FACTORS AFFECTING THE GROWTH OF

PSYCHROPHILIC
MICRO-ORGANISMS
IN FOODS:

A Review

By

R. PAUL ELLIOTT

and

H. DAVID MICHENER

Western Utilization Research and Development Division

Technical Bulletin No. 1320

Agricultural Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE
Acknowledgment

The authors acknowledge the assistance of Anne M. Avakian, Librarian of the Western Utilization Research and Development Division.

Names of companies and trade names are used in this publication solely to provide specific information. Mention of a company or a trade name does not constitute a guarantee or warranty of the company or the product by the U.S. Department of Agriculture or an endorsement by the Department over other companies or products not mentioned.
## Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>INTRODUCTION</strong></td>
<td>1</td>
</tr>
<tr>
<td>- Psychrophilic micro-organisms in food spoilage</td>
<td>1</td>
</tr>
<tr>
<td>- Definitions</td>
<td>1</td>
</tr>
<tr>
<td><strong>EFFECT OF TEMPERATURE ON MICROBIAL ACTIVITIES</strong></td>
<td>6</td>
</tr>
<tr>
<td>- Lag phase</td>
<td>6</td>
</tr>
<tr>
<td>- Logarithmic phase</td>
<td>8</td>
</tr>
<tr>
<td>- Temperature coefficient ( Q_a ) and temperature characteristic ( (\mu) )</td>
<td>9</td>
</tr>
<tr>
<td>- Lipids</td>
<td>19</td>
</tr>
<tr>
<td>- Enzyme production and activity</td>
<td>20</td>
</tr>
<tr>
<td>- Cell size and cell crop</td>
<td>24</td>
</tr>
<tr>
<td>- Adaptation</td>
<td>25</td>
</tr>
<tr>
<td><strong>EFFECT OF WATER AVAILABILITY ON MICROBIAL ACTIVITIES</strong></td>
<td>28</td>
</tr>
<tr>
<td><strong>SPOILAGE OF MEATS AND FISH</strong></td>
<td>32</td>
</tr>
<tr>
<td>- Effect of low temperature</td>
<td>32</td>
</tr>
<tr>
<td>- Floral changes in spoilage</td>
<td>34</td>
</tr>
<tr>
<td>- Inoculum size</td>
<td>40</td>
</tr>
<tr>
<td>- Freezing and thawing</td>
<td>45</td>
</tr>
<tr>
<td>- Heat</td>
<td>49</td>
</tr>
<tr>
<td>- Type of surface</td>
<td>51</td>
</tr>
<tr>
<td>- Relative humidity</td>
<td>52</td>
</tr>
<tr>
<td>- Air movement</td>
<td>55</td>
</tr>
<tr>
<td>- Oxygen</td>
<td>55</td>
</tr>
<tr>
<td>- Gas storage</td>
<td>55</td>
</tr>
<tr>
<td>- Packaging</td>
<td>59</td>
</tr>
<tr>
<td>- Acid</td>
<td>61</td>
</tr>
<tr>
<td>- Antemortem treatment</td>
<td>61</td>
</tr>
<tr>
<td>- Autolysis</td>
<td>62</td>
</tr>
<tr>
<td>- Antibiotics</td>
<td>62</td>
</tr>
<tr>
<td>- Salt</td>
<td>64</td>
</tr>
<tr>
<td><strong>SPOILAGE OF EGGS</strong></td>
<td>65</td>
</tr>
<tr>
<td>- Penetration of shell eggs</td>
<td>65</td>
</tr>
<tr>
<td>- Shell coatings</td>
<td>67</td>
</tr>
<tr>
<td>- Thermostabilization</td>
<td>67</td>
</tr>
<tr>
<td>- Gas storage</td>
<td>67</td>
</tr>
<tr>
<td>- Internal defenses</td>
<td>67</td>
</tr>
<tr>
<td>- Effect of metals on penetration and growth</td>
<td>67</td>
</tr>
<tr>
<td>- Effect of aging</td>
<td>70</td>
</tr>
<tr>
<td>- Liquid egg</td>
<td>70</td>
</tr>
<tr>
<td><strong>SPOILAGE OF FRUITS</strong></td>
<td>71</td>
</tr>
<tr>
<td><strong>SPOILAGE OF VEGETABLES</strong></td>
<td>72</td>
</tr>
<tr>
<td><strong>summary and conclusions</strong></td>
<td>76</td>
</tr>
<tr>
<td><strong>reviews and bibliographies on low-temperature microbiology</strong></td>
<td>78</td>
</tr>
<tr>
<td><strong>literature cited</strong></td>
<td>79</td>
</tr>
</tbody>
</table>
Factors Affecting The Growth Of Psychrophilic Micro-Organisms In Foods:
A Review

By R. PAUL ELLIOTT* and H. DAVID MICHENER, Western Utilization Research and Development Division, Agricultural Research Service

INTRODUCTION

Psychrophilic micro-organisms in food spoilage

Psychrophilic micro-organisms are of major importance in modern food technology, because their activities limit strictly the time that foods can be stored chilled—that is, at refrigeration temperatures above freezing. However, microbial growth is not a problem in frozen foods held at a suitably low storage temperature. They should be stored well below -12° C., which is about the lowest temperature permitting growth of psychrophiles (327). In fact, to minimize nonmicrobial chemical changes, frozen foods are best held at -17.5° or below (171, 484).

Psychrophilic growth does not result in food poisoning. Food-poisoning organisms rarely grow below 10° C., and none has been reported to grow below 3.3°. A review of minimum temperatures for growth of psychrophilic and food-poisoning micro-organisms has been published elsewhere (327). An earlier review (113) covered microbiological standards and handling codes for chilled and frozen foods.

The purpose of this review is to bring together pertinent facts on growth of micro-organisms during decomposition of refrigerated meats, fish, eggs, fruits, and vegetables. Studies on psychrophilic growth in other foods or menstrua are included only when they apply to psychrophilic spoilage of these commodities.

For the benefit of the reader who wishes to study phases of low-temperature microbiology not covered in this report, a list of reviews and bibliographies is presented on page 78.

Definitions

For the purposes of this review, psychrophiles are defined as micro-organisms that grow relatively rapidly at 0° C. (229). Psychrophiles always grow more rapidly at higher temperatures


Italic numbers in parentheses refer to literature cited, p. 79.
than they do at 0°. Optima for most are at 20° to 30°; for some at 30° to 45°; for a few at about 15° or below (232, 327, 541).

A discussion of bacterial growth can most conveniently begin with a description of the growth curve, shown in figure 1 in its simplest form (334, 538). In food spoilage only the lag and logarithmic phases are important, because foods are spoiled by the time bacterial growth has begun to decelerate to the resting phase.

Lag phase, as here used, is defined as the time elapsed from inoculation to the beginning of logarithmic multiplication. At low temperatures, there is sometimes a drop in count before logarithmic growth begins (326, 348, 409) because some components of the population die before others begin logarithmic growth.

During the logarithmic growth phase, micro-organisms grow at their most rapid rate. A convenient measure of this rate is the "generation time," that is, the time for one cell to become two, calculated as follows (387):

\[ g = \frac{t \log 2}{\log b - \log B} \]

where \( g \) = generation time, \( t \) = observation period, \( B \) = number of bacteria at the beginning of the observation period, and \( b \) = number at the end of the period.

Most yeasts reproduce by budding, which means that the time for one cell to become two is not strictly a generation time. Nevertheless, this term has been used by several investigators as a convenient measure for the rate of yeast growth.

![Figure 1.—A typical bacterial growth curve.](image)
When growth rates of molds or actinomycetes are to be measured, generation time cannot be used because the colony grows by terminal extension and branching of the hyphae rather than by binary fission of individuals. Haines (179, 182) prepared growth curves by plotting the logarithms of the total lengths of hyphae against time. As a measure of growth rate he used the slope of the steepest part of the resulting curve.

Instead of growth rate, some investigators use rates of related microbial activities such as oxygen consumption or carbon dioxide production.

As the temperature changes, growth rates and activity rates change. As a convenient means of measuring the effect of temperature on these rates, three terms are often used: temperature coefficient \(Q_{10}\), temperature characteristic \(\mu\), and Bélehrádek's exponent \(b\).

Temperature coefficient \(Q_{10}\) can be defined as a ratio of growth rate or activity rate at one temperature to that at a temperature 10° C. lower, or:

\[
Q_{10} = \frac{k_2}{k_1}
\]

where \(k_1\) and \(k_2\) are velocity coefficients of growth, activity rates, or generation times at temperatures differing by 10° (55). \(Q_{10}\) can be calculated for any other temperature interval \((\Delta T)\) from the following formula (387):

\[
Q_{10} = \left( \frac{k_2}{k_1} \right)_{10}^{\Delta T} = \left( \frac{k_2}{k_1} \right)^{10}_{\Delta T}
\]

Temperature characteristic \(\mu\) is a related figure and is derived from the van’t Hoff-Arrhenius equation:

\[
\mu = \frac{4.6 \left( \log k_2 - \log k_1 \right)}{T_2 - T_1}
\]

where \(k_1\) and \(k_2\) are growth or activity coefficients at absolute temperatures \(T_1\) and \(T_2\), respectively (55, 387). The temperature characteristic is the energy of activation of the reaction in calories per gram molecule and is represented by the slope of the curve obtained when the logarithm of a growth or activity rate is plotted against the reciprocal of the absolute temperature, as shown in figure 2. As will be discussed later, \(\mu\) is usually constant with respect to temperature over a considerable temperature range. \(Q_{10}\) and \(\mu\) are related as shown in figure 3, so that if either is known, the other can be found provided the temperature range is given. Figure 3 also demonstrates that if \(\mu\) is constant, \(Q_{10}\) will change slightly with temperature in accordance with theory.
Figure 2.—Effect of temperature on growth rate of a mesophile and a psychrophile (E. coli).

Exponent $b$, as described by Böehradek (82) was derived for use in biological systems when $Q_{10}$ and $\mu$ were not sufficiently constant. He used the formula

$$t = \frac{a}{T^b} \quad \text{or} \quad b = \frac{\log a - \log t}{\log T}$$

where $t$ is time, $T$ is temperature above the "biological zero" for the system under study, and $a$ and $b$ are constants that are characteristic of the system. This formula can be used only when the biological zero is known. When the logarithm of $t$ is plotted against the logarithm of $T$, the resultant curve is generally a straight line, of slope $b$, whereas its counterpart in the van’t Hoff-Arrhenius plot might be curved or show distinct breaks.
Figure 3.—Relation of temperature coefficient \( Q_\text{m} \) and temperature characteristic \( \mu \) for certain temperature ranges (calculated from \( Q_\text{m} = e^{\frac{5.4\mu}{T+10}} \) as given by Buchanan and Fulmer (55)).
EFFECT OF TEMPERATURE ON MICROBIAL ACTIVITIES

Lag phase

Numerous investigators have found the lag period of microbial growth to be highly sensitive to temperature; lag is shortest at the optimum temperature (39°C) and is prolonged as temperature is lowered. In the range near the minimum growth temperature, lag increases so rapidly with decreasing temperature that it approaches infinity. Hanson and Fletcher (200) reported considerable mold growth on turkey pies at -6.7°C after 9 months but none after 6 months. Gibbons (152) found a lag period of 50 weeks at -5°C for the natural bacterial flora on fish. Chistyakov and Bocharova (74) found a strain of Oospora with a lag period of 414 days at -8°C, the longest lag period we have found on record. It is possible that other investigators would have recorded similarly long lag periods with other organisms, had they continued observations for prolonged periods.

In the temperature range where both psychrophiles and mesophiles are able to grow (generally between 10° and 30°C), the psychrophiles have a much shorter lag period (fig. 4 and 5). However, lag time varies widely among both the psychrophilic bacteria (fig. 6) and the psychrophilic molds (table 1).

<table>
<thead>
<tr>
<th>TABLE I.—Lag periods of various species and strains of molds at various temperatures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
</tr>
<tr>
<td>-------------------------------------------</td>
</tr>
</tbody>
</table>
| Aspergillus glaucus                      | 1
| Mucor reescomus                          | 1
| Phycyomycetes niten                   | 1
| Fusarium sp. I                         | 1
| Penicillium sp.                         | 1
| Fusarium culinarium                    | 1
| Montilia nigra                          | 1
| Penicillium glaucum                     | 1
| Mucor sp.                               | 1
| Fusarium sp. III                      | 1
| Fusarium sp. IV                       | 1
| Penicillium sp. II                     | 1
| Fusarium sp. V                         | 1
| Botrytis cinerea                      | 1
| Oospora sp.                             | 1
| Cladosporium herbarum           | 1
| Chaetosporium frescin          | 1
| Cladosporium sp.                     | 1
| Montilia nigra                          | 1

1 No growth observed.

Source: Chistyakov, F. M., and Bocharova, Z. Z. (73).
When the mold data of table 1 are plotted in the same way as those in figures 4, 5, and 6, a similar set of curves results. That is, they show the marked effect of low temperature in prolonging lag. Representative data from table 1 were combined in table 2 with similar data of Haines and Smith (192). At 0° to -5° C, the observed $Q_{10}$ of the reciprocal of lag is much greater than the expected $Q_{10}$, calculated using figure 3 and assuming that $\mu$ is
constant. The two exceptions, *Cladosporium herbarum* and *Chaetostylum fresenii*, have been observed to grow at the unusually low temperatures of $-6^\circ$ and $-10^\circ$, respectively (327). Probably $-5^\circ$ was not low enough to produce a very long lag in the strains reported in table 2. Also, it is illogical that the lag periods should be nearly equal at $0^\circ$ or $-2^\circ$ and $-5^\circ$, but precise observation of lag at low temperatures is difficult because the changes are extremely small.

Reviews of the effect of various conditions on lag were made by Penfold (377), Hartsell and others (204), and Hartsell (203).

**Logarithmic phase**

Once the lag phase is passed and the logarithmic phase begins, temperature affects rate of reproduction as shown in figure 7. Although this figure represents yeast growth, similar results are
obtained with bacterial and mold growth. Representative data comparing minimum generation times of bacteria above and below 0°C are presented in figures 8 and 9. These data show that rate of growth, or generation time, is highly sensitive to slight changes in temperature in the lower ranges. Below 0°C, generation time may exceed 100 hours (fig. 9). Similar results have been obtained with a mold and an actinomycete (179, 182).

Neither size of inoculum nor the previous temperature of incubation of the inoculum had any significant effect on the rate of logarithmic growth of the psychrophile Pseudomonas fragi (105).

The effect of temperature on the rate of growth during the logarithmic phase is much more marked in the mesophiles than in the psychrophiles (fig. 2 and 10).

**Temperature coefficient** \((Q_{10})\) and **temperature characteristic** \((\mu)\)

The rate of chemical reactions usually increases twofold to fourfold with each 10°C increase in temperature \((Q_{10} = 2 \text{ to } 4)\). But biological reactions, which are composites of many chemical reactions, do not follow this simple rule except approximately in the middle of their active temperature range. In the previous
### Table 2.—Effect of low temperature on the lag period of molds

<table>
<thead>
<tr>
<th>Species</th>
<th>Lag period at—</th>
<th>Q₀ of 1/ lag, 0° to −5° C.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−10° C.</td>
<td>+5° C.</td>
</tr>
<tr>
<td></td>
<td>Days</td>
<td>Days</td>
</tr>
<tr>
<td>Thamnidium chaetocladiodes</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Thamnidium elegans</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Mucor mucedo</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Botrytis cinerea</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Penicillium glaucum</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Cladosporium herbarum</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Chaetostylum freseii</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>

¹ Expected Q₀ at 0° to −5° C. is calculated from the observed Q₀ at +10° to 0°, assuming that μ is constant.
section it was shown that growth rate was more sensitive to temperature differences in the lower range than in the higher. In other words, the $Q_{10}$ for growth is higher in the lower range than one would expect it to be in a simple chemical reaction. Repeated experimentation on micro-organisms of various kinds has shown this invariably to be true (table 3). The $Q_{10}$ values for biochemical activities of micro-organisms are also higher than expected in the lower temperature range (table 4).

When a growth or activity coefficient is plotted according to the van't Hoff-Arrhenius method, as suggested by Crozier (87, 88) and others, $\mu$ is usually relatively constant over a considerable temperature range (229, 423) (fig. 2) although a few have presented data suggesting that $\mu$ varies continuously with temperature (fig. 11). Typically, $\mu$ becomes very high near the minimum growth temperature (steep slopes at low temperatures in fig. 2). This increase in $\mu$ parallels the unexpectedly high values of $Q_{10}$ usually found in this range, as previously described.

Some authors have ascribed the increase in $\mu$ at low temperatures to a shift in the "master reaction" as the temperature is reduced. Of the many reactions occurring within the cell, some have higher values of $\mu$ than others. As the temperature is reduced, reaction rates are reduced more rapidly for reactions having the higher values of $\mu$, so that they tend to control and limit the rate of any physiological process to which they are essential.
Some have observed a definite break in the van't Hoff-Arrhenius plot and have considered this to be the temperature at which a reaction with a higher value of $\mu$ becomes the master reaction. This point has been termed the "critical temperature" (87). Critical temperatures are shown in figure 12 and in the data of other investigators (446, 477); less distinct ones are shown in figures 11 and 13. However, data can sometimes be drawn on different scales to show critical temperatures or not, as the author desires (55).
Ingraham (230) and Ng and others (356) concluded that the master reaction hypothesis could not account for the fairly well defined minimum growth temperature because \( \mu \) for growth appears to become infinite at the minimum growth temperature. They pointed out that the increase in \( \mu \) at low temperatures could
result from altered cell composition. They subjected cultures of an *Escherichia coli* strain in the logarithmic growth phase to sudden changes in incubation temperature. When the change remained within the temperature range where $\mu$ was constant, the cultures assumed their new growth rate without detectable lag. When they were transferred to or from temperatures in the range where $\mu$ was increasing as the temperature dropped, their new growth rate differed for several hours from that which was normal for the new temperature. It was concluded that this was the result of altered cell composition (or cell damage) caused by growth at the low temperature. Other explanations of minimum growth temperature have been reviewed elsewhere (327).

