temperatures. Australia
Coun. Sci. Ind. Res. J. 9:196-198, 1936.
73. Walker, H. H., and J. C. Ayres.
Characteristics of yeasts isolated from processed
poultry and the influence of tetracyclines on their
growth. Appl. Microbiology. 7:251-255, 1959.
74. Walker, Homer W., and John C. Ayres.
Incidence and kinds of microorganisms associated with
commercially dressed poultry. Appl. Microbiology.
4:345-349, 1956.
75. Weinzirl, John and A. P. M. A. Fellow.
Concerning the relation of the bacterial count to the
putrefaction of meat. Amer. J. Public Health.
14:946-949, 1924.
76. Wells, F. E., and W. J. Stadelman.
Development of yeasts on antibiotic-treated poultry.
Appl. Microbiology. 6:420-422, 1958.
77. Wolin, E. F., James B. Evans and C. F. Niven, Jr.
The microbiology of fresh and irradiated beef. Food
Res. 22:682-686, 1957.
78. Wright, Allister McClean.
Moulds on frozen meats. J. Soc. Chem. Ind. Trans. and
Abs. 42:448T-490T, 1923.

PSYCHROPHILIC MICROBIOLOGICAL DETERIORATIVE CHANGES IN
FRESH BEEF, PORK AND POULTRY AND THEIR PRODUCTS
HELD AT REFRIGERATOR TEMPERATURES

by

SATISH CHANDRA NIVAS

B. V. Sc. & A. H., University of Bihar, India, 1960

AN ABSTRACT OF A MASTER'S REPORT

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Animal Husbandry

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1963

This study was undertaken to review the recent findings on psychrophilic microorganisms that cause deterioration in fresh meat and poultry, and their products held at low temperatures.

Contamination of chicken pies could be brought about by faulty handling, unclean dicing machines, and a long period of storage before refrigeration. The bacteria usually found in these pies belonged to the genera Pseudomonas, Achromobacter and Micrococcus.

Ground meat provided an ideal medium for bacterial growth because of fragmentation of tissues and the liberation of cell juices and the intimate mixing of the bacteria normally found on the moist surface with the macerated tissues.

The bacterial genera encountered in ground meats were Micrococcus, Microbacterium, Bacillus, Pseudomonas, Achromobacter, Alcaligenes, Sarcina, Lactobacillus and Proteus. Spoilage was indicated by a stale sour odor and fading of the color at the surface.

The yeast isolated from fresh sausage belonged to the genus Debaryomyces, and the molds were of the genera Penicillium and Mucor.

The most common viable bacteria on refrigerated beef appeared to be Pseudomonas, Micrococcus, Achromobacter, Flavobacterium and Bacillus. Several strains of the genera Achromobacter and Pseudomonas were lipolytic in character, giving rise to off-odors and flavors commonly designated as rancidity. Off-odors and slime production occurred in beef at storage temperatures.

They occurred earlier at higher temperatures, but after some time at lower temperatures. The bacteria involved were of the genera Pseudomonas and Micrococcus.

The molds thriving on refrigerated beef were of the genera Penicillium, Cladosporium, Thamnidium, Mucor, Rhizopus and Aspergillus. These molds were restricted to the outer surfaces where aerobic conditions were obtained. These molds produced various discolorations on the surface of the beef carcass.

The yeasts encountered on refrigerated beef were of the genera Torulopsis, Candida, Rhodotorula, Mycotorula, Geotrichoides and Blastodendrion.

Ham souring in pork was caused by a species of the genus Clostridia, usually C. putrefaciens. Other bacteria involved were Achromobacter, Bacillus, Pseudomonas, Lactobacillus, Proteus, Serratia, Bacterium and Micrococcus.

An atmosphere of carbon dioxide prevented spoilage for a considerable length of time. Storage in an atmosphere of nitrogen could only prevent aerobic spoilage of pork.

Spoilage in poultry occurred frequently. This was mainly due to the method of killing, bleeding, evisceration and dressing of the fowl. Dry-plucked birds were more resistant to decomposition than semi-scalded or scalded ones because the skin was less likely to be broken. Undrawn or New York dressed poultry were not subject to contamination from the alimentary canal of the fowl but developed off-flavors (visceral taints) as a result of microbial growth in the tract. The following bacterial

genera were represented from eviscerated poultry: Pseudomonas, Micrococcus, Achromobacter, Alcaligenes, Proteus, Bacillus, Sarcina, Streptococcus, Salmonella and Aerobacter. The spoilage organism primarily responsible for a "dirty dishrag" odor and slime formation belonged to the genus Pseudomonas.

The mold spoilage in chicken meat often occurred when it was treated with the tetracyclines. The molds were: Cladosporium, Mucor, Penicillium, Rhizopus, Torula and Trichoderma.

Yeasts too grew in chicken meat treated with chlortetracycline. These yeasts were: Saccharomyces, Rhodotorula, Geotrichum, Torulopsis, Candida, Cryptococcus and Trichosporon.

In general, bacterial spoilage of fresh meat and poultry, and their products, can be attributed to three common genera, namely, Pseudomonas, Achromobacter and Micrococcus. These three genera seem to be the most important bacteria giving rise to off-odors, flavors, discolorations and slime production on meat and meat products.

Cured meats, on the other hand, seem to be greatly affected by Lactobacillus and Leuconostoc species.

The molds involved in the deteriorative changes in fresh meats and their products, in general, seem to belong to the genera Penicillium, Mucor, Cladosporium and Rhizopus.

The yeasts commonly encountered were Rhodotorula and Torulopsis.