Microbiological Problems of Frozen Food Products

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I. INTRODUCTION

Frozen foods are constantly gaining new markets. An account of the microbiological problems raised by this kind of food is, therefore, appropriate at this time. Frozen foods are not sterile in the same way as are canned products, for they are not subjected to the same lethal tempera-
ture treatment as are foods sterilized by heat, though an absolute sterility, as a rule, is not even reached through this treatment. The large number of bacteria in a frozen product is of great importance, especially while thawing.

This report will be concerned mostly with frozen products with one important exception: ice cream, which has been excluded from this survey, for it requires special consideration.

II. The Influence of Freezing Temperatures on Microorganisms

It is a common belief that microorganisms are effectively killed by freezing. They are destroyed to a certain extent but far from completely. Bacteria and fungi in the vegetative stage are sensitive to low temperatures and generally succumb. This effect however is not always immediate, and may require long periods of freezing storage. On the other hand, resistance is greatest in the spore stage.

One decisive factor is the freezing temperature. Especially sensitive are yeasts and molds, which occur commonly on berries and vegetables. Soil bacteria, which as a rule survive the freezing temperatures occurring in nature, are, however, not very susceptible to cold. Several species are extremely psychrophilic and even manage to grow at -7° C. (20° F.) (Prescott et al., 1932), which otherwise exerts a definite lethal effect on most bacteria. A unique observation, to which there is as yet no explanation, is the ability of intestinal bacteria to endure the acid medium of orange juice better at -4° C. (23° F.) than at room temperature (Beard and Cleary, 1932).

It is an often overlooked fact that temperature alone is not the decisive factor with respect to the degree of destruction. It is the formation of ice crystals (actual freezing of the product) which has the more profound and disturbing influence on bacterial growth. Supercooling may take place without any substantial change in the colloidal condition or tissue structure. Studies on supercooling both in fish and in meat have shown very slight effects, irrespective of the retardation of growth.

It may be the desiccation of the substrate and not temperature as such, which sets the lower limit for the growth of microorganisms in tissue. This is indicated in studies on meat and fish, and from many observations in which growth has been observed in media supercooled to as low as -20° C. (-4° F.). When meat is frozen and is allowed to reach equilibrium at a certain temperature, only a certain proportion of the water separates as ice. The amount of bound water and the degree of protein denaturation determines the extent to which ice is formed. Moran (1931) reported the following:

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Percentage of total water present as ice</th>
</tr>
</thead>
<tbody>
<tr>
<td>-3° C. (27° F.)</td>
<td>70</td>
</tr>
<tr>
<td>-5° C. (23° F.)</td>
<td>82</td>
</tr>
<tr>
<td>-10° C. (14° F.)</td>
<td>94</td>
</tr>
</tbody>
</table>

Most papers in this field make no clear distinction between freezing rate and freezing temperature. The most efficient way of accelerating freezing rate—the advance of the freezing front in the product—is to use low temperature. This implies the need of distinguishing between the freezing temperature as it is measured outside the product—the external temperature at which freezing takes place on one hand, and on the other hand the internal temperature at which actual freezing takes place in the product. A similar distinction should be made regarding thawing temperature, which in publications has a two-fold meaning: either the internal temperature at which defrosting of the product actually takes place, or the external temperature condition under which thawing is carried through.

A critical scrutiny of available publications in the field showed that very few studies could be accepted as providing information on the effect of freezing (thawing) temperature and freezing (thawing) rates.

The corresponding increase in the concentration of the solutes may on the other hand obviously exercise some kind of a protective influence on surviving bacteria enabling them to avoid actual freezing. More studies are needed to clarify this fundamental point.

Several studies have also indicated that far more microorganisms are destroyed at -4° C. (25° F.) than at -15° C. (5° F.) or -24° C. (-11° F.). In most cases for temperatures below -10° C. (14° F.) the lower the temperature, the less effectively are the bacteria killed. It can also be said that temperatures below -24° C. (-11° F.) and even as low as -193° C. (-315° F.) have no additional effect (Luyet and Gehenio, 1940; Swift, 1937).