The $\mu$ values for oxygen consumption (fig. 13) and for growth (fig. 2) are higher for the mesophiles than for the psychrophiles (48, 229, 477). Table 5 shows that sonication to break up cells brought the $Q_{10}$ of glucose oxidation of a mesophile closer to that of a psychrophile. From this it was concluded that the higher

---

<table>
<thead>
<tr>
<th>Organism</th>
<th>Upper range</th>
<th>Middle range</th>
<th>Lower range</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temperature, °C</td>
<td>Observed $Q_{10}$</td>
<td>Expected $Q_{10}$</td>
<td>Observed $Q_{10}$</td>
</tr>
<tr>
<td>Cladosporium herbarum</td>
<td>5 to 15</td>
<td>4.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thamnidium chaetocladiodes</td>
<td>5 to 15</td>
<td>1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thamnidium elegans</td>
<td>5 to 15</td>
<td>2.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sporotrichum carnis</td>
<td>5 to 25</td>
<td>2.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T. chaetocladiodes</td>
<td>10 to 20</td>
<td>1.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas flourescens</td>
<td>5 to 20</td>
<td>3.7</td>
<td>0 to 5</td>
<td>4.1</td>
</tr>
<tr>
<td>Aerobacter aerogenes</td>
<td>20 to 30</td>
<td>1.6</td>
<td>10 to 20</td>
<td>1.65</td>
</tr>
<tr>
<td>Pseudomonas 92</td>
<td>20 to 30</td>
<td>1.8</td>
<td>10 to 20</td>
<td>1.9</td>
</tr>
<tr>
<td>Pseudomonas 69</td>
<td>20 to 30</td>
<td>2.5</td>
<td>10 to 20</td>
<td>2.7</td>
</tr>
<tr>
<td>Streptococcus fecalis</td>
<td>20 to 30</td>
<td>2.4</td>
<td>5 to 10</td>
<td>2.7</td>
</tr>
<tr>
<td>Achromobacter sp.</td>
<td>7 to 25</td>
<td>2.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For comparison, the expected $Q_{10}$ values in the lower and middle ranges have been calculated from the observed $Q_{10}$ in the upper range, with data from figure 3.

1 Calculated from curve.

2 Average of 4 tests.
Q₁₀ of mesophilic activity is connected in some way with the intact cell (281). For a more comprehensive discussion of differences between mesophiles and psychrophiles, see Michener and Elliott (327).

**TABLE 4.** Temperature coefficients of the growth and biochemical activity of three typical psychrophiles

<table>
<thead>
<tr>
<th>Temperature range, °C</th>
<th>Aerobacter aerogenes</th>
<th>Pseudomonas sp.</th>
<th>Pseudomonas sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Growth Q₁₀</td>
<td>Acid production Q₁₀</td>
<td>Growth Q₁₀</td>
</tr>
<tr>
<td>0-10</td>
<td>9.1</td>
<td>4.2</td>
<td>4.8</td>
</tr>
<tr>
<td>10-20</td>
<td>3.1</td>
<td>2.1</td>
<td>3.2</td>
</tr>
<tr>
<td>20-30</td>
<td>1.6</td>
<td>1.8</td>
<td>1.8</td>
</tr>
</tbody>
</table>

*Source:* Greene, V. W., and Jezeski, J. J. (165)

**TABLE 5.** Effect of sonication of cells on Q₁₀ of glucose oxidation by a psychrophile and a mesophile

<table>
<thead>
<tr>
<th>Organism</th>
<th>Cell condition</th>
<th>Q₁₀ of glucose oxidation ¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas perolens (a psychrophile)</td>
<td>Intact</td>
<td>1.78</td>
</tr>
<tr>
<td></td>
<td>Sonicated</td>
<td>1.58</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa (a mesophile)</td>
<td>Intact</td>
<td>3.16</td>
</tr>
<tr>
<td></td>
<td>Sonicated</td>
<td>1.91</td>
</tr>
</tbody>
</table>

¹ Q₁₀ between 10° and 30° C.

² Average of two determinations.


The relation between temperature and growth rate is influenced by the nature of the carbon substrates and the degree of aeration (267, 387). As shown in table 6, the Q₁₀ of growth of *Pseudomonas fluorescens* in stationary cultures with casamino acids decreased regularly with temperature rise, but there was an increase at 20° to 25° C. with glucose and citrate. This has been interpreted to mean that more than one temperature-influenced metabolic system is involved with glucose and citrate but that primarily only one is involved with casamino acids (287). When the cultures were aerated, those on casamino acids showed lower...

*Figure 11.* A van't Hoff-Arrhenius plot showing the effect of temperature on growth rate of *Sporotrichum canis*. $k = \frac{\log L_2 - \log L_1}{t_2 - t_1} \times 2.303$ where $L_1$ and $L_2$ are total length of hyphae at times $t_1$ and $t_2$, respectively (179).
Figure 12.—Effect of temperature on intensity of light from luminous bacteria (333).

$Q_{10}$ values at 4° to 10° than did the other cultures. These data show that aeration and utilization of organic nitrogen-containing compounds is related to metabolic transformations that permit low-temperature growth of \textit{Pseudomonas fluorescens} (267).
19 PSYCHROPHILIC MICRO-ORGANISMS IN FOODS

TEMPERATURE, °C.

Figure 13.—Effect of temperature on oxygen consumption by Pseudomonas aeruginosa (X) and a psychrophile (0) (48).

Table 6.—Effect of temperature, nutrition, and aeration on the Q10 of growth of Pseudomonas fluorescens

<table>
<thead>
<tr>
<th>Media</th>
<th>Culture</th>
<th>Q10 values at various temperature intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4° to 10° C.</td>
</tr>
<tr>
<td>Glucose</td>
<td>Stationary</td>
<td>4.04</td>
</tr>
<tr>
<td></td>
<td>Shake</td>
<td>3.62</td>
</tr>
<tr>
<td>Citrate (Na)</td>
<td>Stationary</td>
<td>4.25</td>
</tr>
<tr>
<td></td>
<td>Shake</td>
<td>3.89</td>
</tr>
<tr>
<td>Casamino acids</td>
<td>Stationary</td>
<td>4.49</td>
</tr>
<tr>
<td></td>
<td>Shake</td>
<td>2.25</td>
</tr>
</tbody>
</table>

Source: Jezeski, J. J., and Olson, R. H. (267).

Lipids

Kates and Baxter (278) have reviewed the literature and confirmed by their own investigation on Candida that organisms growing at low environmental temperatures have more highly unsaturated lipids than do organisms growing at higher temperatures. Rose (402) suggested that this phenomenon might account for the diminished growth rate of micro-organisms at low temperatures.

Wells and others (532) have suggested that the minimum growth temperature can be explained by decreased absorption of
nutrients caused by an increase in the amount of fat in the membranes. They described one strain of *Breitbacterium linens* that produced 7.2 percent of fat at 25°C, where it grew well; but it produced 16.7 percent at 4°C, where it grew poorly. Two typical psychrophilic bacteria displayed no such temperature-induced differences. Eklund confirmed that bacteria that grew poorly at 4°C produced more fat there than at 9.4°C or at 22°C. However, one can do no more than speculate that higher fat production or greater unsaturation may explain minimum growth temperature, for much more convincing data are needed.

**Enzyme production and activity**

Enzymes reduce the energy of activation required to cause chemical reactions under physiological conditions at rates useful to the cell. Although some strains of bacteria cannot ferment carbohydrates near their minimum growth temperatures (20), more commonly microorganisms produce a greater quantity of enzyme at a low than at a high temperature (table 7 and fig. 14). On the other hand, the activities of the isolated enzymes themselves often have high temperature maxima—indeed, even above the optimum growth temperature of the organism (fig. 15). The data in figures 14 and 15 are combined in table 8 with those of other workers to show that this relation is commonly found. Precht and others (388) have summarized similar results of German investigations on *Streptococcus lactis* and a parasitic mold. A psychrophilic bacterium failed to produce formic hydrogenlyase at 35°C but produced this enzyme at temperatures between 0° and 20°C (517).

**Table 7.** Catalase activity in psychrophilic bacteria harvested at various ages during incubation at 2°C and at 30°C C.

<table>
<thead>
<tr>
<th>Incubation temperature, °C</th>
<th>Harvest age</th>
<th>Specific catalase activity (×10⁵)¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>Pseudomonas</em> strain 92</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>256</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>198</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>153</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>877</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>656</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>382</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>433</td>
</tr>
<tr>
<td></td>
<td></td>
<td>329</td>
</tr>
</tbody>
</table>

¹ Catalytic reaction rate constant at 10°C C. per minute per 10⁶ cells.


A psychrophilic *Cryptococcus* failed to grow at 30° C. until it had first been grown at 16° (figure 16). At 30° it was unable to synthesize α-oxoglutarate, but this was not the full explanation because there were also unknown temperature-sensitive metabolic processes (177). Jezeski and Olson (267) found that with the psychrophile *Pseudomonas fluorescens*, the temperature at which a culture was grown influenced the respiratory response to several substrates.

Spoilage while bacterial counts are still low could be the result of high enzyme production by bacteria at low temperatures.
Peterson and Gunderson (382) reported spoiled poultry pies with counts of $10^6$. Michener and others (326) reported off-flavor of vegetables before the bacterial count had increased significantly at $-3.9^\circ \text{C}$ to $+4.4^\circ \text{C}$, but attributed this to enzymatic or chemical changes unrelated to bacteria. Low counts at time of evident decomposition are relatively unusual, for most foods have counts of $10^7$ and $10^8$ per gram at this time (113).

Enzyme activity continues at temperatures too low to allow growth. Lipases from fish spoilage bacteria (216) and from other bacteria and molds (3, 4) have been found active at temperatures below the temperature range of growth of the bacteria which pro-
TABLE 8.—Relations between enzyme production, enzyme activity, and optimum growth temperature for various organisms and enzyme systems

<table>
<thead>
<tr>
<th>Organism and enzyme system</th>
<th>Maximum enzyme production</th>
<th>Maximum enzyme activity</th>
<th>Optimum growth rate</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>6 strains of psychrophiles (dehydrogenase)</td>
<td></td>
<td>44</td>
<td>24 to 30</td>
<td>(71)</td>
</tr>
<tr>
<td>Pseudomonas fluorescens (lipase)</td>
<td>20</td>
<td>60</td>
<td>ca 29</td>
<td>(2)</td>
</tr>
<tr>
<td>“K-10” (urease)</td>
<td></td>
<td></td>
<td></td>
<td>(283)</td>
</tr>
<tr>
<td>Pseudomonas fluorescens (proteases)</td>
<td>0</td>
<td>25 or above</td>
<td>20</td>
<td>(383)</td>
</tr>
<tr>
<td>Pseudomonas fragi (lipase)</td>
<td>15</td>
<td>40</td>
<td></td>
<td>(351, 352)</td>
</tr>
<tr>
<td>Streptococcus lactis (fermentation)</td>
<td></td>
<td>40</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>Streptococcus thermophilus (fermentation)</td>
<td></td>
<td>47</td>
<td>37</td>
<td></td>
</tr>
</tbody>
</table>

- Enzyme activity and growth temperatures for various organisms and enzyme systems are listed in the table.
- Maximum enzyme production, enzyme activity, and optimum growth rates are indicated for each organism.
- The reference number for each entry is provided in parentheses.

Invertedase activity has been reported (273) at -16° to -12° C., but not within 55 days at -40°. Enzyme activity con-

![Figure 16](image-url)
tinues in soils after bacterial growth has been inhibited by cold (514).

Activity rates of enzymes at low temperatures above freezing can be predicted by extrapolation from their activity rates at higher temperatures (305). The activities of the enzymes with low \( \mu \) become increasingly important as temperature is lowered. However, when the substrate freezes, the temperature characteristics of enzyme activities usually change abruptly, and thus it is impossible to predict enzymatic reaction rates below 0\(^\circ\) C. from studies made above that temperature (305, 446). A high \( \mu \) in the frozen material shows the efficacy of lowering freezer temperatures to slow enzymatic action (446).

Many investigations have shown that enzymes are little injured by freezing. Indeed some (28, 95, 96, 280) have claimed they were more active for having been frozen and thawed. Young (554) used 18 cycles of freezing and thawing to prepare cell-free extracts of *Escherichia coli* for enzyme studies. Luyet and Gehlenio (311) reviewed the early literature, which showed that many enzymes can withstand freezing and storage at very low temperatures.

In practical storage tests, various foods have been adversely affected by microbial enzymes at temperatures below the growth range (table 9). On frozen vegetables, however, quality was unrelated to the bacterial count (223). It is common knowledge that naturally occurring plant enzymes must be inactivated by blanching before vegetables can be stored in the freezer. Although relatively few studies have been made of this subject, it is clear that enzymes of either microbial or nonmicrobial origin may cause food deterioration at temperatures too low for microbial growth.

<table>
<thead>
<tr>
<th>Food product</th>
<th>Storage temperature</th>
<th>Result of high microbial level</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacon</td>
<td>-17.8</td>
<td>Rancidity</td>
<td>(264)</td>
</tr>
<tr>
<td>Butter</td>
<td>-10</td>
<td>Lipolysis</td>
<td>(353)</td>
</tr>
<tr>
<td>Pork sausage</td>
<td>-18</td>
<td>Deleterious odor</td>
<td>(317)</td>
</tr>
<tr>
<td>Chicken pies</td>
<td>-12.2</td>
<td>Rancidity</td>
<td>(299)</td>
</tr>
<tr>
<td>Fats</td>
<td>-7 to -29</td>
<td>Lipolysis</td>
<td>(3, 4)</td>
</tr>
</tbody>
</table>

**Cell size and cell crop**

Bacterial cell size is larger at low temperatures than at high temperatures, but the converse is true with yeasts (213, 288). In addition, cell size changes during the various phases of growth. The largest cells occur in late lag phase and during the logarithmic phase.
Cell crop often has been described as largest below the temperature where growth is most rapid. Hess (211) found total crops of marine bacteria at $-3^\circ$ and $0^\circ$ C. to be larger than those at $20^\circ$ or $37^\circ$, and he recommended maximum cell crop as a criterion of optimum growth temperature. Dorn and Rahn (103) reported that the streptococci do not always produce their largest number of cells at the temperature of most rapid growth, as follows:

<table>
<thead>
<tr>
<th></th>
<th>Fastest rate of growth of cells at $^\circ$C.</th>
<th>Largest number of cells at $^\circ$C.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus lactis</em></td>
<td>34</td>
<td>25-30</td>
</tr>
<tr>
<td><em>Streptococcus thermophilus</em></td>
<td>37</td>
<td>37</td>
</tr>
</tbody>
</table>

Higher cell crops at low temperatures were reported in two investigations on milk (fig. 17). However, data in figure 18 indicate the opposite effect. Also, in the genus *Arthrobacter*, the number of generations produced (equivalent to cell crop) is not greatly affected by incubation temperature within the temperature range of growth of the organism (407).

Most of the foregoing authors failed to consider the oxygen relations of their cultures. Sinclair and Stokes (445) showed that higher cell crops at low temperatures can be explained by the greater solubility of oxygen. With continuous aeration of cultures at both high and low temperatures the difference is erased (table 10). Upadhyay and Stokes (510) found that a facultatively anaerobic psychrophilic rod produced maximal cell crop aerobically at $5^\circ$ C. but anaerobically at $25^\circ$.

**Table 10.**—Effect of temperature and aeration on maximal cell yields of *Pseudomonas aeruginosa*.

<table>
<thead>
<tr>
<th>Temperature, $^\circ$C.</th>
<th>Stationary</th>
<th>Aerated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maximal count per milliliter</td>
<td>Time to reach maximum</td>
</tr>
<tr>
<td></td>
<td>Billions</td>
<td>Hours</td>
</tr>
<tr>
<td>20</td>
<td>6</td>
<td>25</td>
</tr>
<tr>
<td>30</td>
<td>11</td>
<td>75</td>
</tr>
</tbody>
</table>

*In trypticase soy broth containing 1 percent glucose in 1.4 percent phosphate buffer.*


### Adaptation

Reports of adaptation of micro-organisms to low temperature growth are few. Hess (210, 214) found that by cultivating bacterial psychrophiles at $5^\circ$ C. he was able to produce "adapted" strains that were more active at $0^\circ$ and at $-3^\circ$ than were strains cultured at $20^\circ$. Also one strain of *Pseudomonas fluorescens* pro-
duced more colonies at 0° and -8° after having been grown at 5°. Others (164, 221, 534) similarly showed that the incubation temperature of the mother culture markedly influenced speed of response at low temperatures. Chistyakov and Noskova (75) successfully adapted various bacterial strains to growth at -2° by growing them at 0° to -8° for 2 years. As shown in table 11, there were more colonies at -2° from adapted strains than there were from control strains previously grown at 20°. Similarly, lag was shorter in adapted strains; however, minimum

---

generation time was very little different (23 to 24 hours and 25 to 26 hours, respectively).

**TABLE 11.—Effect of adaptation by growth at 0° to −8° C. for 2 years on growth of bacteria at −2°**

<table>
<thead>
<tr>
<th>Species and strain No.</th>
<th>Controls</th>
<th>Adapted</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Percent</td>
<td>Percent</td>
</tr>
<tr>
<td><em>Achromobacter</em> sp.:</td>
<td>80</td>
<td>85</td>
</tr>
<tr>
<td>1</td>
<td>59</td>
<td>98</td>
</tr>
<tr>
<td>2</td>
<td>91</td>
<td>132</td>
</tr>
<tr>
<td>3</td>
<td>78</td>
<td>93</td>
</tr>
<tr>
<td>4</td>
<td>84</td>
<td>350</td>
</tr>
<tr>
<td><em>Flavobacterium</em> sp.:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>55</td>
<td>108</td>
</tr>
<tr>
<td>6</td>
<td>83</td>
<td>132</td>
</tr>
<tr>
<td>7</td>
<td>97</td>
<td>193</td>
</tr>
<tr>
<td><em>Pseudomonas</em> fluorescens</td>
<td>82</td>
<td>105</td>
</tr>
<tr>
<td><em>Pseudomonas</em> herbicola:</td>
<td>89</td>
<td>109</td>
</tr>
<tr>
<td>13</td>
<td>85</td>
<td>105</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Serratia</em> rubra/faciens</td>
<td>86</td>
<td>111</td>
</tr>
</tbody>
</table>

1 As percentage of those developing at 20° C.

SOURCE: Chistyakov, F. M., and Noskova, G. (75).

They obtained similar results with several molds. Adapted strains of *Penicillium glaucum* showed a more rapid growth at −5° to 2° C. (table 12). A relatively quick adaptation to rapid growth at 0° was shown by *Monilia nigra* (fig. 19).

Certainly in geologic time, mutants capable of growth in continuous cold must occur, and these would become predominant in such an environment by selectivity (7, 101). But laboratory studies always cover too brief a period to give data showing an impressive degree of adaptation, and artificially induced mutations of this type have not been reported. A review of adaptation was made by Precht and others (388).

**TABLE 12.—Effect of adaptation by growth at −5° C. on growth of *Penicillium glaucum* at −5° to 2°**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Time required for visible growth at—</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−5° C.</td>
</tr>
<tr>
<td></td>
<td>Days</td>
</tr>
<tr>
<td>Unadapted</td>
<td></td>
</tr>
<tr>
<td>Adapted</td>
<td>96</td>
</tr>
</tbody>
</table>

SOURCE: Chistyakov, F. M., and Noskova, G. (75).

We have found no report that a mesophile has ever been adapted to grow below its normal minimum growth temperature. On
EFFECT OF WATER AVAILABILITY ON MICROBIAL ACTIVITIES

In addition to being dependent on temperature, microbial activities are also highly dependent on availability of water, and their water and temperature requirements are interdependent. The term usually used to designate availability of water is "water activity" ($a_w$), defined as $P/P_o$, where $P$ and $P_o$ are vapor pressures of the solution or system under consideration and of pure water, respectively. $P/P_o \times 100$ is also the relative humidity (RH) of the atmosphere in equilibrium with the solution. Thus, pure water will have an $a_w$ of 1.00 and be in equilibrium with an RH of 100 percent; a molar concentration of an ideal non-ionized solute will have an $a_w$ of 0.9823 (426).

If the solution begins to freeze, pure water freezes out and the solution becomes more concentrated. This process continues as the temperature is lowered. Under these conditions, the solute...
particle concentration depends only on temperature; therefore, the
\( a_w \) of a partly frozen system depends only on its temperature,
again assuming that it is in equilibrium (327, 426, 427). The
following values of \( a_w \) occur at temperatures below freezing
(426):

\[
\begin{array}{c|c}
\text{Temperature} & a_w \\
{^\circ C.} & \\
-5 & 0.9526 \\
-10 & 0.9074 \\
-15 & 0.8642 \\
\end{array}
\]

These relations hold for foods and for microbiological media as
well as for solutions. However, foods and media usually contain
colloidal materials that bind water. Under specific conditions the
amount of water bound by a colloid depends on the colloid and the
\( a_w \) of the system (158). For this and other reasons, the \( a_w \) of a
system does not exactly parallel its water content.
Bacteria require a higher a_w than yeasts, which in turn require a higher a_w than molds. Any condition that lowers a_w inhibits bacteria first, then yeasts, then molds (295, 426, 427). This explains why molds can grow at lower temperatures, in drier foods, and in higher salt concentrations than can bacteria. The growth of a yeast and two species of bacteria on thin slices of beef at various relative humidities is shown in figure 20. The ability of the yeast to grow at much lower values of RH is evident.

Whenever the minimum growth temperature, minimum a_w, or other minimum is determined for growth of an organism, all other factors should be optimum. As the temperature is reduced, a higher a_w is required (table 13). Achromobacter will grow at a lower RH at 4° C than at 2° or -1° (fig. 21). The lowest a_w at which growth of nonhalophilic bacteria has been reported is 0.86 on food (425) and 0.84 on high sodium chloride media (76). The lowest at which mold growth has been reported is 0.62 (453).

Wodzinski and Frazier (542, 543, 544), using Pseudomonas fluorescens, Aerobacter aerogenes, and Lactobacillus viridescens, confirmed the effect of temperature and added the following: (1) At unfavorable pH the tolerance to low a_w was less; (2) when both pH and temperature were unfavorable, the tolerance to low
a_w was less than if only one condition was adverse; and (3) less than optimal quantities of essential nutrients also lessened the tolerance to low a_w. Scott (424) reported that at -1° C., 10-percent carbon dioxide inhibited bacteria and yeast more effectively at low a_w than at high a_w. Wodzinski and Frazier (545) obtained similar results at 15° but not at 20°. Insofar as they affect minimum growth temperatures of micro-organisms, these relations are discussed more fully elsewhere (327, 427).

Fluctuating storage temperatures during defrost cycles can encourage mold growth in frozen foods because water migrates from the food to adjacent package surfaces. This then provides the necessary humidity, while the top of the temperature cycle provides adequately high temperatures, so that mold growth can occur in packages of frozen foods whose a_w ordinarily would be too low to permit growth (172).
TABLE 13.—Effect of temperature on the minimum water activity permitting growth of various organisms

<table>
<thead>
<tr>
<th>Organism</th>
<th>Temperature °C</th>
<th>Minimum water activity (aw or R.H./100)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternaria citri</td>
<td>37</td>
<td>0.876</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.876</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>0.838</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>0.838</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.838</td>
<td></td>
</tr>
<tr>
<td>Penicillium sp.</td>
<td>10</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>Cladosporium sp.</td>
<td>10</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td>Mucor sp.</td>
<td>10</td>
<td>0.86</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.88</td>
<td></td>
</tr>
</tbody>
</table>

1 Optimum for this organism.

SPOILAGE OF MEATS AND FISH
Effect of low temperature

The effect of storage temperature on the spoilage rate of foods is illustrated for chilled poultry meat in figures 22 and 23, for chilled beef in figure 24, and for chilled fish in figure 25. Decreas-
The effect of ambient temperature during thawing on the lag period of natural flora in frozen foods is shown in figure 26. These data, however, were obtained by incubating plates at $32^\circ$ C. It is possible that many of the psychrophilic bacteria growing in the food at the lower temperatures were not recovered on plates incubated at $32^\circ$. If this was so, a falsely long lag period is shown at the low temperatures in this figure. The occurrence of false lag periods in chilled foods from incubation of plates at high temperatures has been shown for coconut pies (387) and chicken meat (114).

Cooling milk from $5^\circ$ to $0^\circ$ C. increased the keeping time as much as did cooling from $30^\circ$ to $5^\circ$ (165). The keeping time of processed meats was more than twice as long at $1.1^\circ$ as it was...
at 7.2° (6). All these data point to the advantage of bringing the temperature of chilled products as low as possible without actually freezing them.

Attempts to bring the temperature below 0° C. to enhance storage, but still without freezing the product, have been singularly successful with fish. Refrigerated sea water at −1° or slightly lower keeps fish twice as long as ice at 0° (507, 399), and this increase is due entirely to reduced temperature (466). However, the advantage of the lower temperature is lost if heavy bacterial loads are allowed to grow or accumulate in the brine (464). Storage at −5° to −10° with sugar or salt to reduce the freezing point has been suggested (502), but such treatment does not yield as good a product as quick-freezing.

**Floral changes in spoilage**

When foods are held at refrigeration temperature, it is obvious that mesophiles will not grow and may perhaps drop in numbers,
whereas the psychrophiles will grow to predominance as spoilage progresses. The flora on the meat of recently killed warm-blooded animals is always predominantly mesophilic, whereas that on fish is predominantly psychrophilic. For this reason, there is a distinct floral change during low-temperature spoilage of poultry and red meats, but a lesser change during low-temperature spoilage of fish.

In poultry spoilage, the pseudomonads are favored (fig. 27). Nagel and others (350) reported that of 108 cultures from spoiled poultry, 88 were *Pseudomonas*, 2 were *Aeromonas*, and 13 were either *Achromobacter* or *Alcaligenes*. The pseudomonads present at the spoiled stage are predominately nonpigmented strains (28, 493). Molds or yeasts will predominate in spoilage of poultry.
Figure 26.—Effect of ambient temperature on lag period of flora in thawing foods. Plates were incubated at 32° C. (224, 225).
meat only when growth conditions are somewhat adverse for bacteria—for example, at high freezer temperatures (molds), after irradiation (yeasts), or in the presence of antibiotics (yeasts or molds) \((441, 493, 526, 552)\).

The floral changes during spoilage of refrigerated red meat are similar to those for poultry. Pseudomonads are favored as spoilage progresses at chill temperatures \((16, 194, 284, 400)\) (table 14), although sometimes a few lactobacilli are present \((194, 284)\). Floral changes are least marked when the predominating flora at the beginning of the storage period is composed of typical psychrophilic slime organisms (fig. 28). Ayres \((16)\) found that at \(15^\circ C.\) or above, *Micrococcus* and *Pseudomonas* were present in about equal numbers.

Like poultry, red meats support the growth of yeasts or molds only if there is a condition that stops or slows bacterial growth. For example, Whitehill \((536)\) found that bacterial growth on
meats impeded yeast growth but that the application of antibiotics to prevent bacterial growth favored the growth of yeasts. Storage in the range -5°C to -10°C usually favors mold growth on carcases, probably because it is near or below the minimum growth temperature for most psychrophilic bacteria (827) but also because the surfaces are usually dry. (See “Relative humidity,” p. 52.)
TABLE 14.—Changes in bacterial flora during spoilage of ground beef at 7°C.

<table>
<thead>
<tr>
<th>Micro-organism</th>
<th>Proportion of total isolates after</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 days</td>
</tr>
<tr>
<td></td>
<td>percent</td>
</tr>
<tr>
<td>Pseudomonas or Achromobacter, or both</td>
<td>4</td>
</tr>
<tr>
<td>Bacillus</td>
<td>28</td>
</tr>
<tr>
<td>Microbacterium</td>
<td>0</td>
</tr>
<tr>
<td>Micrococci</td>
<td>26</td>
</tr>
<tr>
<td>Yeasts</td>
<td>42</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>

1 From plates at 7°C.


Spoilage of sausages falls into a special category because they are usually heated in impervious casings where there is low oxygen tension and fairly high salt concentration, and are then refrigerated. Few heat-resistant organisms can grow under these conditions. In one investigation (465), the predominant organisms in freshly made liver sausage were aerobic micrococci and sporulating rods, neither of which grew at 5°C. An organism similar to a Lactobacillus and a facultative anaerobic yeast finally spoiled the product after several weeks. Processed vacuum-packed meats have been spoiled by lactobacilli (6). On the other hand, the slime that commonly forms on the surface and between the layers of sausage casings is due to the growth of yeasts, micrococci, and aerobic gram-negative rods (104, 465). A review of sausage spoilage has been made by Jensen (263).

Floral changes during decomposition of fish caught in the temperate or arctic zones are not so marked as they are in meat spoilage because the organisms predominating naturally on the surface of the live or recently killed fish, particularly after it contacts ice and deck surfaces, are the same psychrophiles as the ones that cause decomposition (11, 65, 228, 437, 482). However, the Pseudomonas-Achromobacter group is favored during spoilage in ice (69, 162, 202, 439, 440, 549). In tropic zones the floral change in favor of the Pseudomonas group during low-temperature spoilage is more marked because of the lower percentage of psychrophiles on the recently killed fish (437). The nonpigmented pseudomonads contribute most of the tissue breakdown and odors of decomposition (440). Flavobacterium plays a minor role (435, 439).

*Many organisms that in earlier work were identified as Achromobacter because they lacked pigments would now, according to the 5th, 6th, and 7th editions of Bergey’s Manual (33, 44, 45) be classed as Pseudomonas on the basis of flagellation (50, 249).
Floral changes in favor of the *Pseudomonas-Achromobacter* group also occur in crabmeat and in shrimp during spoilage (tables 15 and 16) (5, 60, 495).

### TABLE 15.—Changes in flora of Louisiana crabmeat stored at 3° to 5° C.

<table>
<thead>
<tr>
<th>Days in storage</th>
<th>Colonies picked</th>
<th>Proportion of total isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cocci</td>
</tr>
<tr>
<td></td>
<td>Number</td>
<td>Percent</td>
</tr>
<tr>
<td>1</td>
<td>525</td>
<td>60.4</td>
</tr>
<tr>
<td>5-8</td>
<td>420</td>
<td>30.9</td>
</tr>
<tr>
<td>11-15</td>
<td>598</td>
<td>3.0</td>
</tr>
</tbody>
</table>


The type of organism growing in milk during storage depends on the storage temperature, as shown in table 17. *Pseudomonas* predominates at refrigeration temperatures. However, some of the coliforms also grow readily in milk at 3° to 5° C. (371). On the basis of 586 isolations, Schultze 6 reported the bacterial content of pasteurized milk stored at 4° for 1 to 2 weeks as follows:

<table>
<thead>
<tr>
<th>Percent</th>
<th>Pseudomonas</th>
<th>Alcaligenes</th>
<th>Achromobacter</th>
<th>Flavobacterium</th>
<th>Coliforms</th>
<th>Yeasts</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>170.6</td>
<td>7.9</td>
<td>9.2</td>
<td>.7</td>
<td>10.8</td>
<td>.8</td>
<td></td>
<td>100.0</td>
</tr>
</tbody>
</table>

65.2 percent of these were fluorescent.

**Inoculum size**

It is well established that high initial contamination results in more rapid spoilage of chilled foods than does low initial contamination. Duncan and Nickerson (105), however, showed that inoculum size did not affect growth rate in the logarithmic phase of a pure culture of *Pseudomonas fragi*. Essentially the same observation can be made with the natural inoculum on beef and on chicken meat (figs. 28 and 29) with the exception of the lowest inoculum in figure 29, which may not have been followed long enough to reach logarithmic growth. In the experiments represented by these figures, the cultures grown from the smaller inocula had longer *lag* periods. These observations confirm the early

---

### Table 16.—Changes in bacterial flora of Gulf shrimp during storage in crushed ice

<table>
<thead>
<tr>
<th>Days in storage</th>
<th>Proportion of total isolates</th>
<th>( \text{%} )</th>
<th>( \text{%} )</th>
<th>( \text{%} )</th>
<th>( \text{%} )</th>
<th>( \text{%} )</th>
<th>( \text{%} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Achromobacter</td>
<td>Bacillus</td>
<td>Flavobacterium</td>
<td>Micrococcus</td>
<td>Pseudomonas</td>
<td>Miscellaneous</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>27.2</td>
<td>2.0</td>
<td>17.8</td>
<td>33.6</td>
<td>19.2</td>
<td>.2</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>31.3</td>
<td>.6</td>
<td>13.1</td>
<td>23.0</td>
<td>26.5</td>
<td>.5</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>46.0</td>
<td>2.0</td>
<td>18.0</td>
<td>5.7</td>
<td>28.0</td>
<td>.3</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>67.0</td>
<td>0</td>
<td>2.0</td>
<td>.8</td>
<td>30.1</td>
<td>.1</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>82.0</td>
<td>0</td>
<td>1.5</td>
<td>0</td>
<td>16.5</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*Source:* Campbell, L. L., Jr., and Williams, O. B. (60).
TABLE 17.—Bacteria predominating in milk held at various temperatures

<table>
<thead>
<tr>
<th>Temperature, °C.</th>
<th>Bacterial types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Under 5</td>
<td>Pseudomonas</td>
</tr>
<tr>
<td>5-10</td>
<td>Proteus, Micrococcus, alkali-forming rods.</td>
</tr>
<tr>
<td>10-15</td>
<td>Aerobacter and lactic streptococi.</td>
</tr>
<tr>
<td>15-30</td>
<td>Lactic streptococci.</td>
</tr>
<tr>
<td>30-40</td>
<td>Escherichia and Aerobacter, various types of strepto-</td>
</tr>
<tr>
<td></td>
<td>cocci and lactobacilli.</td>
</tr>
<tr>
<td>Above 40</td>
<td>Lactobacilli and high temperature streptococci.</td>
</tr>
</tbody>
</table>

Source: Davis, J. G. (91).

work of Penfold (377), who reported that an increased inoculum size diminished lag time.

Ayres and others (18) stored cut-up chicken with initial contamination at three levels. The chicken with the highest contami-

![Figure 29.—Effect of initial bacterial load on shelf life of chicken meat at 4.4° C. (18).](image-url)
nation reached the spoiled stage faster than the cleaner meat (fig. 29). When spoilage time of chicken meat is plotted against log of the initial count, the relation appears to be linear, as shown in figure 30. The position and slope of the line depends on storage temperature. Corresponding data for beef are presented in figure 31 and for fish in figure 32. Similar results were obtained with veal and milk (265) and with sliced processed meats (8).

Fromm (135) chilled chickens in slush ice containing chlorine and attributed increased subsequent keeping time to the decreased bacterial numbers during chilling (fig. 33). He also reported (136) that even repeated use of slush ice as many as five times had no deleterious effect on shelf life of birds chilled. However, others (26, 118, 119) found that extended chill time reduced subsequent shelf life because bacterial numbers increased during chilling. These differing results could have been due to Fromm’s use of chlorinated chill water.

If initial contamination is composed largely of mesophiles, however, their numbers will not affect the spoilage rate. Only the level of the psychrophiles is significant in spoilage of foods held at chill temperatures. Castell and others (67) inoculated Escherichia coli, Aerobacter cloacae, Bacillus subtilis, B. mycoides, B. mesentericus, 11 species of Micrococcus, and other mesophiles onto sterile fish muscle at 2° to 3° C. None spoiled the product. However, when he used Proteus and Pseudomonas isolated from fish, the fish spoiled in 5 to 6 days at this temperature.

![Graph](image-url)
In a similar way, Ayres (14) showed that the initial level of the typical psychrophilic slime organisms on beef determined keeping time, and that other bacteria were not significant (fig. 28).