The finding that low freezing temperatures -20° C. (-4° F.) are less harmful to microorganisms than the medium range of temperatures such as -10° C. (14° F.) (Campbell, 1932; Berry, 1934; Haines, 1934-38; McFarlane, 1940a, b; Weiser, 1951; Gottlib, 1951; Hacker et al., 1952) is highly important to an understanding of the microbiology of frozen food (see Tables I and II). Thus for example, less than 1% of microorganisms in beans survived a storage temperature of -10° C. (14° F.) while 6% survived -21° C. (-6° F.). This also implies the strange consequence that freezing is bactericidal to the greatest extent if it takes place slowly and the products are afterwards stored at a comparatively high temperature (Haines, 1938). These findings are quite contrary to the demand of
TABLE I
Per Cent Survival of Bacteria at Different Subzero Temperatures

<table>
<thead>
<tr>
<th>Storage temperature</th>
<th>Length of freezing storage (days)</th>
<th>per cent survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>115</td>
</tr>
<tr>
<td>-10° C. (14° F.)</td>
<td>100</td>
<td>6.1</td>
</tr>
<tr>
<td>-15° C. (7° F.)</td>
<td>100</td>
<td>16.8</td>
</tr>
<tr>
<td>-20° C. (4° F.)</td>
<td>100</td>
<td>50.7</td>
</tr>
</tbody>
</table>

* From Gotlib (1951).

TABLE II
Survival Time of Certain Pathogenic Bacteria in Sliced, Sweetened Strawberries Stored at -18° C. (4° F.)*

<table>
<thead>
<tr>
<th>Culture</th>
<th>Bacteria per gram before freezing</th>
<th>Bacteria per gram after freezing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without culture</td>
<td>With culture</td>
</tr>
<tr>
<td><em>Eberthella typhosa</em></td>
<td>112,000</td>
<td>194,000</td>
</tr>
<tr>
<td><em>Salmonella paratyphi</em></td>
<td>248,000</td>
<td>214,000</td>
</tr>
<tr>
<td><em>Salmonella schottmuelleri</em></td>
<td>80,000</td>
<td>232,000</td>
</tr>
<tr>
<td><em>Salmonella aerolyke</em></td>
<td>151,000</td>
<td>335,000</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>135,000</td>
<td>810,000</td>
</tr>
</tbody>
</table>

* From McCluskey and Christopher, 1941.

† Plate counts were made after the berries had been kept in crushed ice for 5 hrs.

‡ Showed the existence of pathogenic bacteria in the berries.

Modern freezing technique, which dictates a rapid freezing and subsequent storage at low temperature.

In spite of the pronounced lethal effect of temperatures in the range of 0° C. (32° F.) down to almost -10° C. (14° F.), there are substantial data to testify to the capacity of several microorganisms to grow even under these conditions. The cause for this dualism in the reaction of the living protoplast has so far not been unveiled. A great number of bacteria, generally termed psychrophilic, can grow at subzero temperatures even down to -8° C. (18° F.) (Haines, 1934; Tchistjakow and Botscharowa, 1938). In most cases, growth is checked by temperatures below -10° C. (14° F.). Some molds even manage to grow at this temperature (Bidault, 1922).

In 1941, McCleskey and Christopher proved that *Staphylococcus aureus* as well as some *Salmonella* species still survived in unsliced strawberries after being stored for fourteen months at -18° C. (0° F.). Freezing storage consequently does not “pasteurize” a product, if it contains bacteria. A vast number of papers prior to this discovery, and subsequently, have given overwhelming proof of the wide range of organisms which manage to survive in frozen storage, even for extremely long periods.