The presence of mesophiles might explain the interesting results of Yamamura (553), who found that the $Q_{10}$ of bacterial growth on fish was higher when the fish had come from warm waters than when they had come from cold waters. The temperature coefficient of growth for natural flora on fish (comparing $5^\circ$ and $17^\circ$ C.) was as follows:

<table>
<thead>
<tr>
<th>Species tested (Number)</th>
<th>Average $Q_{10}$</th>
<th>Temperature of water from which fish came</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>5.34</td>
<td>Warm</td>
</tr>
<tr>
<td>13</td>
<td>3.8</td>
<td>Temperate</td>
</tr>
<tr>
<td>8</td>
<td>2.87</td>
<td>Cold</td>
</tr>
</tbody>
</table>

He attributed these differences to the types of bacteria in the waters of different temperatures. Shewan (437) has reviewed literature demonstrating that such differences do exist.
Freezing and thawing

That bacteria are injured by freezing and thawing is well established (452), so that their ability to grow and decompose their substrate may be impaired. On the other hand, it is known also that the tissues of foods are damaged by freezing and thawing so that juices may become more easily available to surviving bacteria. Furthermore, there are reports that frozen-thawed bacteria in pure culture and in egg melange sometimes grow faster than those that have never been frozen (203, 204). Thus, counteracting influences are at work. Even before frozen foods came into general use, the effect of freezing on subsequent chilled
shelf life had been investigated repeatedly. The early investigations have been mentioned by Marginesu (322). A re-evaluation is important because some products, such as poultry and fish, are often frozen for shipment, then thawed for retail sale.

Table 18 lists the investigations that have been undertaken in this field. Although the belief that frozen-thawed foods spoil faster than their fresh counterparts is widespread, there seem to be few data that confirm this, and four investigations indicate a slower spoilage in frozen-thawed foods. The overwhelming evidence, however, is that there is no effect or an insignificant effect.

The results of five investigations warrant further discussion. Stewart (469) made an extensive study of the effect of freezing and thawing on shelf life of haddock. She included variables such as air- and brine-freezing; 0, 6, and 12 weeks of frozen storage; and storage at -21° and -12° C. In all six experiments, there was no significant difference in the rate of bacterial growth nor in the rate of increase of volatile nitrogen in the fresh and thawed muscle. In all experiments, there was a lag period of 6 to 9 days before the organisms began to increase rapidly. She reported that differences that do exist between fresh and thawed fish (i.e.

---

**Figure 33.**—Half chickens: Effect of chill time in slush ice on bacterial numbers and shelf life (135).
### Table 18. Review of investigations showing the effect of freezing and thawing on the subsequent spoilage rate of foods

<table>
<thead>
<tr>
<th>Effect of freeze-thaw (investigators' conclusions)</th>
<th>Product</th>
<th>Reviewers' comments</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faster spoilage.</td>
<td>Fats, proteins</td>
<td>An enzyme study; no bacteriological data.</td>
<td>(23)</td>
</tr>
<tr>
<td></td>
<td>Meat</td>
<td>No data presented.</td>
<td>(120)</td>
</tr>
<tr>
<td></td>
<td>do</td>
<td>Claim of faster spoilage based on inadequate data.</td>
<td>(167)</td>
</tr>
<tr>
<td></td>
<td>Chickens</td>
<td>No data presented.</td>
<td>(374)</td>
</tr>
<tr>
<td></td>
<td>do</td>
<td>Thawed and nonfrozen chickens were separate lots; direct comparison not warranted.</td>
<td>(524, 525)</td>
</tr>
<tr>
<td>Slower spoilage.</td>
<td>Eggs</td>
<td>E. coli growth rate...</td>
<td>(203, 204)</td>
</tr>
<tr>
<td></td>
<td>Beef, pork</td>
<td>Longer lag, slower growth</td>
<td>(472)</td>
</tr>
<tr>
<td></td>
<td>Fish</td>
<td>Longer lag after 3 to 6 months storage.</td>
<td>(1)</td>
</tr>
<tr>
<td></td>
<td>do</td>
<td>Slightly longer lag period</td>
<td>(310)</td>
</tr>
<tr>
<td></td>
<td>Meat</td>
<td>Investigator's conclusion that rate of thaw or length of storage influenced spoilage seems based on inadequate data.</td>
<td>(322)</td>
</tr>
<tr>
<td>Insignificant effect or none.</td>
<td>do</td>
<td>Growth slightly faster on frozen-thawed, but author considered the difference insignificant.</td>
<td>(277)</td>
</tr>
<tr>
<td></td>
<td>Horse, pork</td>
<td>See table 17</td>
<td>(286, 287)</td>
</tr>
<tr>
<td></td>
<td>Beef</td>
<td></td>
<td>(472)</td>
</tr>
<tr>
<td></td>
<td>Chickens</td>
<td></td>
<td>(114, 355, 456, 457)</td>
</tr>
<tr>
<td></td>
<td>Fish</td>
<td></td>
<td>(115, 471)</td>
</tr>
<tr>
<td></td>
<td>Herring</td>
<td></td>
<td>(527)</td>
</tr>
<tr>
<td></td>
<td>Haddock</td>
<td></td>
<td>(86, 469, 470)</td>
</tr>
<tr>
<td></td>
<td>Halibut</td>
<td>See figure 34</td>
<td>(431)</td>
</tr>
<tr>
<td></td>
<td>Peas</td>
<td>Slightly longer lag</td>
<td>(535)</td>
</tr>
</tbody>
</table>


Browning of blood, gills, and flesh; diffusion of pigment; softening of flesh; and change in appearance of the eyes were correlated instead with mechanical and physicochemical effects of freezing, storage, and thawing. Tarr (181) found that the growth rate of spoilage bacteria was about the same in fresh minced halibut as in defrosted minced halibut (fig. 34). Kitchell and Ingram (286) reported a slightly longer lag time for bacteria in frozen-thawed horse meat and pork, which resulted in a slight but unimportant increase in keeping time at 10° (table 19).
(535) reported a 2- to 9-hour increase in lag time at 30° C. in broth cultures of *Streptococcus lactis* and *Leuconostoc mesenteroides* but an insignificant increase in lag in frozen-thawed peas, indicating a protective effect by the food product. Freezing and thawing did not affect rate of reproduction in the log phase.

Kitchell and Ingram (287) failed to demonstrate a difference in growth rate on the slightly wetter surfaces of chunks of meat that had been frozen and thawed.

It may be concluded that frozen-thawed foods spoil at about the same rate as those not frozen. If freeze-thaw has any effect at all, it is to increase slightly the lag period. Disagreements in the literature could be due to variation in experimental conditions. In any case, freezing has the advantage of delaying microbial growth until the thaw period and thus permits a longer storage than is possible at chill temperatures.

**Figure 34.**—Effect of freezing and thawing on rate of bacterial growth in minced halibut muscle at 1.5° C. (481).
TABLE 19.—Effect of freezing on subsequent bacterial spoilage of meats at 10° C.

<table>
<thead>
<tr>
<th>Product</th>
<th>Treatment</th>
<th>Frozen-thawed Hours</th>
<th>Not frozen Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pork</td>
<td>Minced</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>Horse</td>
<td>do</td>
<td>40</td>
<td>33</td>
</tr>
<tr>
<td>Do</td>
<td>Sliced, post rigor</td>
<td>41</td>
<td>43</td>
</tr>
<tr>
<td>Do</td>
<td>Sliced, pre-rigor</td>
<td>50</td>
<td>51</td>
</tr>
<tr>
<td>Do</td>
<td>Natural surfaces</td>
<td>40</td>
<td>38</td>
</tr>
<tr>
<td>Do</td>
<td>Stored 7 days at -20° C.</td>
<td>32</td>
<td>29</td>
</tr>
<tr>
<td>Do</td>
<td>Stored 28 days at -20° C.</td>
<td>35</td>
<td>29</td>
</tr>
<tr>
<td>Do</td>
<td>Stored 56 days at -20° C.</td>
<td>38</td>
<td>29</td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>37</td>
<td>33.8</td>
</tr>
</tbody>
</table>


Semipreserved canned hams and luncheon meats will keep well, even though they have received only a pasteurization treatment. However, after they have been chilled or frozen and then held above a chill temperature, their keeping time is relatively short. Halvorson and others (197) have reviewed the literature and offer three possible explanations for this phenomenon:

1. Low-temperature storage alters the food to make it a more favorable medium for spore germination and growth.
2. Low-temperature storage may alter spores so that they germinate more rapidly when brought to a higher temperature.
3. The spores may actually germinate at low temperatures.

Heat

Experience in marine microbiology (561) and in milk products (see p. 79) has established the fact that most psychrophiles are easily destroyed by relatively mild heat. Bacteria surviving pasteurization cannot grow in milk at refrigerator temperatures (91, 491, 541). Lawton and Nelson (297) found that when part of a population of a psychrophilic bacterium was killed by heat, the survivors had a slightly extended lag period at 25° C., a greatly extended lag at 10°, and there was no growth at 5° in a 7-day period (fig. 35). By dilution techniques it was determined that the absolute level of viable organisms at the beginning of the experiment did not affect the lag period in the range of numbers used. Also, chlorine did not result in the same prolonged lag at 5°. Sublethal heat also narrows the pH range of growth of Pseudomonas and makes them more fastidious in their requirements for nutrients (207, 208).

The presence of psychrophiles in pasteurized milk products is due to postpasteurization contamination, and scrupulous sanitation after pasteurization is necessary to ensure good keeping time (370).
Psychrophiles in meat are also easily killed. Most organisms that survive heat treatment cannot grow in the low oxidation-reduction potential and 5-percent salt of liver sausage at chill temperatures (485). The keeping quality of canned hams also depends on the inability of heat-resistant organisms to grow at low temperatures. Spoilage occurs when heat is inadequate during processing or when refrigeration is inadequate (120).

Scalding chickens to remove feathers destroys any psychrophiles that may be on the feathers and skin, but the birds are reinfected by psychrophiles during processing and heated skin is a better medium for subsequent growth. Scalding therefore results in reduced keeping quality (378). Scalding at 53°C gives a product with better shelf life than scalding at 60°C (119, 459, 557), although the shelf life can be equalized by reducing the exposure time at the higher temperature (119). A high scald temperature decreases costs of processing because feathers are more easily removed (459, 557).
Type of surface

Because micro-organisms need moisture and nutrients, they are inhibited by any film or membrane that keeps these things away from them. Thus, the unbroken skin of a flesh food, a dried surface, or a layer of fat reduces the rate and extent of microbial growth.

Spoilage of fresh beef is primarily a surface phenomenon (219). The growth in deep portions, if any occurs, is very slow (335). Bone taint is a deep spoilage due to clostridia (191) but this does not occur at refrigeration temperatures (58, 240). Haines (180) reported growth on lean meat at -5° C. but not on connective tissue covering the surface fat. Uncut meat lasts longer than cut meat (192); thus growth of bacteria in ground meat is rapid, and very high counts may be expected (284, 530). Fat in ground meat enhances growth (196).

The primary spoilage in poultry, as in red meat, is a surface growth of bacteria whose colonies become visible and coalesce to form slime when spoilage is advanced (18, 306). Bacteria are not carried through the skin to muscle nor to the cavity unless the skin is broken (346). Once bacteria have access to a cut surface or the body cavity they multiply more rapidly than on the skin (92, 517), although one group of authors (325) reported a more rapid growth on excised skin than on any other excised tissue.

Bacterial decomposition is slower in undrawn (New York dressed) poultry than in eviscerated poultry because the tissues in the former are protected by uncut skin surfaces (355, 379). In earlier years, carcasses were shipped undrawn and evisceration occurred at the retail level. However, it has been found that diffusion of decomposition products from the gut causes an off-flavor in the meat of New York dressed birds (468). Thus when the criterion is flavor, the New York dressed bird has a shorter keeping time, even though bacterial growth is less (22):

<table>
<thead>
<tr>
<th></th>
<th>Keeping Time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In ice 17°C.</td>
</tr>
<tr>
<td>New York dressed</td>
<td>11</td>
</tr>
<tr>
<td>Eviscerated, ready-to-cook</td>
<td>6</td>
</tr>
</tbody>
</table>

However, the British still prefer the stronger flavor of meat from uneviscerated chickens (442).

Fish spoilage is likewise primarily due to growth of micro-organisms on the surface, for the flesh of sound living fish is sterile (54, 227). Unopened fish generally keep better than eviscerated fish (527) unless they have been feeding heavily, in which case the opposite is true because of the high level of bacteria and enzymes in the gut (254, 463). Whole fish keep longer than fillets, which in turn keep longer than mince fish (481). Thus to hold fish for the longest time, they should be left in the round or eviscerated, and fillets should be cut late in the storage period (table 20).
TABLE 20.—Total storage time required for iced fish and fillets to reach a trimethylamine (TMA) value of 15

<table>
<thead>
<tr>
<th>Gutted fish stored on ice</th>
<th>Time for fillet stored at 3°C to reach TMA of 15</th>
<th>Total storage time to reach TMA of 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days</td>
<td>Days</td>
<td>Days</td>
</tr>
<tr>
<td>1</td>
<td>4.0</td>
<td>5.0</td>
</tr>
<tr>
<td>2</td>
<td>4.0</td>
<td>6.0</td>
</tr>
<tr>
<td>3</td>
<td>4.0</td>
<td>7.0</td>
</tr>
<tr>
<td>4</td>
<td>4.0</td>
<td>10.0</td>
</tr>
<tr>
<td>5</td>
<td>4.0</td>
<td>11.5</td>
</tr>
<tr>
<td>6</td>
<td>3.5</td>
<td>13.5</td>
</tr>
<tr>
<td>7</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1.0</td>
<td>14.0</td>
</tr>
</tbody>
</table>


Although chemical products of decomposition diffuse to the inside (107), penetration of organisms into fish flesh itself is slow. Even fish that are decomposed with heavy slime and odor may still be sterile within the flesh (106, 279, 548). On this basis, chemical and bacteriological tests for fish spoilage are most sensitive if sampling is limited to the surfaces (548).

Niven has described three types of sausage decomposition (359, 369, 361): (1) surface slime, (2) green ring, and (3) green core. Surface slime forms only when insanitation is combined with excess moisture. Green ring and green core are caused by lactobacilli growing before and after the cook, respectively. The color forms only after the sausage is exposed to the oxygen of the air. Adequate sanitation, heat processing, dry storage conditions, and refrigeration control all three types of decomposition. Preslicing increases the contaminated surfaces in packaged precooked meats (304).

Relative humidity

As explained previously, relative humidity (RH) and water activity (a_w) are equivalent when the system is in equilibrium. Scott (426) described the a_w of most fresh foods as 0.98 to above 0.99, so that they offer ideal growth conditions to micro-organisms. Meat, however, is frequently stored at a relatively low RH to suppress surface growth of micro-organisms. Under these conditions, although the meat surface presumably dries slightly, the a_w within the meat is never in equilibrium with the RH of the surrounding air.

Schmidt (416) investigated bacterial growth on large pieces of meat at humidities down to 73 percent. Of course, moisture moving from the interior to the surface of the meat by capillarity and by diffusion presented to the organisms a higher a_w than would have occurred in equilibrium conditions. Scott (428), however, studied spoilage of thin slices whose a_w came promptly into equilibrium with the RH of the surrounding atmosphere. Despite this difference their results are similar and can be presented together to show the effect of RH and temperature on storage life of beef (fig. 36).
Figure 36.—Effect of relative humidity and temperature on the storage life of beef: A, Thin slices, time for Achromobacter No. 7 to reach $10^8$/cm.$^2$ (423); B, large pieces (416).
Jepsen (265) stored sides of pork for 8 days at 4°C, at two levels of humidity and found the following:

<table>
<thead>
<tr>
<th>Case</th>
<th>Left sides stored at 75 to 85 percent RH Thousands</th>
<th>Right sides stored at 100 percent RH Thousands</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>490</td>
</tr>
<tr>
<td>2</td>
<td>31</td>
<td>130</td>
</tr>
<tr>
<td>3</td>
<td>94</td>
<td>110</td>
</tr>
<tr>
<td>4</td>
<td>17</td>
<td>58</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>190</td>
</tr>
</tbody>
</table>

Allowing cold meat to "sweat" in a warm room enhances bacterial growth on the surface after it is returned to the cold (192).

Even though surface drying is a major factor in controlling bacterial development in meats, holding them below 85 percent RH causes serious weight loss by evaporation (417). As a compromise, meat is generally stored commercially in atmospheres between 85 and 90 percent RH (29, 558).

In meat-storage rooms with this range of humidity, mold growth becomes the major problem. Kaess (274) related growth of Mucor, Penicillium, and Cladosporium on meats to temperature and RH. Rate of growth increased directly as temperature and RH increased, but maximum growth occurred at 95 percent RH, rather than at 100 percent RH, because bacterial growth at 100 percent RH interfered with growth of the molds.

The reported investigations on the effect of RH or a* on poultry spoilage are less comprehensive and less conclusive. When compared with ice-chilled poultry, air-chilled poultry has been reported to have a longer (289, 290, 378), an equal (21), and a shorter (313) storage life. However, in the tests that showed a shorter storage life, the air-chilled poultry was held at a slightly higher temperature, which may have speeded spoilage somewhat. Ice chilling has been recommended because of the more rapid heat transfer (358), and several investigators have mentioned weight loss by evaporation from air-chilled chickens, which gives the ice chilling a competitive advantage from a profit standpoint. However, prolonged storage in slush ice causes loss of flavor (384).

There is a belief in the fishing industry that water from melting ice has a washing action that removes bacteria from the surfaces, thus enhancing keeping time (484). The Department of Scientific and Industrial Research of Great Britain (159, 160) has reported that well-iced fish last longer at a high ambient temperature than at a low ambient temperature. They attributed this phenomenon to the greater washing action at the higher temperature. However, no data are presented and no other reports have been found that would prove or disprove this generally held belief. Green (163) reported higher counts on shrimp held in contact with ice than on controls held at the same temperature without contact with ice. She ascribed this difference to the availability of water on the surfaces of the iced shrimp.
Air movement

At relatively low humidities, movement of air over the surface of the meat increases the moisture gradient from the interior of the meat to the air surrounding it. As a result, the aw of the meat surface may be low enough to retard or prevent microbial growth (275). The moving air must be dry (30, 275); otherwise the moisture gradient will not be steep enough and the meat surface will not be dry. If the air is above 90 or 96 percent RH, the rate of mold or bacterial growth, respectively, is not materially altered by air movement. Scott and Vickery (428) have pointed out that air movement speeds the chilling of meat and thereby slows bacterial growth during the initial stages of storage. But they indicated that in the absence of surface desiccation, even rapid cooling is not an effective means of bacterial control.

Oxygen

The psychrophilic bacteria that spoil flesh foods grow best aerobically. However, because some are facultative anaerobes, they can grow on foods held in the absence of air, but more slowly (45, 64, 299, 510).

Molds are generally strict aerobes, and in food spoilage their growth is usually limited to surfaces exposed to air. In fact, some film laminates can prevent mold growth on tightly wrapped products by preventing the passage of oxygen (146). However, some strains of molds have been reported that can grow with very small amounts of oxygen; indeed a strain of *Oospora lactis* grew in the total absence of measurable amounts of oxygen (328).

Gas storage

Either hydrogen or nitrogen appears valueless for preserving flesh foods at low temperatures, but carbon dioxide increases keeping time markedly (37, 83). The mechanism of the effect of carbon dioxide is not known. Although the pH change that it induces has been suggested (513), others (83, 183) have argued against this theory, and one author (183) suggested that the effect may be due instead to interference with a hydrogenating enzyme.

Susceptibility varies with the organism. Psychrophilic microorganisms causing spoilage of flesh foods at low temperatures are particularly sensitive to CO₂, whereas some of the mesophiles such as *Proteus* and *Aerobacter* are less so (83, 183).

It has been suggested that inhibitors are most effective when growth is slow (496, 498). It can thus be anticipated that CO₂ will be more inhibitory near the minimum temperatures for spoilage organisms than near the optimum. This was shown for meat spoilage molds (490), for *Achromobacter* (figs. 37 and 38), and for the flora on chicken meat (fig. 39).

Carbon dioxide is more effective in slowing bacterial growth on meats when aw is low than when it is optimal (424). However, in one investigation (545) at temperatures of 20° C. or above, 5 or
Figure 37.—Effect of 20.6 percent of CO₂ on growth of *Achromobacter* at 0.1° C. (183).

Figure 38.—Effect of 20.2 percent of CO₂ on growth of *Achromobacter* at 21.9° C. (183).
10 percent of CO\textsubscript{2} had a stimulatory effect on the growth of *Pseudomonas fluorescens* and *Aerobacter aerogenes*. This allowed them to grow in CO\textsubscript{2} at a lower a\textsubscript{w} than they could in air.

Red meats, poultry, and fish have all been stored successfully under partial atmospheres of carbon dioxide. Sometimes this has doubled or trebled shelf life. No attempt will be made to treat the subject exhaustively, for it has been reviewed by others. (See p. 78.) Studies conducted 30 years ago by British and Australian...
investigators (57, 116, 183) culminated in the use of CO₂ in commercial transport of red meats from Australia to Britain. This practice has continued to the present time. It has also been applied successfully to chicken meat (365), to fish (83, 84, 85, 462), and to frankfurters (366).

Within certain limits, the ratio of the shelf life in carbon dioxide to that in air is directly proportional to the percentage of carbon dioxide in the atmosphere (fig. 40). On the other hand, concentrations over 50 percent are of no greater value than 50 percent (85, 366), and concentrations over 25 percent have been reported to discolor chicken (365), beef (300), and some self-service meats, but not frankfurters (291).

Investigators disagree on the efficacy of ozone as a preservative. Micro-organisms are indeed destroyed or inhibited by ozone. Ewell (123), in a review of the subject, has recommended using it in storage rooms to inhibit mold growth on the surfaces of eggs, meat, fruit, and cheese, and to destroy odors in the air. Ingram and Haines (247), however, reported that (1) *Achromobacter* and *Pseudomonas* from chilled meats are relatively resistant; (2) bacterial growth, once established, is not easily inhibited by ozone; (3) nutrients in aqueous suspensions interfere with its antibacterial action so that while 10 p.p.m. will kill bacteria in water, 100 p.p.m. are required in nutrient broth and 1,000 p.p.m. in agar media; (4) it combines with foods, causing their spoilage;

![Figure 40](image)

**Figure 40.—Effect of carbon dioxide concentration on shelf life of chicken at 4.4° C. (365).**

---

and (5) inhibitory concentrations are higher than human beings can tolerate. Ingram and Barnes (243) reviewed the use of ozone more critically and reported that it deteriorated rapidly in storage rooms, especially when foods were present; that low RH encouraged such deterioration; and that it combined with fats, causing rancidity. They concluded that the effects of relative humidity and temperature on the efficacy of ozone are unclear and require more investigation, although there is some reason to believe that low temperature increases its inhibitory action.

Thus ozone appears to have limited value in inhibiting surface microbial growth on stored foods primarily because of its toxicity to human beings and because of its enhancement of rancidity. However, storage-room air might be treated to remove odors by circulating it through a separate chamber containing ozone and then through charcoal or metal oxides to remove the ozone before it is returned to the storage area (243).

Packaging

The effects of humidity, air movement, oxygen, and carbon dioxide on bacterial growth are all involved in the effects of packaging. Halleck and others (195) found that when impermeable films were used for packaging meat, the atmosphere within the package influenced the growth of bacteria. The RH was high in this entrapped atmosphere, and therefore surface drying was minimized. Despite this they found that growth was marked by a longer lag phase under these films than under permeable films. Under permeable films, growth of bacteria occurred at maximum rates until dehydration or mold growth interfered. Evacuation of the atmosphere from the package wrapped in impermeable film enhanced shelf life. Many others have also found increased shelf life of meats, vegetables, poultry, and crabmeat packaged in impermeable films or cans, especially when evacuated (8, 13, 64, 77, 260, 291, 385, 455, 533). This effect has been attributed to retention of CO₂ formed by respiration of either the food or the micro-organisms or both (291), or to exclusion of oxygen (77, 443, 533), as shown in table 21.

Only a few authors have reported impervious packaging to be of little value for fresh meats. Results of this kind were obtained on sliced meats (304) and on chicken meat (252, 315). However, better color was maintained in the sliced meats in the impervious packages. In one of the chicken meat investigations, (315) air was not evacuated from the packages and they were held at a slightly higher temperature than the unpackaged controls. In the other (252), the meat was treated with an antibiotic, which may have introduced a special factor. The organisms that grow under impermeable films are facultative anaerobes (385). In some instances their growth has been reported only slightly inhibited by low oxygen tension (299, 300).

*See footnote 7, p. 58.
TABLE 21.—Effect of film permeability to oxygen and carbon dioxide on spoilage rate of packaged chicken meat at 1°C.

<table>
<thead>
<tr>
<th>Time at 1°C (days)</th>
<th>Composition of gas within package</th>
<th>Viable bacteria per cm.²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Permeable film</td>
<td>Impermeable film</td>
</tr>
<tr>
<td></td>
<td>O₂</td>
<td>CO₂</td>
</tr>
<tr>
<td>--------------------</td>
<td>------------</td>
<td>------------</td>
</tr>
<tr>
<td>0</td>
<td>20.8</td>
<td>0.2</td>
</tr>
<tr>
<td>8</td>
<td>20.2</td>
<td>0.5</td>
</tr>
<tr>
<td>12</td>
<td>17.1</td>
<td>2.1</td>
</tr>
<tr>
<td>14</td>
<td>20.3</td>
<td>1.0</td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>6.7</td>
</tr>
</tbody>
</table>

¹ Combined results from 3 packing stations.
² Each value is the mean from 3 carcasses from 1 packing station.
³ Definite odor of spoilage.

Shelf life of cured meat is generally greatly increased by proper packaging. Prepackaged meats such as liver sausage are cooked in the skin, and the skin then protects against further contamination. Films that have low permeability to water and oxygen keep sausages many weeks, whereas highly permeable films permit growth as shown in the following data obtained with liver sausage (192):

<table>
<thead>
<tr>
<th>Casing</th>
<th>Bacteria per gram</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before storage</td>
<td>After storage at 5°C for 58 days</td>
</tr>
<tr>
<td>High permeability</td>
<td>5,200</td>
<td>2,400,000</td>
</tr>
<tr>
<td>Low permeability</td>
<td>5,300</td>
<td>1,400</td>
</tr>
</tbody>
</table>

Various films or laminates have been recommended for cured meat because they inhibit growth by retaining carbon dioxide (291) or by preventing entry of oxygen (146, 198). Vacuum-packaged cured meats finally spoil from lactic acid bacteria, whereas those permitted access to oxygen also support the growth of yeasts and molds (431).

Impervious films have likewise extended the shelf life of smoked fish at chill temperatures. Unfortunately, absence of the normal psychrophilic spoilage flora has permitted the growth of *Clostridium botulinum* type E in some commercial shipments of smoked fish from the Great Lakes Area, and food poisonings have occurred (509). Type E has grown at temperatures as low as 3.3°C in laboratory studies (reviewed by Michener and Elliott 327).

Stewart (467) and Ayres (15) have reviewed poultry packaging. Gross (169) has reviewed shelf-life problems of packaged foods in general, and Hannan (198) and Ingram (242) have reviewed microbiological aspects of packaged foods.

**Acid**

Psychrophilic food spoilage bacteria do not grow readily at low pH. Fish spoilage has been inhibited by using acetic or tartaric acids to obtain pH 6 (420), or hydrochloric acid to obtain pH 5 (449). Spoilage of crabmeat has been inhibited by using phosphoric acid to obtain pH 5 to 6 (496), and growth of bacteria in minced beef was slowed by using hydrochloric acid to obtain pH 4.5 (49). In two reviews, Ingram (234, 241) has described the effect of pH on bacteria, particularly as applied to meat spoilage. Witter (541) has reviewed the effect of low pH in inhibiting psychrophilic spoilage of dairy products. The effect of acids in preserving various cured and fermented foods is well known but is outside the scope of this review.

**Antemortem treatment**

Meat from a hog properly rested and fed before slaughter keeps well, whereas that from a febrile or overfatigued hog decomposes rapidly. The tired animal bleeds less well, and in addi-
ation contains less muscle glycogen and goes into and out of rigor more rapidly. The pH of muscles containing high quantities of glycogen falls farther to the acid side than does that of muscles containing less glycogen. These factors have been discussed in more detail by various authors (155, 186, 234, 241, 318).

The situation is similar in other mammals; however, beef is somewhat less susceptible to such differences than is pork (234). Brown and others (49) found that when rigor was slower in onset, beef kept better, but the effect was separate from that of pH.

On the other hand, Koonz (289) reported that withholding feed before slaughter of poultry had no effect on subsequent spoilage rate. Thus, there is no advantage in feeding birds before slaughter. In fact, an empty intestinal tract makes for better sanitation during poultry evisceration.

Fish that have been feeding heavily before they are removed from the water will keep less well than those that have empty stomachs. This is because the intestines of a fish full of feed contain many bacteria and in addition the digestive enzymes of the fish are present in high concentration. On the other hand, a fish that has been fasting has few if any bacteria in its intestines (323). For this reason, "feedy" fish are often kept alive in captivity until their stomachs are empty (216).

Fish that are physically exhausted when they die will go into and out of rigor more rapidly than otherwise, and the pH will remain low a shorter time. Thus, bacterial decomposition is most rapid in gill-net caught fish, somewhat less so in line-caught fish, and least in seined or trapped fish (90, 216).

**Autolysis**

Before bacteria begin to grow on fish or beef muscle, an autolytic breakdown to peptides and amino acids occurs. These simple compounds are not only the first products used by bacteria growing on fish (154), but they actually are required before bacteria can build enzymes to attack pure proteins (178). However, the amount of autolytic material present does not affect growth rate on fish tissue because once the proteins are attacked, a sufficient amount of simple nitrogenous material is immediately available (282).

**Antibiotics**

This review will be limited to a few interesting developments in the field of antibiotic preservation of food. More extensive reviews are listed on page 78.

Some of the antibiotics, primarily chlortetracycline (CTC), enhance the shelf life of chilled meats, poultry, and fish to a remarkable degree. The effectiveness of antibiotics is greatly enhanced at low temperatures—more so than the additive effects of the antibiotic and low temperature would be separately (fig. 41).
Weinberg (529) in a comprehensive review has related chelation of metal ions to the effectiveness of antibiotics in general. CTC may owe its antibacterial action in beef to chelation of trace metal ions required by the organisms for growth (255–259). In a similar way, the high magnesium content of sea water interferes with the action of the tetracyclines against fish spoilage bacteria (454).

Wells and others (532, 534) have reported that CTC increases the fat content of microbial cells, and they suggest this phenomenon to be a possible explanation of its effectiveness. Such a possibility cannot be discounted, although metal chelation appears to be a reasonable explanation.

The suppression of bacterial action on flesh foods usually (162, 441, 526, 552), but not always (444), encourages the growth of yeasts or molds, with resultant musty odors. Another antibiotic, mycostatin, has been suggested to combat the yeast and mold growth (552).

Resistant bacterial strains have appeared in poultry plants where there was continuous contact between equipment surfaces and the antibiotic (162, 357, 518, 519), but the problem can be
controlled by chlorination and other stringent hygienic precautions. On the other hand, chlorine will destroy CTC if it is applied simultaneously. Highly resistant strains develop by a process of selection of the more resistant individuals. These are primarily *Achromobacter* and the less sensitive pigmented pseudomonads.

CTC has been reported to retard the growth of *Staphylococcus aureus*, *Clostridium botulinum*, and spoilage organisms in fish at 10° C. However, one investigation revealed large numbers of the pathogenic yeast *Candida parapsilosis* in CTC-treated chicken meat; other reports described antibiotic-resistant strains of *Salmonella typhimurium*, one of which grew on carcasses of chickens in the absence of the normal spoilage that would have rendered them inedible. Another investigation failed to confirm that CTC-treated poultry meat represented a health hazard from yeasts or bacteria; however, failure to isolate a given organism does not constitute proof of safety.

The commercial use of antibiotics to preserve fish and poultry has decreased in recent years. One reason is that some producers and handlers have tried to use the antibiotics as a substitute for good sanitation, refrigeration, and rapid handling. If antibiotics are to prove of value, all of these other practices should be continued at the same high level in order to place a product of high quality in the hands of the consumer. In addition, some consumers have not been convinced of the safety and usefulness of such additives despite U.S. Food and Drug Administration approval.

**Salt**

Psychrophilic bacteria are sensitive to sodium chloride. The organisms capable of growth in high salt concentrations are rarely able to grow below 5° C. Salted foods are outside the scope of this review. However, small amounts of salt on the surfaces of flesh foods can inhibit bacterial growth by creating a microclimate of moderately high salt content without changing the nature of the foods or contributing enough saltiness to make them unpalatable.

Salt brine dips with acid have been suggested for fish. Shrimp at -1.1° C. in 6-percent brine remained in good condition longer than that in 3-percent brine. Salt brine dips are used regularly for crabmeat and reduce bacterial loads.

Tracheal injection of salt brine was suggested to preserve chickens in areas of the U.S.S.R. where refrigeration was unavailable, and a carageenin gel coating containing 6 percent of salt enhanced the keeping time of frozen-thawed chicken.
However, salt has been only partially successful in enhancing preservation of dairy foods, for many psychrophiles resistant to 4 to 6 percent of salt have been found in such products (541).

SPOILAGE OF EGGS

Penetration of shell eggs

Only part of the available information on bacterial decomposition of eggs in the shell will be presented. We refer to the important reviews listed on page 78.

Lorenz and others (508) have quoted nine authors who agree that spoilage organisms are very rarely inside the egg before it is laid. Even the outside of the egg shell is free of bacteria as it leaves the oviduct, but bacterial contamination occurs after the egg is laid (475). Eggs that are obviously dirty, usually with fecal material from the nest, have been found to contain more bacteria after a short period than have obviously clean eggs (139) and in addition they spoil more readily in storage (406, 506).

To cause spoilage of the interior, micro-organisms must penetrate the shell and membranes and overcome the natural inhibitory materials within the albumen. The shell contains several hundred small holes filled with mucin, which effectively prevents bacterial entry when it is clean and dry (401). However, abrasion or moisture may remove this mucin, whereupon the shell offers little resistance to the passage of bacteria (137, 138, 151, 156). There is still a question whether shell porosity, which varies with the hen, does (293) or does not (342) affect the penetration rate. There is no correlation between shell thickness and permeability to bacteria (138).

The outer shell membrane likewise offers little resistance to bacterial penetration, but the intact inner membrane is an effective physical barrier to bacteria (110, 151, 292, 321, 475, 523) so that penetration to the albumen is delayed hours (185, 523) or even days (47, 110, 151, 333). Colonies may grow on the surface of this membrane before the bacteria break through to the albumen (110, 156). Florian and Trussell (129) have listed organisms according to their capabilities of penetration of the inner membrane as “primary” or “secondary” invaders.

Although some investigators (475) have reported that the membranes have an antibacterial activity, subsequent work has shown this to be incorrect (474). In fact, membranes may actually support bacterial growth (47, 108, 151, 161).

Wet eggs are much more susceptible to bacterial invasion than are dry eggs (185, 190, 321). Thus, wetwashing enhances spoilage more than does dry abrasion (505). Similarly, removing cold eggs to a warm, moist atmosphere may result in condensation (sweating) on the shells, which encourages surface growth and entry of bacteria, especially if the eggs are again chilled (131,
High humidity in storage rooms also encourages growth of molds on the shell (188, 432) (table 22) and enhances penetration of bacteria (342). If eggs must be wetwashed, they should then be dried thoroughly (41, 314).