The reason why temperatures higher than -10° C. (14° F.) are more lethal to microorganisms is most likely connected with the processes of protein denaturation being far less disastrous at lower temperatures. In freezing mammalian and fish muscles, Moran (1935) and Reay (1933) drew attention to the rapid denaturation of the protein which occurs at temperatures just below the freezing point of the muscle and in the region of -2 to 4° C. (28-39° F.). They attributed the rapid death of microorganisms in this temperature range to the fact that, within 8 days at -2° C. (28° F.), half of the coagulable protein of the organisms was precipitated. At -20° C. (-4° F.) no such change took place. Death of frozen microorganisms consequently may be ascribed to the denaturation of the protein and subsequent flocculation of the cellular proteins. This concept entirely refutes the idea that death is due to the mechanical action of ice crystals. Nor is it likely that intracellular ice crystals cause destruction as death is most effective at -2° C. (28° F.) where such
intracellular crystals should not form because of the salts in the cell contents (Weiser and Osterud, 1945).

III. Influence of the Freezing Rate

When freezing extremely rapidly (e.g., by immersion in liquid air), the effect on bacteria is so small that their count is at the most only slightly diminished (van Eseltine et al., 1948). Some sensitive species of certain Pseudomonas may in this respect represent important exceptions (Ingram, 1951; Borgström, unpublished data). This is explained by the discovery that even a living tissue can be kept in a frozen state because of the fact that no ice crystals are formed when freezing is sufficiently rapid (Luyet and Geheno, 1940). The protoplasm is transformed into a vitreous state.

In water solutions a slow freezing may sometimes cause an unusual result. During this process practically pure water in the form of ice crystals is extracted from the cell solution, and the bacteria become concentrated in the remaining solution. In this case the microorganisms survive and even continue to grow in the remaining unfrozen solution. Slow freezing consequently might allow far more bacteria to survive than rapid freezing, in clear contradiction to what happens in most cases (Hess; 1934a,b). Along such lines, an explanation may be given to why Haines (1938) has been able in his studies to establish that the freezing rate is of no importance.

The rate of freezing at temperatures as low as $-20^\circ$ C. ($-4^\circ$ F.) affect the mortality rates of bacteria and yeasts, but there are certain differences with respect to the effect. Escherichia coli and Lactobacillus casei were found to be more readily destroyed by slow than quick freezing, whereas the opposite effect was observed with Micrococcus pyogenes var. aureus and some yeasts (Devik and Ulrich, 1949).

The freezing rate has a great influence on the number of bacteria developing before the temperature inhibits further growth. Packaging in highly insulated material or freezing in still air, as often happens in lockers or home freezers, might give products with a higher bacterial count (van Eseltine et al., 1948). Generally, however, this is no reason for alarm. The bacterial count is seldom sufficiently high to seriously impair the quality. Mortality curves for frozen foods, stored at a constant, defined temperature, indicate that during the initial stage, when the bacterial count is decreasing comparatively quickly, there is a definite relationship between death rate and viable bacteria count. During later stages of frozen storage the bacterial count decreases less rapidly. Gundersen and Ross (1948b) found that the number of bacterial colonies (bacterial populations) remained more or less constant when stored at $-25^\circ$ C. ($-13^\circ$ F.). It is of special interest that pathogenic bacteria seem to have less resistance to freezing storage than saprophytic types (Anonymous, 1946a).

Greater numbers of viable cells are destroyed in the first 24 hr. of storage after freezing than in any other similar period, except for Staphylococcus aureus which may remain unchanged for at least 55 days (Ulrich and Halvorson, 1947).

Of greatest importance in connection with precooked frozen foods is the circumstance that substrates containing fats and sugar seem to protect bacteria (James, 1933a; McFarlane, 1942; Gotlib, 1951). Table III, shows the protective effect of 20% sucrose as compared with 2%. In an unsweetened frozen orange juice 97% of the organisms were destroyed within 48 hr., whereas large numbers of cells were still viable after 26 weeks of storage in juice containing 40-50% sucrose. In frozen eggs bacteria are able to survive for a very long time (McFarlane and Goreline, 1943; Wallace and Baumgartner, 1936). Freezing temperatures, although not necessarily lethal to microorganisms, may markedly influence the action of deleterious agents present. There is an appreciable enhancement of the bactericidal effect of the hydrogen ion by low temperatures (Beard and Cleary, 1932). Thus in an acid reaction the mortality rate is higher than in an alkaline one (Stille, 1950). This is favorable in so far as frozen fruits, berries and their juices are concerned (Berry, 1932a,b and c; Hahn and Appleman, 1952b).