### TABLE 22.—Effect of temperature and humidity on the growth of mold on eggs as judged by eye

<table>
<thead>
<tr>
<th>Humidity (percent)</th>
<th>0° C.</th>
<th>10° C.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DAYS FOR VERY SLIGHT GROWTH</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>78</td>
<td>17</td>
</tr>
<tr>
<td>95</td>
<td>24</td>
<td>11</td>
</tr>
<tr>
<td>98</td>
<td>18</td>
<td>3</td>
</tr>
<tr>
<td>100</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>DAYS FOR DISTINCT GROWTH</td>
<td></td>
</tr>
<tr>
<td>95</td>
<td>41</td>
<td>16</td>
</tr>
<tr>
<td>98</td>
<td>49</td>
<td>13</td>
</tr>
<tr>
<td>100</td>
<td>28</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>DAYS FOR HEAVY GROWTH</td>
<td></td>
</tr>
<tr>
<td>98</td>
<td>41</td>
<td>17</td>
</tr>
<tr>
<td>100</td>
<td>35</td>
<td>11</td>
</tr>
</tbody>
</table>

**Source:** Haines, R. B. (188).

A temperature drop, particularly when the shell is wet, may encourage penetration by pulling bacteria through the shell. This may occur when the egg drops into a dirty or wet nest, when the warm egg is washed in cool water (table 23), or when storage temperature changes during handling. As a result of work such as this, the use of wash water warmer than the egg has been recommended by many investigators and is now commercial practice (41, 131, 418). Eggs spoil faster when stored at high temperatures than when stored at low temperatures (188, 547) (table 22).

### TABLE 23.—Effect of relative temperature of egg and wash water on rotting during subsequent storage

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rots</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water and eggs same temperature</td>
<td>12</td>
</tr>
<tr>
<td>Eggs, 10° C.; water, 25° C</td>
<td>7</td>
</tr>
<tr>
<td>Eggs, 37° C.; water, room temperature</td>
<td>100</td>
</tr>
<tr>
<td>Immersed 1 hour immediately after being held</td>
<td>100</td>
</tr>
<tr>
<td>Cooled 1 hour, then immersed 1 hour</td>
<td>79</td>
</tr>
<tr>
<td>Cooled 24 hours, then immersed 1 hour</td>
<td>19</td>
</tr>
</tbody>
</table>

1 Between 27 and 119 eggs were used for each test.
2 Average of 3 tests.

**Source:** Haines, R. B. (189).
Despite the dangers of washing, most eggs are now wetwashed because the trade prefers a clean product. In many operations all eggs are washed to avoid the need for sorting (41). Washing of dirty eggs is advisable, provided they are washed before bacteria have had time to enter and provided care is taken in the washing procedure (137). Funk (142) and Winter and others (539) have reviewed the use of detergents and detergent-sanitizers and have concluded that they have little value in preventing subsequent spoilage. However, detergent-sanitizers containing a quaternary ammonium compound give 1/3 to 1/2 the amount of spoilage as do those without (41, 329, 331, 332). Funk (142) and Winter and others (539) have reviewed the use of detergents and detergent-sanitizers and have concluded that they have little value in preventing subsequent spoilage. However, detergent-sanitizers containing a quaternary ammonium compound give 1/3 to 1/2 the amount of spoilage as do those without (41, 329, 331, 332). Iodine in concentrations of 50 to 100 p.p.m. is an effective sanitizer when used following a non-sanitizer detergent (413). Antibiotics are valueless in preventing spoilage (32, 330), and may even increase spoilage (418). (See also “Effect of metals on penetration and growth.”)

**Shell coatings**

Oiling retards evaporation of egg contents, but there is still a question as to whether it retards (143), has no effect on (406), or enhances (52, 281) spoilage of shell eggs.

**Thermostabilization**

Thermostabilization—a dip in hot oil or hot water long enough to coagulate a thin layer of albumen—retards bacterial entry (125, 126, 141, 143, 145, 410). This subject has been reviewed by Funk (144).

**Gas storage**

Carbon dioxide storage inhibits spoilage organisms on shell eggs as it does on meats and fish (187, 190, 336) and prevents mold growth as shown in figure 42.

Ozone, at a concentration of 1.5 to 3 p.p.m., successfully prevents mold growth on the exterior of the shells of eggs but is ineffective in preventing internal spoilage (122, 186, 319).

**Internal defenses**

Once penetration is achieved, the egg still has natural defenses in the albumen. Garibaldi (147) has listed and discussed the factors in albumen that have been reported to inhibit growth: High pH, lysozyme, avidin, ovomucoid, conalbumin, and the presence of only native proteins. Gram-positive bacteria are sensitive to lysozyme, and bacteria requiring biotin or iron are inhibited by the binding action of avidin and conalbumin, respectively. Ovomucoid is an antitrypsin factor, but it does not affect growth of gram-negative bacteria. Garibaldi (147) and later, Brooks (47) questioned the inhibitory effect of high pH, per se, explaining that the pH effect may be due instead to the greater power
Figure 42.—Effect of carbon dioxide on growth of mold on shell eggs at 10° C. over water (888).
of conalbumin to bind iron at high pH. Garibaldi described conalbumin as the most important of the protective factors against gram-negative bacteria in the albumen.

**Effect of metals on penetration and growth**

A low level of iron in water used to wash eggs is enough to overcome the chelating power of conalbumin at the site of entry into the albumen, and to allow rapid penetration and growth of bacteria (148, 149, 150) (fig. 43). More rapid growth of pseudomonads in egg albumen can be encouraged either by adding 20 µg. of iron per ml. of albumen, or by adjusting the pH from the normal 9.3 to 6.5, thus releasing iron from the conalbumin (47). A concentration of 10 p.p.m. of magnesium ion similarly enhances penetration and growth of bacteria (205).

![Graph](image_url)

**Figure 43.**—Effect of iron in wash water on spoilage of shell eggs by *Pseudomonas ovalis* (148).
Effect of aging

Aging of shell eggs tends to diminish their natural protective mechanisms against microbial penetration. Shell porosity increases (9) and with it, rate of penetration of bacteria through the inner membrane (110, 140, 161). The protective mechanisms within the albumen may also diminish. This was suggested by more rapid growth in aged albumen after separation from the shell (342) although Brooks (47) failed to confirm this but instead found that the inner membrane became more favorable for growth and penetration as it aged.

As shell eggs age, iron passes from the yolk into the albumen (401). However, this iron appears insufficient to affect bacterial growth, since growth in albumen removed from the yolk is not different from that in albumen stored with the yolk (47).

Liquid egg

Once the albumen is mixed with the yolk, the natural protective mechanisms of the albumen are overcome and bacteria can grow rapidly (19, 110), as shown in figure 44. To prevent gross con-
tamination by micro-organisms, shell eggs should be washed (246, 380, 555) or even pasteurized (141) before they are broken for liquid egg. Also, the magma should be refrigerated quickly (301, 302, 555) to prevent growth, particularly if it has been previously subjected to heavy contamination (419). Even in a well-managed plant, the average total count in liquid egg before freezing or drying often exceeds $10^6$ per gram, because of the good medium it offers to bacteria (246). The liquid egg magma may also be pasteurized to reduce the bacterial count and thereby enhance keeping quality and kill pathogens (246, 347, 479, 540).

Similarly, frozen eggs should be defrosted rapidly to prevent bacterial multiplication (53, 276, 390). Liquid egg has a low oxidation-reduction potential during spoilage (171) so that only obligate and facultative anaerobes can grow in it, except in surface layers.

**SPOILAGE OF FRUITS**

Spoilage of fresh fruits stored at chill temperatures without other processing is outside the scope of this review and has been reviewed elsewhere (see p.79). Frozen fruits are normally stored at temperatures too low to permit growth, so that microbial spoilage can occur only before or after storage.

Spoilage of fruits is usually by molds or yeasts, rarely by bacteria, for the pH of fruits is generally sufficiently low to prevent growth of all bacteria except the lactobacilli. However, the edible organic acids do not owe their antibacterial action solely to their low pH; the anion, too, has antibacterial activity (117). Sugar is usually added before freezing; and the yeasts and molds, which are also more tolerant to high osmotic pressure than are bacteria (239), are again favored. Fermentation by yeasts is a common problem in fruits during delays before freezing.

Before freezing has begun, the added sugar in fruit is a good preservative because of its dehydrating action (27). After freezing has started, the sugar may have a protective action but will not affect the $a_w$ of the system (327). The preserving and germicidal action of various sugars against bacteria is in the following order of decreasing efficacy: levulose, dextrose, sucrose, lactose. Yeasts are considerably more tolerant of sugars than are bacteria (117).

Interpretation of the effect of sugar is complicated by the fact that a high sugar content increases the rate of heat transfer, probably because the mass becomes liquid (100, 272). This reduces the time during which the product is at a temperature favorable for microbial growth.

Early investigators of frozen fruit discovered that molds do not grow if oxygen is excluded (35, 98, 100, 320). Therefore, they do not grow in the interior of barrels of frozen berries nor in sealed containers of fruit. Sealed containers of berries did not spoil below $-2.2^\circ$ C, because molds were inhibited by lack of oxygen, bacteria by the acid, and yeasts by the combined effect
of these with low temperature (35). However, organisms grew in berries at \(-2^\circ\) and \(-4^\circ\) when the package was not airtight (34).

Fruits may become contaminated with micro-organisms while still on the plant; and they may start to break down even before harvest. Breakdown, often due to the same micro-organisms, continues during storage at a rate dependent on temperature (206, 405). If fruits or purees are to be frozen in large containers such as barrels, it is important that the product be cooled to \(10^\circ\) or lower before it is placed in the container, to prevent growth during the slow freezing period (504).

Among the yeasts that grew on grapejuice at low temperatures, Lawrence and others (296) isolated strains that grew well at 0\(^\circ\) C., poorly at 17\(^\circ\), and had optima between 7\(^\circ\) and 13\(^\circ\). These same strains had maximum growth temperatures below 20\(^\circ\) when tested on a temperature gradient incubator (112).

Growth of these yeasts on grapejuice at \(-2.2^\circ\) to \(-5.5^\circ\) C. can be suppressed by adding sorbic, benzoic, capric, or caprylic acid in concentrations of 0.01 to 0.015 percent, although these levels are too low to prevent growth of these yeasts at room temperature (376).

Microbiological spoilage of citrus juice and juice concentrate has not been reported at subzero temperatures. Berry and others (38) reported no growth of *Lactobacillus* species at 4.4\(^\circ\) C. in orange concentrate in the range of 12\(^\circ\) to 58\(^\circ\) Brix, although these organisms grew in orange concentrate at higher temperatures. These authors observed growth of yeast at 4.4\(^\circ\) in 42\(^\circ\) Brix concentrate, and Rushing and others (408) reported growth of yeast at 1.7\(^\circ\) in concentrate of 40\(^\circ\) to 55\(^\circ\) Brix. Microbial populations (largely yeasts) in commercial orange concentrate decreased at 4.4\(^\circ\) until the third week of storage, after which they rose rapidly (348). Wolford (546) found no growth in orange concentrates at 4.4\(^\circ\) in 187 days. The microbiology of citrus juices and juice concentrates has been reviewed by Patrick and Hill (373) and that of noncitrus juices by Lüthi (309).

Other than juices, few processed fruit products are stored at chill temperatures, but Rushing and Senn (409) stored citrus salad at temperatures down to \(-1.1^\circ\) C. At this temperature, an initial drop in total count of about 80 percent during the first 25 days was followed by growth. This behavior resembled the behavior of microbial populations on vegetables at similar temperatures (fig. 45). Growth was prevented in the salad by potassium sorbate at 0.066 percent but not at 0.033 percent, while sodium benzoate was ineffective at 0.066 percent. These chemicals were thus less effective than in grapejuice at low temperatures (376).

**SPOILAGE OF VEGETABLES**

The parasitic micro-organisms that damage fresh vegetables are generally capable of attacking living tissue and are considered plant pathogens. This subject has been reviewed elsewhere (p. 79) and is outside the scope of this review.
Figure 45.—Bacterial growth in vegetables at −3.9° C. (326).
Vegetables become contaminated with saprophytic micro-organisms from the soil and from harvesting operations. Some contain small to moderate numbers of bacteria within the tissues (345, 411).

However, frozen vegetables differ from most other frozen foods in that they are blanched during processing. Blanching greatly reduces the microbial population (36, 80, 268, 375, 448, 520, 521). The vegetables before processing have a natural resistance to decay (78) which disappears after blanching (36, 99, 268, 375, 449) or after freezing (31), presumably as a result of heat killing of the plant tissue with accompanying increase in permeability and leakage of nutrients from the plant cells. Recontamination and growth occur during subsequent processing, and the product may enter the freezer with a large and rapidly growing bacterial population (268, 375, 460, 461, 520, 521).

Since blanched vegetables are very susceptible to microbial spoilage, they are always stored at temperatures intended to prevent microbial growth. Most of the available information on the microbiology of frozen vegetables, therefore, merely gives the minimum temperature at which microbial growth has been observed under particular circumstances (327), or describes the survival of micro-organisms at temperatures too low for growth.

There are a few reports on the microbiology of frozen or thawed vegetables in the bacterial growth range. Growth on vegetables has been reported occasionally down to about $-8^\circ$ C. and once at $-12^\circ$ (327). Berry (34) found that counts on vegetables rose to over $10^6$ per gram in 2 months at $-4^\circ$. Michener and others (326) stored several lots of peas, green beans, cauliflower, and spinach at $-3.9^\circ$, $-1.2^\circ$, and $-4.5^\circ$, and found growth within 8 weeks at the first two temperatures but within 1 week at the third. As shown in figure 15, growth was often preceded by an initial drop in count, presumably caused by death of some species while the others were still in their lag phase. The population of one lot of peas stored at $-3.9^\circ$ reached $10^9$ per gram (fig. 15). Similarly, Hucker and David (225) incubated frozen-thawed vegetables at $+7^\circ$, and obtained counts after 70 hours of $10^{10}$ per gram (spinach), $10^6$ per gram (peas), and $10^7$ to $10^8$ per gram (corn, lima beans, string beans).

The natural microflora of frozen vegetables grows slowly near the freezing point (figs. 26 and 45). The advantage of permitting frozen vegetables to thaw at low temperatures is evident from the time required for growth to begin. However, these platings were incubated at $30^\circ$ and $32^\circ$ C., respectively. Platings at lower temperatures might have shown a somewhat shorter lag time (114, 381).

Hucker and David (225) followed the total count of the naturally occurring microflora on several frozen vegetables during five successive freeze-thaw cycles but failed to find significant changes. The thaw phase of each cycle lasted 8 hours at $2^\circ$ or $7^\circ$ C., which was less than the lag period at these temperatures. While the count varied more at $7^\circ$ than at $2^\circ$, the variations were irregular and presumably were sampling differences.
The process of freezing vegetables may break up clumps of bacteria, thus causing an apparent increase in the number of bacteria (226, 344, 375). In one case these bacterial clumps were observed microscopically before freezing but were not seen in the same sample after freezing (375). When peas or corn were frozen slowly so that they remained for 10 hours or more at their freezing point, bacterial counts increased up to threefold after the product reached this temperature (516). Although these authors considered this a result of breaking up of bacterial clumps, Michener and others (326) found that sampling variations of this magnitude are not unusual.

The microbial flora of frozen vegetables is for the most part that which contaminates them after blanching and survives freezing. The common types have been listed (447-451). During spoilage of previously frozen vegetables at 10° to 32° C., initial souring by streptococci was followed by growth of lactobacilli, although these groups originally made up only about 5 percent of the flora (412). At 30°, Streptococcus lactis and Leuconostoc mesenteroides predominated in spoilage of peas (535).

When peas were held at -4° C., they were spoiled by Pseudomonas fluorescens and a Lactobacillus, while at -6.7° they were below the growth range of their bacterial flora and were spoiled by Sporobacterium (57). In the same series of experiments, okra and kale at -6.7° were spoiled by Cladosporium. At 0°, a gram-negative organism resembling Flavobacterium predominated in spoilage of frozen-thawed vegetables (222).

Michener and others (326) failed to find any correlation between microbial growth and development of off-flavors in vegetables. During storage at -3.9°, -1.2°, and +4.5° C., off-flavors developed before the bacterial count had doubled, and the onset of off-flavor was not correlated with the initial bacterial count. It was concluded that the onset of off-flavor was not due to bacterial growth, although bacterial enzymes could be involved. Furthermore, off-flavor developed at a measurable rate even at and below -18° (431) although bacterial growth is prevented by these temperatures.

Prepeeled potatoes are especially subject to microbial deterioration. Although they are not cooked, they are frequently heat- or lye-peeled and treated with sodium bisulfite or other agents to prevent darkening. The cells on the surfaces are killed, and are therefore subject to rapid attack by saprophytic micro-organisms during storage at 0° to 5° C. The methods of processing prepeeled potatoes have been described (166, 201). The relation between temperature and shelf life of prepeeled potatoes was investigated by Ceponis and Friedman (70). They found that the peeled potatoes had to be cooled in water before they were bagged. Otherwise they took 30 to 36 hours to cool to refrigeration temperature. Total counts after processing were 6,000 to 51,000 per gram and included bacteria, yeasts, and sometimes molds. The shelf life at 2° was 8 to 11 days for whole potatoes, and 9 to 13 days for cut strips. Microbial spoilage causing off-odors and flavors was the principal factor limiting shelf life. Bisulfite inhibited microbial
growth, but its concentration was very low near the end of shelf life. Greig and Smith (166) were unable to extend shelf life by treatments with calcium or sodium propionate or chlortetracycline.

Kohman (288) recommended "Frigi-Canning" of vegetables—a process using only enough heat to kill vegetative cells followed by refrigeration above freezing. Refrigeration would be controlled at a temperature low enough to prevent growth of Clostridium botulinum and other food-poisoning organisms. The psychrophiles that could grow are sensitive to heat. This procedure, however, has the serious disadvantage that cans accidentally stored at a higher temperature may become hazardous.

SUMMARY AND CONCLUSIONS

An understanding of factors affecting microbial growth at low temperatures will assist the food industry in minimizing spoilage losses in chilled foods. Microbial growth is not a problem in frozen foods unless they are mishandled by being held above -12° C.

Lag period of microbial growth is shortest at the optimum temperature and becomes progressively longer and approaches infinity as the temperature is lowered. For this reason, growth on frozen foods stored at high freezer temperatures may become evident only after very long periods. Most investigators limit their observations of lag time to a few weeks, but one worker found that Oospora began growth at -8° C. after a 414-day lag. Lag periods vary widely with the species and strain of organism. Psychrophiles have a shorter lag period than mesophiles in the temperature range where both grow.