Of course the more concentrated the syrup or the juice, the lower is the freezing point, but more bacteria survive the lower freezing temperatures (Woodroof, 1931; Lutza et al., 1932; McFarlane, 1940a,b).

Keith (1913) showed that if certain substances such as sugar, milk and glycerol were added to the suspending medium, bacteria were partially protected from the damage of freezing at $-20^\circ$ C. Recent observations on a similar effect of glycerol on spermatozoa (Polge et al., 1949; Smith and Polge, 1950) is (is) the basis of the present new developmental trend in artificial insemination.

Glycerol is also able to prevent the damage to bacteria (4 species studied) in freezing and thawing, but does not seem to exert any influence during storage (Hollander et al., 1954). This protective effect may be due to its interference with the expansion of water, when freezing to ice (Hollander et al., 1954).

The recent discovery that bacteria, particularly E. coli, inactivated by ultraviolet radiation, may be reactivated later seems to be of little importance to the frozen food industry even though it has been proved that such an inactivation may take place at subfreezing temperatures. In this case reactivation occurs only in the liquid phase (Heinmets and Taylor, 1951). These peculiar reactions may in special cases affect small samples...
treated in the daylight. In most cases, however, all light influences can be ruled out with respect to frozen food packages.

In summary, it may be stated that the bacteria and spore killing effect of low temperatures implies that frozen foods generally contain fewer bacteria than the corresponding fresh products. This, however, is valid only if the frozen products are actually stored at a temperature guaranteeing an unthawed condition.

IV. Freezing Death of Bacteria

Critical reviews on the death of bacteria at low temperatures are few, and much data remain widely scattered in the literature among other subjects. Only a brief summary of some of the most important publications will be given here, for an extensive review of the subject is beyond the scope of this paper. Some of the authors who have presented the most extensive bibliographies on the subject are Prudden, 1887; Sedgwick and Winslow, 1902; Smith and Swingle, 1905; Keith, 1913; Hilliard and Davis, 1918; Vass, 1919; Hampil, 1932; Wallace and Tannor, 1933; Turner and Brayton, 1938; Kyos and Potter, 1939; Luyet and Gheenio, 1940; and Stille, 1942. Different phases of frozen food microbiology have been reviewed by Mattek (1952), Ingram (1951), and Davis (1951).

As the result of investigations involving experiments on supercooling versus freezing, repeated freezing, and storage of frozen water suspensions of various bacteria, Prudden (1887) concluded that the marked initial killing following freezing is due to the immediate killing of the "more feeble" bacteria. He reported that supercooling was even more destructive than freezing and that very low temperatures were more destructive than the higher freezing temperatures. He also noted the gradual death of organisms during storage, a higher mortality with repeated freezing, and also a low resistance of old cultures of *Staphylococcus aureus* to freezing. Most of these observations are contrary to present conceptions and may be due to deficient experimental conditions.

The most common belief for a long period of time was that bacteria died when crushed by the formation of extracellular ice crystals, that is to say, mechanical death (Keith, 1913). This was disproved chiefly by the studies of Haines (1938) showing that a slow freezing rate, giving larger ice crystals, was not more destructive than rapid freezing. Only the temperature of subsequent storage (in the frozen state) had a decisive influence on survival. This mechanical theory received a late supporter in Gaebelien (1940) claiming that ice crystals at -4°C (25°F.) caused a maximum disruption of the cells.

Recently evidence was presented to the effect that the death of bac-

teria by freezing involves a rapid action or "immediate" death, caused by freezing and thawing *per se*, and a "storage" death, which is a direct function of time and temperature. The immediate death seems to result principally from the mechanical action of extracellular ice (Weiser and Osterud, 1945). No ice is formed within the bacterial cells, even at very low temperatures. In such cases water solidifies to a vitreous state.

Hollander et al. (1954) suggest that death which occurs during the freezing and thawing of bacteria is due to mechanical compression, a consequence of the fact that water expands 9% when it changes into ice.

The killing effect of freezing temperature is complicated by the diverse reactions of water to subzero temperatures. Under ordinary circumstances it crystallizes to form ice. However, if cooled to very low temperatures under special conditions, it may solidify without crystallizing, a state analogous to glass and termed vitreous. The vitrification of pure water is very difficult to accomplish, but has been reported by Hawkes (1929) and by Burton and Oliver (1935).

Any extensive discussion of the factors influencing the crystallization, vitrification, and devitrification of water is not within the scope of this review. These subjects are well treated in such works as those by Luyet and Gheenio (1940) and Dorsey (1940). Briefly, vitrification of any aqueous solution can only be accomplished by reducing the temperature through the zone at which freezing occurs so rapidly that there is insufficient time for crystals to form. With ordinary water, the velocity of the formation of ice crystal nuclei, and of crystal growth, is so great that vitrification is seldom accomplished. The vitrification point is at -110°C. (-166°F.) and consequently has no relevance to ordinary frozen foods, but most likely explains the survival even at temperatures quite close to the absolute limit (120°K.) as was proved by Bbeequerel (1960).

As already stated denaturation of the protein must be taken into serious consideration as a causative factor of the death of the microorganisms (van den Broek, 1949) whatever the mechanism of this denaturation is.

If the destruction of bacteria proceeds in the same way, in subsequent long-range storage, it is so far not established. An indication that this might be so is the fact that most frozen products, when stored, show a greater reduction in viable counts when held at temperatures between -2°C. (28°F.) and -10°C. (14°F.) than between -15°C. (5°F.) and -20°C. (-4°F.) (Hartcell, 1949; Hucker et al., 1952). This gradual destruction may have importance to sanitary control and to the hygienic evaluation of a product. In the following, many examples will be given of survival of bacteria during freezing storage. There are several indications of a differing resistance to such conditions. At any rate freezing storage
is now used as a method of maintaining stable infectious bacterial collections, which are still fully viable after two years of such storage (Yurchenko et al., 1954).

V. OCCURRENCE OF BACTERIA IN FROZEN FOODS

1. Survey of Problems

The factors influencing the bacterial content of prepackaged frozen foods may be summarized in the following manner: (1) The treatment of the raw product including the number of bacteria originally present in the raw product, the manner and rapidity of handling between harvesting (or slaughter) and processing, the processing methods, and the hygienic conditions in factories with machines and equipment. (2) The freezing rate. (3) The amount of oxygen present in the package. (4) The microbiological conditions in packages for frozen foods. (5) The storage temperature. (6) The pH of the product. (7) The presence of osmotic substances in frozen foods. (8) Defrosting.

An unexpected high bacterial content in frozen foods may under certain conditions be apprehended. On the whole this may be due to the following four causes:

(1) High original content of bacteria, possibly an initial decomposition in the raw products.

(2) Delay in the freezing process.

(3) Slow or imperfect freezing.

(4) Thawing or partial heating above 0°C (32°F.) induced by fluctuating temperatures reaching dangerous levels [heating to temperatures in the vicinity of -2 to -6°C (28 to 21°F.)] should nevertheless diminish the bacterial count (p. 165).

In principle, we may distinguish between two categories of investigations on these problems: studies on the microbiological flora of frozen foods and its character under different conditions, and direct infection studies concerned with bacterial inoculation, particularly pathogenic forms.