Growth rate during the logarithmic phase is similarly sensitive to temperature. The growth rate of mesophiles increases faster with temperature rise than does that of psychrophiles.

The temperature coefficient (Q_{10}) and the mathematically related temperature characteristic (\( \mu \)) of microbial growth and activity increase more than one would expect in simple chemical reactions when the temperature approaches the lower limits for growth. For this reason the storage life of chilled foods can be doubled or trebled by keeping them near the freezing point instead of at 5° to 10° C. where they are often held.

Bacteria often produce extracellular enzymes in greater quantity at temperatures below their optimum than at the optimum or above it. However, the enzymes themselves may alter the substrate most rapidly above the optimum or even above the maximum temperature for growth of the organism. A few investigators have reported that some foods held at temperatures near 0° C. spoiled before counts became high. This may have been due to high enzyme production at low temperatures. But usually counts are 10^7 or 10^8 per gram when spoilage is evident. Microbial enzymes are active below the minimum growth temperature. This is one reason for good sanitation and rapid chilling, which prevent high bacterial levels in frozen foods.
The optimum temperature for cell crop is usually lower than that for growth rate. This can be explained by the greater solubility of oxygen at low temperatures. The temperature range of growth is narrow when conditions are adverse but widens when conditions are otherwise satisfactory. Sufficient adaptation to permit rapid growth at low temperatures has been accomplished to only a limited extent. We have found no report of a successful attempt to adapt a mesophile to grow below its normal minimum growth temperature.

A high level of initial contamination results in more rapid spoilage of foods than does a low level, when the foods are held at temperatures suitable for growth. At chill temperatures or below only the level of psychrophiles is significant in this regard.

Frozen-thawed foods spoil at about the same rate as do foods not frozen.

Micro-organisms require high water activity for growth. They must compete with solutes and colloidal water-binding compounds for the water content of the food. Bacteria require more water than do yeasts, which in turn require more than molds.

During spoilage of products of warm-blooded animals at low temperatures, floral changes occur in favor of psychrophiles, which are present originally only as a minority group. A less distinct change occurs in fish products because the initial flora is predominantly psychophilic, at least in temperate and arctic zones.

Spoilage of meats and fish is primarily a surface phenomenon. Growth is much more rapid on cut surfaces than on skin, fat, or connective tissue.

Psychrophilic spoilage of meats is commonly prevented or reduced by storage in circulating dry air to dry the surfaces on which spoilage occurs. However, too much surface drying may result in undesirable weight loss.

Psychrophiles are predominantly aerobic, but some are facultative anaerobes, so that exclusion of air slows but does not stop spoilage. Storage in atmospheres containing carbon dioxide is eminently successful in increasing shelf life of chilled flesh foods. Packaging with impermeable films inhibits surface growth of spoilage organisms somewhat by limiting access to oxygen and by allowing an increased carbon dioxide content within the entrapped atmosphere, but this effect is limited because the humidity is high.

Psychrophilic spoilage bacteria are inhibited by low pH, and thus by the conditions in red meats from animals properly fed and rested before slaughter. These bacteria are sensitive to heat and to moderately high concentrations of salt.

Antibiotics, primarily the tetracyclines, greatly increase keeping time of flesh foods, especially at low chill temperatures. This may be the effect of chelation of trace metals essential to bacterial growth. Sometimes the inhibition of bacterial growth allows yeasts or molds to grow, and frequently the use of an antibiotic is followed by appearance of a resistant microbial population.
Eggs are usually sterile when laid. Dirt, moisture, and drop in temperature encourage microbial penetration. Penetration can be inhibited by good sanitation, by washing with warm water (when washing is necessary), by thermostabilization, or by storage in carbon dioxide or ozone. The natural defenses of the albumen are high pH, lysozyme, avidin, ovomucoid, and conalbumin. Iron in wash water enhances microbial growth in the albumen by saturating the chelating ability of conalbumin at the site of entry. Aging reduces the effectiveness of the natural protective mechanisms of shell eggs. Once the yolk is mixed with the albumen, as in liquid egg, the protective mechanisms of the albumen are neutralized and growth is rapid; therefore, eggs for breaking should be clean, and the liquid egg should be refrigerated quickly.

Plant pathogens and saprophytes from the field are responsible for rotting of fresh fruits and vegetables. The microflora of fruits consists primarily of yeasts and molds, because the low pH inhibits bacterial growth. Sugar inhibits growth before freezing. Likewise anaerobic conditions within impervious packages or in the centers of large containers inhibit mold growth.

Blanching of vegetables greatly reduces their microbial population, but it also removes their natural resistance to decay so that very rapid bacterial growth occurs on recontamination before freezing. Part of the resulting flora survives freezing and can grow to the point of spoilage at temperatures at or above -4° C. If spoilage occurs at lower temperatures, it is usually caused by molds. For prepeeled potatoes, which are refrigerated but not frozen, shelf life is about 13 days at 2° C. and is limited by microbial spoilage.

REVIEWS AND BIBLIOGRAPHIES ON LOW-TEMPERATURE MICROBIOLOGY

General Reviews.—Psychrophilic bacteria: 1959 (232); 1960 (173); 1961 (541); 1962 (402). Effect of cold on food microorganisms: 1944 (480); 1951 (246); 1955 (40); 1962 (61).

Food spoilage and the microecology: 1949 (541); 1952 (339); 1955 (70, 340); 1959 (415). Temperature and life: 1935 (12); 1955 (58).

Bibliographies.—Effect of cold: 1937 (253); 1946 (528); 1947 (174); 1948 (10, 173, 175); 1961 (476). Fish: 1946 (109); 1949 (56, 59).

Reviews of specific fields.—Antibiotics: 1953 (482); 1954 (531); 1955 (59, 244); 1956 (24, 62, 92, 245); 1957 (25, 94, 170, 372, 501, 529, 550); 1958 (298); 1959 (124, 486); 1960 (157, 362 (pp. 305–327), 487). Baked goods: 1960 (480). Gas storage: 1936 (128); 1938 (337); 1944 (480); 1950 (123); 1951 (365, 366); 1954 (243). Chemical preservatives: 1941 (269); 1942 (270, 271); 1943 (551); 1954 (483); 1957 (484, 497); 1959 (415, 485); 1961 (488). Eggs: 1939 (188); 1940 (180); 1944 (480, 550); 1945 (312); 1948 (142); 1949 (401); 1950 (143); 1952 (246); 1955 (144, 539); 1960 (177). Fish and shellfish: 1937 (168); 1949 (397, 436); 1954 (398);
PSYCHROPHILIC MICRO-ORGANISMS IN FOODS

1957 (424, 497); 1960 (354); 1961 (48, 127, 437); 1962 (438). Fruits and vegetables, fresh: 1938 (394); 1941 (393); 1943 (403); 1949 (396); 1950 (404); 1951 (405, 500); 1952 (395); 1959 (392); 1960 (206); 1961 (250); 1963 (251). Fruits and vegetables, processed: 1951 (414); 1959 (399, 373). Halophilic micro-organisms: 1957 (239). Irradiation: 1957 (389); 1960 (493); 1961 (97). Marine environment: 1946 (559); 1959 (294); 1962 (560). Meats and poultry: 1937 (186); 1944 (268); 1955 (265, 289); 1958 (240, 249); 1959 (15, 161); 1960 (17, 192); 1962 (179). Milk products: 1943 (81); 1947 (368); 1948 (72); 1951 (102); 1953 (217); 1954 (266); 1955 (218, 369); 1957 (124); 1958 (490, 491); 1960 (492); 1961 (541). Minimum growth temperature: 1962 (402); 1961 (327). Packaging: 1958 (467); 1959 (15, 169); 1962 (193, 242). Soils: 1957 (434).

LITERATURE CITED


(22) --- Naylor, H. B., Pfund, M. C. and others. 1956. KEEPING QUALITY OF READY-TO-COOK AND DRESSED POULTRY. Poultry Sci. 35: 399-406.


(30) Bates, P. K., and Highlands, M. E.

(31) Bedford, R. H.

(32) Bélehrádek, J.

(33) Bergey, D. H., Breed, R. S., Murray, E. G. D., and Hitchens, A. P.

(34) Berry, J. A.

(35) ——

(36) ——

(37) —— and Magoon, C. A.

(38) Berry, J. M., Witter, L. D., and Folinazzo, J. F.

(39) Bluem, H. M., and Tarir, H. L. A.

(40) Borgström, G.

(41) Botwright, W. E.

(42) Boyd, J. W., and Tarr, H. L. A.

(43) Bramstedt, F., and Auerbach, H.

(44) Breed, R. S., Murray, E. G. D., and Hitchens, A. P.

(45) —— Murray, E. G. D., and Smith, N. R.


(47) Brooks, J.


(51) **BROWN, E. B.** 1933. BACTERIAL STUDIES OF DEFROSTED PEAS, SPINACH, AND LIMA BEANS. Jour. Home Econ. 25: 887-892.

(52) **BROWN, H. J., and GIBBONS, N. E.** 1954. AIR CELL MOLD IN OILED EGGS. Food Technol. 8: 307-311.

(53) **BROWNLEE, D. S., and JAMES, L. H.** 1939. BACTERIAL CONTAMINATION OF FROZEN WHOLE EGGS AND AN IMPROVED METHOD OF DEFROSTING. World's Poultry Cong. Proc. 7: 488-492.


(58) **and INGRAM, M.** 1956. BONE-TAINT. Food 24: 52-55.

(59) **CAMPBELL, L. L., Jr., and O'BRIEN, R. T.** 1956. ANTIMICROBIAINS IN FOOD PRESERVATION. Food Technol. 9: 461-465.


(61) **CAMPBELL SOUP COMPANY.** 1952. PROCEEDINGS OF LOW TEMPERATURE MICROBIOLOGY SYMPOSIUM, 1951. 322 pp. Camden, N.J.


(64) **CARLSON, C. J.** 1954. KEEPING QUALITY OF CHILLED DUNGENESS CRABMEAT PACKED IN HERMETICALLY-SEALED CONTAINERS. Com. Fisheries Rev. 16 (11): 20-21.


(85) Coyne, F. P.

(86) Cross, G. C., and Ritchie, W. S.
1938. A STUDY OF RATE OF DECOMPOSITION OF HADDOCK MUSCLE AT VARIOUS TEMPERATURES AS INDICATED BY AMMONIA CONTENT. Food Res. 3: 589–598.

(87) Crozier, W. J.

(88) Dassow, J. (A).

(89) Davis, J. G.

(90) Davis, J. C.

(91) Deatherage, F. E.

(92) DeSeres, F. J.

(93) Diehl, H. C.
1929. THE COLD PACK METHOD OF PRESERVING BERRIES IN THE PACIFIC NORTHWEST. Glass Packer 2: 115–120, 129.

(94) Dorn, F. L., and Rahn, O.
1939. DEFINITION VERSUS MEASUREMENT OF OPTIMAL TEMPERATURE. Arch. f. Mikrobiol. 10: 6–12.


EWELL, A. W.
1936. THE USE OF OZONE IN COLD STORAGE PLANTS. Ice and Refrig. 91: 235-236.

---

FARBER, L.

TEENY, R. E., MACDONNELL, L. R., and LORENZ, F. W.

---
MACDONNELL, L. R., and LORENZ, F. W.
1954. HIGH TEMPERATURE TREATMENT OF SHELL EGGS. Food Technol. 8: 242-243.

FISCH, N. R.

FLORIAN, IV!., and TRUSSELL, P. C.
1957. BACTERIAL SPOilage OF SHELL EGGS. IV. IDENTIFICATION OF SPOilage ORGANISMS. Food Technol. 11: 56-60.

FORSTER, J.

FORSYTHE, R. H., AYRES, J. C., and RADO, J. L.
1953. FACTORS AFFECTING THE MICROBIOLOGICAL POPULATIONS OF SHELL EGGS. Food Technol. 7: 49-56.

FOTER, M. J., and RAHN, O.

FRANK, H. A., ISHIBASHI, S. T., REID, A., and ITO, J. S.

FROMM, D.
1957. BACTERIAL CONTAMINATION AND SHELF LIFE OF FRESHLY EVISCERATED BROILERS AS INFLUENCED BY HOLDING TIME IN SLUSH ICE. Poultry Sci. 36: 1006-1009.

---
1958. INFLUENCE OF REUSING CHILL TANK SLUSH ICE ON MARKET QUALITY OF EVISCERATED BROILERS. Food Technol. 12: 257-269.

---

---

---
and MARGOLF, P. H.

---
and MONROE, R. J.


(162) [Great Britain] Department of Scientific and Industrial Research.


(166) GRESSEL and GruFlE. 1929. ZUR FRAGE DER HALTBARKEIT VON HACKFLEISCH AUS FRISCHFLEISCH UND GERIERFLEISCH. Berlin Tierärztl. Wochnsch. 45: 430-432.


(196) HALLECK, F. E., BALL, C. O., and STIER, E. P.

(197) HALVORSON, H. O., WOLF, J., and SRINIVASAN, V. R.

(198) HANNAN, R. S.

(199) HANSEN, N. and RIEMANN, H.

(200) HANSON, H. L., and FLETCHER, L. R.

(201) HARRINGTON, W. O., MAYER, P. C., OLSON, R. L., and OTHERS.

(202) HARRISON, F. C., and SADLER, W.

(203) HARTSEL, S. E.

(204) --- CHANCE, H. L., JACOBSTEIN, H. C., and HALVORSON, H. O.

(205) HARTUNG, T. E., and STADELMAIER, W. J.

(206) HARVEY, J. M., and PENTZER, W. T.

(207) HEATHER, C. D., and VAN DER ZANT, W. C.

(208) --- and VAN DER ZANT, W. C.

(209) HENDRICKSON, R. L.

(210) HESS, E.

(211) ---

(212) ---

(213) ---
(214) Hess, E.

(215) ———

(216) ———
1950. BACTERIAL FISH SPOILAGE AND ITS CONTROL. Food Technol. 4: 477-480.

(217) Hiscox, E. R., and Briggs, C. A. E.

(218) ——— and Briggs, C. A. E.

(219) Hoagland, R., McBride, C. N., and Powick, W. C.

(220) Horne, B. C., Reeves, L. C., Ganside, J. S., and Others.

(221) Horowitz-Wlassowa, L. M., and Grinsberg, L. D.

(222) Huicke, G. J.
1954. LOW TEMPERATURE ORGANISMS IN FROZEN VEGETABLES. Food Technol. 8: 79-82.

(223) ——— Brooks, R. F., and Emery, A. J.
1952. THE SOURCE OF BACTERIA IN PROCESSING AND THEIR SIGNIFICANCE IN FROZEN VEGETABLES. Food Technol. 6: 147-155.

(224) ——— and David, E. R.

(225) ——— and David, E. R.

(226) ——— and Robinson, W. B.

(227) Hunter, A. C.

(228) ———

(229) Ingraham, J. L.

(230) ———

(231) ——— and Bailey, G. F.

(232) ——— and Stokes, J. L.


(253) Jacobson, M., Spencer, J., Hard, M., and Weller, M.
1959. THE EFFECTS OF ANTIBIOTIC TREATMENTS, PACKAGING FILMS, AND
STORAGE UPON FLAVOR, ODOR, AND APPEARANCE OF CUT-UP FRYERS.
Poultry Sci. 38: 40-47.

(253) James, L. H.
1937. ABSTRACTS OF LITERATURE ON REFRIGERATION. Refrig. Engin.
34: 237, 244, 246, 248.

(254) Jarvis, N. D.
1948. PRINCIPLES AND METHODS IN THE CANNING OF FISHERY PRODU­

(255) Jay, J. M., Weiser, H. H., and Deatherage, F. E.
1956. THE EFFECT OF ANTIBIOTICS ON THE MICROFLORA OF BEEF AND
STUDIES ON THE MODE OF ACTION OF THIS ANTIBIOTIC IN THE

(256) --- Weiser, H. H., and Deatherage, F. E.
1957. STUDIES ON THE MODE OF ACTION OF CHLOROTETRACYCLINE (AURE­
OMYCIN) IN THE PRESERVATION OF BEEF. (Abstract A37) Soc. Amer.

(257) --- Weiser, H. H., and Deatherage, F. E.
1957. STUDIES ON THE MODE OF ACTION OF CHLOROTETRACYCLINE IN THE
PRESERVATION OF BEEF. Appl. Microbiol. 5: 400-405.

(258) --- Weiser, H. H., and Deatherage, F. E.
1957. FURTHER STUDIES ON THE PRESERVATION OF BEEF WITH CHLOR­
OTETRACYCLINE. Food Technol. 11: 563-566.

(259) --- Weiser, H. H., and Deatherage, F. E.
1958. THE INHIBITION IN BEEF OF CHLOROTETRACYCLINE-RESISTANT BAC­
TELA BY SUB-BACTEROSTATIC CONCENTRATIONS OF THE ANTI­

(260) Jaye, M., Kitaaka, R. S., and Oral, Z. J.
1962. THE EFFECT OF TEMPERATURE AND PACKAGING MATERIAL ON THE
STORAGE LIFE AND BACTERIAL FLORA OF GROUND BEEF. Food
Technol. 16 (4): 95-98.

(261) Jensen, L. B.
1944. MICROBIOLOGICAL PROBLEMS IN THE PRESERVATION OF MEATS.
Bact. Rev. 8: 161-188.

(262) ---
1949. MEAT AND MEAT FOODS. 218 pp. The Ronald Press, N.Y.

(263) ---
1954. MICROBIOLOGY OF MEATS. Ed. 3. 422 pp. The Garrard Press,
Champaign, Ill.

(264) --- and Grettin, D. P.
1937. ACTION OF MICRO-ORGANISMS ON FATS. Food Res. 2: 97-120.

(265) Jespersen, A.
1945. BIOLOGISKE PROBLEMER I FORINDSELSE MED KØLING OG FRYSNING
AF KÆLED. [BIOLOGICAL PROBLEMS IN CONNECTION WITH CHILLING
AND FREEZING OF MEAT]. 1 AND II. Dansk Tekniks Tidsskr.

(266) Jezeski, J. J.
8: 429-448.