When discussing the microbiology of frozen foods it may be appropriate to point out that there are essentially three groups of microbes which are of significance, namely: pathogenic, toxigenic, and saprophytic types. This classification serves all practical purposes although certain exceptions may be easy to state, as *Clostridium botulinum* being a pathogenic saprophyte (Frobisher, 1953). It is particularly important that the methods for the examination of frozen foods primarily aim at detecting the organisms that are dangerous to the gastro-intestinal system.

2. MICROBIOLOGICAL METHODS OF ANALYSIS

It is likely that too much attention has been given to the quantitative point of view in the bacterial control of frozen foods. The number of colonies on a plate, as a matter of fact, does not always give an adequate picture of the quality or of the risks. This does not only refer to frozen foods but may be valid for foods in general (Wilson, 1935). In some cases a wrong conception of the bacterial condition is obtained by relying on per cent decrease or increase figures. As pointed out above large changes may mean little from the bacteriological point of view. For example, Nickerson (1943) found that the bacterial count in different packages of frozen broccoli might vary within a range of 200% and still be not very different in quality from the bacteriological point of view. Consequently no fixed figures corresponding to quality grades can be established or should be promulgated.

The standard methods valid for bacterial control of other preserved foods have been elaborated on the basis of long experience, principally from the standpoint of the errors that can occur. These accepted methods, however, can be used without modifications for frozen foods only in exceptional cases. Frozen foods vary to such an extent from other types of preserved foods that different sets of standards must be established (Humphrey, 1950).

The sampling technique may influence considerably the result of a determination of the microbial content, particularly if the sample is not thawed under defined conditions. The substrate always influences the result obtained. The methods in this field have been inadequately elaborated (Humphrey, 1950). In particular, observations made on frozen meat, using the plate count method, have shown the necessity for emphasizing great care in arriving at conclusions with respect to the lethal effect of freezing. The occurrence of conglomerates which, according to temperature conditions, become separated into smaller units might thus produce an increase in the number of colonies without any real growth taking place.

Contrary to what might be supposed, taking samples for bacteriological investigation from the unthawed product is in most cases to be preferred. If the product has thawed, the water frozen in the product melts, as well as the water which has been retained as a coating during the preparation. This water generally contains more bacteria per unit weight than the product itself. Therefore, a sample which includes too much water will result in erroneous counts. It is essential, therefore, to get proportionate amounts of water; this however is very difficult. Hence, sampling of unthawed products is preferable. Special sample-taking instru-
ments have been invented which cut out representative samples from the frozen product (Anonymous, 1946b).

In the United States a committee (Committee on Microbiological Examination of Foods) has been developing methods intended to be used for the preparation of samples of frozen foods. The following is a summary of the recommendations of this committee (Anonymous, 1946b): A mechanical blender (Waring Blender, Oster Blender, Turnix, etc.) should be used in the preparation of samples of frozen fruits and vegetables. This conclusion has been arrived at after trying many different methods of preparation with various frozen products. In comparison with hand grinding, mechanical disintegration as a rule yields higher bacterial counts, since conglomerates are more efficiently separated. At any rate more uniform results are obtained and the method is less laborious.

Similarly, the choice of tryptone-glucose agar (with meat extract) rests on the demonstrated superiority of this medium over nutrient agar or glucose agar as a medium for frozen peas.

The influence of the plating media is profound. In many cases negative results do not indicate the absence of microorganisms. Hartsell (1951) warns against this risk, particularly due to his experience with pathogenic Salmonella.

The coliform test appears to be more efficient for detecting contamination in foods prior to freezing and storage, while fecal Streptococci are superior indicators in frozen foods, since coliform organisms seem less able to survive the low storage temperatures. The presumptive enterococcus test, using the "SF" medium of Hajna and Perry (1943), seems to be reliable and practical for the routine examination of frozen foods (Burton, 1949a and b). Larkin et al. (1955) do, however, recommend other media as more reliable indicators of the number of enterococci.