(267) --- and Olson, R. H.
1962. THE ACTIVITY OF ENZYMES AT LOW TEMPERATURES. Campbell

(268) Jones, A. H. and Ferguson, W. E.
1956. THE EVALUATION OF FROZEN VEGETABLES FOR QUALITY. Canad.
Food Indus. 27 (9): 24, 27, 29, 31.

(269) Jones, C.

(270) ---

(271) ---


(283) Kimura, T. 1958. Studies in the bacteriological chemistry of shark muscle spoilage. VI. On the effects of temperature upon the urea decomposing ability of the urea-splitting bacteria isolated from shark muscle. Hokkaido Univ. (Japan), Faculty Fisheries Bul. 8: 310-313.


FACTORS AFFECTING THE GROWTH OF PSYCHROPHILIC MICRO-ORGANISMS IN FOODS

ELLIOTT, R. P.; MICHENER, H. D.


(291) KRAFt, A. A., and AYRES, J. C. 1952. POST-MORTEM CHANGES IN STORED MEATS. IV. EFFECT OF PACKAGING MATERIALS ON KEEPING QUALITY OF SELF-SERVICE MEATS. Food Technol. 6: 8-12.

(292) KRAFt, A. A., and AYRES, J. C. 1952. POST-MORTEM CHANGES IN STORED MEATS. IV. EFFECT OF PACKAGING MATERIALS ON KEEPING QUALITY OF SELF-SERVICE MEATS. Food Technol. 6: 8-12.

(293) KRAFT, A. A., and AYRES, J. C. 1952. POST-MORTEM CHANGES IN STORED MEATS. IV. EFFECT OF PACKAGING MATERIALS ON KEEPING QUALITY OF SELF-SERVICE MEATS. Food Technol. 6: 8-12.


(306) Lochhead, A. G., and Landerkin, G. B.
1935. BACTERIOLOGICAL STUDIES OF DRESSED POULTRY. I. PRELIMINARY INVESTIGATIONS OF BACTERIAL ACTION AT CHILL TEMPERATURES.

(307) Long, S. K., and Williams, O. B.
1959. GROWTH OF OBLIGATE THERMOPHILES AT 37 C. AS A FUNCTION OF THE CULTURAL CONDITIONS EMPLOYED.
Jour. Bact. 77: 545-547.

(308) Lorenz, F. W., Starr, P. B., Starr, M. P., and Ogasawara, F. X.
1957. THE DEVELOPMENT OF PSEUDOMONAS SPOILAGE IN SHELL EGGS. I. PENETRATION THROUGH THE SHELL.
Food Res. 17: 361-369.

(309) Lüthy, H.

(310) Lutjen, A. F. M. C.
1958. OBJECTIVE SPOILAGE TESTS FOR FISH STORED UNDER CONDITIONS OTHER THAN NORMAL CHILLING IN ICE.

(311) Luyet, B. J., and Greeno, P. M.
1938. THE LOWER LIMIT OF VITAL TEMPERATURES: A REVIEW.
Biodynamics 1: 1-62.


(313) McKee, R. C., Conkey, J., and Carlson, J. A.
1959. A STUDY OF THE COMPARATIVE SHELF LIFE OF WET AND DRIED PACKED POULTRY.

(314) McNally, E. H.
1952. EFFECT OF DRYING AFTER WASHING ON THE INCIDENCE OF EGGS INFECTED BY SPOILAGE MICRO-ORGANISMS. Poultry Sci. 31: 1102-1104.

(315) McGVICKER, R. J., Dawson, L. E., Mallmann, W. L., and others.
1959. THE EFFECT OF CHLORTETRACYCLINE ON SHELLFLESH OF FRESH POULTRY MEAT TREATED IN A COMMERCIAL PROCESSING PLANT.
Poultry Sci. 38: 337-343.

(316) McWhorter, A. C., Murrell, M. C. and Edwards, P. R.
1963. RESISTANCE OF SALMONELLA ISOLATED IN 1962 TO CHLORTETRACYCLINE.

(317) Macy, H., and Steele, G. H.
1934. BUTTER AS A SUBSTRATE FOR MOLD GROWTH.

(318) Margolis, L.

(319) Margolies, P.

(320) Glass, E. H., and Frazer, W. C.
1957. BACTERIOLOGY OF MILK HELD AT FARM MILK COOLING TANK TEMPERATURES. III. PSYCHROPHILES FOUND AND THEIR GROWTH.
(325) MAY, K. N., IRBY, J. D., and CARMON, J. L.  
1962. SHELF LIFE AND BACTERIAL COUNTS OF EXCISED POULTRY TISSUE.  
Food Technol. 16 (2): 65-68.

(326) MICHENER, H. D., DIETRICH, W. C., and THOMPSON, P. A.  
1960. TIME-TEMPERATURE TOLERANCE OF FROZEN FOODS. XXII. RELATIONSHIP OF BACTERIAL POPULATION TO TEMPERATURE.  

(327) --- and ELLIOTT, R. P.  

(328) MILLER, D. D., and GOLDING, N. S.  

(329) MILLER, W. A.  

(330)  

(331)  

(332)  
1955. DRY CLEANING SLIGHTLY SOILED EGGS VERSUS WASHING TO PREVENT PENETRATION OF SPOILAGE BACTERIA. Poultry Sci. 34: 906-913.

(333) --- and CRAWDUM, L. B.  

(334) MONOD, J.  

(335) MORGAN, T.  

(336)  

(337)  
1938. GAS STORAGE OF MEAT AND EGGS. Food Res. 3: 149-154.

(338) MORGAN, T. F.  

(339) MOESSEL, D. A. A.  

(340) --- and INDIAN, M.  

(341) --- and WESTERDUIJK, J.  

(342) MOUTNEY, G. J. and VAN DER ZANT, C.  
(343) MÜLLER, M.  

(344) MULCOCK, A. P.  

(345) MUNDT, J. O.  

(346) --- STOKES, R. L. and GOFF, O. E.  

(347) MURDOCK, C. R., CROSSLEY, E. L., ROBB, J., and OTHERS.  

(348) MURDOCK, D. I. and BROKAW, C. H.  

(349) NADEAU, P. A.  

(350) NAGEL, C. W., SIMPSON, K. L., NG, H., and OTHERS.  

(351) NASHIFF, S. A., and NELSON, F. E.  

(352) --- and NELSON, F. E.  

(353) --- and NELSON, F. E.  

(354) NETHERLANDS MINISTRY OF AGRICULTURE, FISHERIES, AND FOOD.  

(355) NEWELL, G. W., CWIN, J. M., and JULI, M. A.  
1948. THE EFFECT OF CERTAIN HOLDING CONDITIONS ON THE QUALITY OF DRESSED POULTRY. Poultry Sci. 27: 251-256.

(356) NG, H., INGRAHAM, J. L., and MARR, A. G.  

(357) --- VAUGHN, R. H., STEWART, G. F., and OTHERS.  

(358) NILSSON, T., and REINJUS, L.  

(359) NIVEN, C. F., Jr.  

(360) ---  
(361) NIVEN, C. F., JR.

(362) and CHESSON, W. R.


(364) NOTEVARE, O., and HJORTH-HANSEN, S.

(365) O'GISLY, W. S. and AYRES, J. C.

(366) and AYRES, J. C.
1951. POST-MORTEM CHANGES IN STORED MEATS. III. THE EFFECT OF ATMOSPHERES CONTAINING CARBON DIOXIDE IN PROLONGING THE STORAGE LIFE OF FRANKFURTERS. Food Technol. 5: 309-323.

(367) OLSEN, R. H., and JEZESKI, J. J.

(368) OLSON, J. C., JR.

(369) PARKER, R. B., and MUELLER, W. S.

(370) WILLOUGHBY, D. S., THOMAS, E. L., and MURRIS, H. A.

(371) PAINES, J. J., and THOMAS, S. E.

(372) PARMANN, W.

(373) PATRICK, R. and HILL, E. C.

(374) PEARCE, J. A., and LAVERS, C. G.

(375) PEDERSON, C. S.

(376) ALBURY, M. N., and CHRISTENSEN, M. D.

(377) PENFOLD, W. J.

(378) PENNINGTON, M. E.
100 TECHNICAL BULLETIN 1320, U.S. DEPT. OF AGRICULTURE


PSYCHROPHILIC MICRO-ORGANISMS IN FOODS


(400) ROGERS, R. E., and McCLESKEY, C. S. 1957. BACTERIOLOGICAL QUALITY OF GROUND BEEF IN RETAIL MARKETS. Food Technol. 11: 318-320.


(409) ——— and SENN, V. J. 1962. EFFECT OF PRESERVATIVES AND STORAGE TEMPERATURES ON SHELF LIFE OF CHILLED CISUS SALADS. Food Technol. 16: 77-79.


(415) ——— 1959. KOMBINATION VON METHODEN ZUR VERHINDERUNG DES MUKRO- 
(416) SCHMID, W.  
1931. INFLUENCE OF TEMPERATURE AND FEEDING ON THE BACTERIAL GROWTH ON CURED MEAT. Ztschr. f. die Gesamte Kältetechnik 38: 1-5.

(417) SCHMIDT, W.  

(418) SCHMIDT, F. J., and STADELMAN, W. J.  

(419) SCHMIDT, E., BARTRAM, M. T., and LEPPER, H. A.  

(420) SCHÖNBERG, F., and DSEBULIC, S.  

(421) SCHWARTZ, W., and ZEISER, Th.  

(422) SCOTT, W. J.  

(423) SCOTT, W. J.  

(424) SCOTT, W. J.  

(425) SCOTT, W. J.  

(426) SCOTT, W. J.  

(427) SHANK, J. L., and LUNDQUIST, R. B.  

(428) SEAGRAN, H., COLLINS, J., and IVIERS, J.  

(429) SEAGRAN, H., COLLINS, J., and IVIERS, J.  

(430) SELUHIN, G. L., and PUMPIANSKI, A. Ya.  

(431) SHANK, J. L., and LUNDQUIST, B. R.  
(432) Shannon, W. G., and Stadelman, W. J.
1957. THE EFFICACY OF CHLORTETRACYCLINE AT SEVERAL TEMPERATURES IN CONTROLLING SPOILAGE OF POULTRY MEAT. Poultry Sci. 36: 121-123.

(433) Sharp, P. F., and Stewart, G. F.

(434) Shepherd, A. D.

(435) Shewan, J. M.

(436) Shepherd, A. D.

(437) ---

(438) ---

(439) --- and Georgala, D. L.

(440) Hobbs, G., and Hodckett, W.

(441) Shrimpton, D. H.

(442) ---

(443) --- and Barnes, E. M.

(444) Simpson, K. L., Nagel, G. W., Ng, H., and others.


(446) Sizer, I. W., and Josephson, E. S.
1942. KINETICS AS A FUNCTION OF TEMPERATURE OF LIPASE, TRYPSIN, AND INVERTASE ACTIVITY FROM -70 TO 50° C. (−94 to 122° F.) Food Res. 7: 201-209.

(447) Smart, H. F.

(448) ---
1937. TYPES AND SURVIVAL OF SOME MICRO-ORGANISMS IN FROZEN-PACK PEAS, BEANS, AND SWEET CORN GROWN IN THE EAST. Food Res. 2: 515-528.
(449) Smart, H. F.
1939. Microbiological studies on commercial packs of frozen fruits and vegetables. Food Res. 4: 293-298.

(450) --- and Brunstetter, B. C.

(451) --- and Brunstetter, B. C.

(452) Smith, A. U.

(453) Snow, D.

(454) Southcott, B. A., and Tarl, H. L. A.


(456) --- Sauter, E. A., and Stadelman, W. J.

(457) --- Sauter, E. A., and Stadelman, W. J.

(458) --- and Stadelman, W. J.

(459) --- Zeigler, F., and Stadelman, W. J.


(461) --- Wettergreen, W. P., and Pederson, C. S.

(462) Stanishy, M. E., and Griffiths, F. P.

(463) --- and Lemon, J. M.

(464) Steiner, C., and Tarl, H. L. A.

(465) Steinke, P. K. W., and Foster, E. M.

(466) Stein, J., and Dassow, J. A.

(467) Stewart, C. F.
(468) STEWART, G. F., LOWE, R., and MORGH, M.  
1941. POST-MORTEM CHANGES IN NEW YORK DRESSED POULTRY AT 35° F.  

(469) STEWART, M. M.  

(470) ———  
1935. THE KEEPING QUALITY OF HADDOCK FROM COLD STORAGE. Soc.  

(471) STILIE, B.  

(472) ———  

(473) STOKES, J. L.  
1960. GROWTH OF MICRO-ORGANISMS AT LOW TEMPERATURES. Amer.  

(474) ——— and OSBORNE, W. W.  
1956. EFFECT OF THE EGG SHELL MEMBRANE ON BACTERIA. Food Res.  
21: 264-269.

(475) STUART, L. S., and McNALLY, E. H.  
1943. BACTERIOLOGICAL STUDIES ON THE EGG SHELL. U.S. Egg &  
Poultry Mag. 49: 28-33, 45-47.

(476) STUTTS, H. P.  
865, 208 pp.

(477) SUSTER, B. M.  
1962. OXIDATIVE ACTIVITY OF PSYCHROPHILIC AND MESO-PHILIC BAC-  
TERTIA ON SATURATED FATTY ACIDS. Jott. Bact. 82: 492-497.

(478) SZECHYNSKI, W. L.  
1952. EFFECT OF FREEZING AND THAWING ON THE GROWTH RATE OF  
BACTERIA IN GROUND MEAT. Food Technol. 6: 341-343.

(479) SZCZEPULKA, W., and Szczerbula, J.  
1956. EFFECT OF PASTEURIZATION ON QUANTITY AND MICROFLORA OF  
(Abstract in Chern. Abs. 52: 18947a. 1958.)

(480) TANNER, F. W.  
1944. THE MICROBIOLOGY OF FOODS. Ed. 2, 1196 pp. Garrard Press,  
Champaign, Ill.

(481) TARR, H. L. A.  
1943. RISE AND FALL OF BACTERIAL POPULATIONS IN FISH MUSCLE.  

(482) ———  
8th SIX-Pub. 100, ch. 5, 7 pp.

(483) ———  

(484) ———  

(485) ———  
1959. NEW HORIZONS IN FOOD PRESERVATION. PART 2. Canad. Food  

(486) ———  
1950. ANTIBIOTICS AS A PRESERVATIVE MEASURE. Canad. Inst. Food  

(487) ———  
1960. ANTIBIOTICS IN FISH PRESERVATION. Canada Fisheries Res. Bd.  
(3): 866. 1961.)
(488) TARR, H. L. A.  

(489) THATCHER, F. S., and LORD, A.  

(490) THOMAS, S. B.  

(491) ———  

(492) ———  

(493) THORNLEY, M. J., INGRAM, M., and BARNES, E. M.  

(494) THORNTON, H. G., and MEIKLEJOHN, J.  


(496) ——— and MCCLESKEY, C. S.  
1941. BACTERIOLOGICAL STUDIES OF FRESH CRABMEAT. Food Res. 6: 157-157.

(497) TOMIYASU, Y., and ZENITANI, B.  

(498) TOMKIN, R. G.  

(499) ———  

(500) ———  

(501) TOMLINSON, N.  
1957. ANTIBIOTICS IN FOOD PRESERVATION. Sanitarian 1 (9): 15-22.

(502) TÖRÖK, G., and ALMÁSI, E.  

(503) TORRES RÖTELKO, A.  

(504) TRESSLER, D. K., and EVERS, C. F.  

(505) TRUSSELL, P. C., FULTON, C. 0., and CAMERON, C. J.  
1955. BACTERIAL SPOILAGE OF SHELL EGGS. II. INCIDENCE OF SPOILAGE IN EGGS FROM NINETY-FOUR FARMS. Food Technol. 9: 130-134.
(506) TRUSSELL, P. C., TRIGGS, R. E., and GREER, B. A.
1955. BACTERIAL SPOILAGE OF SHELL EGGS. III. FARM PRACTICES PROMOTING SPOILAGE. Food Technol. 9: 134-137.

(507) UNITED STATES FISH AND WILDLIFE SERVICE.

(508) UNITED STATES FOOD AND DRUG ADMINISTRATION.

(509) UNITED STATES PUBLIC HEALTH SERVICE.

(510) UPADHYAY, J., and STOKES, J. L.

(511) —— and STOKES, J. L.

(512) USPENSKY, A. A.

(513) VALLEY, G., and REITZER, L. F.

(514) VANDERLECK, J.

(515) VAN DER ZANT, W. C., and MOORE, A. V.


(517) VAUGHN, R. H., NAGEL, C. W., SAWYER, F. M., and STEWART, G. F.

(518) —— No., H., STEWART, G. F., and OTHERS.

(519) —— No., H., STEWART, G. F., and OTHERS.

(520) —— and STADTMAN, T. C.

(521) STADTMAN, T. C., and KUENEMAN, R. W.

(522) VENKATARAMAN, R.

(523) WALDEN, C. C., ALLEN I. V. F., and TRUSSELL, P. C.

(525) ——— Taylor, J. H., and Davis, J. G.

(526) Walker, H. W., and Ayres, J. C.

(527) Wall, S.

(528) Well, B. H., and Sterne, F.

(529) Wernberg, E. D.

(530) Weinrich, J., and Newton, E. B.

(531) Weisser, H. H., Kunkle, L. E., and Deathержage, F. E.

(532) Wells, F. E., Hartsell, S. E., and Stadelman, W. J.

(533) ——— Spencer, J. V., and Stadelman, W. J.

(534) ——— Stadelman, W. J., and Hartsell, S. E.

(535) White, A., and White, H. R.

(536) Whitehill, A. R.

(537) Williams, L. L., and Yung, F. D.


(539) Winter, A. R., Burkart, B., Clements, P., and MacDonald, L.

(540) ——— Greco, P. A., and Stewart, C. F.


(558) Ziegler, P. T.

(559) Zo Bell, C. E.
1946. MARINE MICROBIOLOGY, A MONOGRAPH ON HYDRO BACTERIOLOGY.

(560) ---

(561) --- and Conn, J. E.
1940. STUDIES ON THE THERMAL SENSITIVITY OF MARINE BACTERIA.