Nickerson (1943) modified the Frost little-plate technique and on comparison with the Petri-plate method found counts to be of the same general magnitude. Wolford (1943) described a modification of the direct- microscopic method stained by the Gram method, thereby achieving a differentiation between live and dead organisms. This opens the possibility of presenting a more complete sanitary history of the sample being examined. Berry (1946b) is of the opinion that a direct microscopic count is a better gauge of the sanitary history than is the culture method.

a. Methods for Frozen Vegetables:

These methods were taken from Anonymous, 1946b and Goreline, 1948. Select from the lot to be examined a suitable number of packages (3 or 4 from each package). Transport in dry ice to the laboratory for analysis and place the samples in a refrigerated (−18 to −20°C, 0 to −4°F) storage chest until they are to be analyzed. The temperature preferably should not rise above −18°C (0°F) in the handling of the material.

Samples of peas, green beans, etc. are prepared in the following way for microscopic examination. Open the package and note the color, structure and consistency. Especially state if ice crystals are present on the inner wall of the package and if the vegetables show a tendency toward shriveling. Such a condition is indicative of thawing and subsequent refreezing. Record observations and the occurrence of abnormalities such as unnatural color or odor or the occurrence of pink colonies of Toruloides, which are indicative of improper handling.

Samples are then taken for microbiological analyses. The sample, if not loose-frozen, should be broken up into small units. This can be done by tapping the unopened package sharply against the table edge or by striking the package with a dull instrument, being careful not to break it open. After opening remove portions with a sterile spoon from various parts of the package (i.e., centers and corners) in order to obtain a composite sample.

A 50 g. sample is aseptically weighed into a sterile, glass, mechanical blender jar; 450 ml. of sterile water are added and the contents blended for 2 min. If the blender is equipped with a variable transformer it is advisable to increase the speed of the motor gradually. Allow the samples to stand for 2 to 3 min, to permit the foam to subside. Pipette 1 ml. of the mixture into a 99 ml. sterile water blank. Replace the cap on the dilution bottle and shake the bottle briskly. Pipette 1 ml. aliquots of this mixture into each of 2 Petri dishes (1:1000 dilution) and also 0.1 ml. aliquots into each of 2 more Petri dishes (1:10,000 dilution). A 1:100 dilution may be obtained by pipetting 0.1 ml. aliquots of the original mixture into each of two Petri dishes.

Four melted tryptone-glucose extract agar (pH 7.0) cooled to 45°C (113°F.) into the Petri dishes immediately, and thoroughly mix the dilution water with the agar by gently rotating the plates in a figure 8 motion with slight tilting of the Petri dish. Cool to harden, and incubate at 32°C (90°F.) for 4 days.

Dilutions of 1:100, 1:1000, and 1:10,000 will usually suffice for commercially packed frozen vegetables, although further dilutions should be made if the history or the appearance of the samples warrant it.

It is of primary importance that the agar be poured immediately after the inoculum is introduced; otherwise many bacteria will adhere to the glass and an inaccurate count will result.

The direct microscopic method has certain advantages over the plate count method; it is quicker and requires less equipment and glass. It also detects dead microorganisms, and indicates sanitary history, irrespective of the viable count.

Weigh 50 g. of the vegetable into a 250 ml. flask. Add 100 ml. water, stopper the flask and shake it briskly 50 times through a wide arc. Using a Breded pipette, transfer 0.01 ml. of the washings to a microscope slide, and with a needle, spread the drop evenly over a 1 sq. cm. area of the slide. Dry and fix with heat or methyl alcohol. Stain with Grays' double dye stain or with North's aniline oil-methylene blue stain, rinse, dry, and examine under the microscope, using oil immersion. Use an ocular micrometer (such as a Whipple or Howard disk) with the microscope tube so adjusted that the side of the graduations is equal to 0.1 mm. (area of field = 0.01 sq. mm.). Count the cells in 100 fields and multiply the number by 20,000 to bring to a gram sample basis. Express results as "direct microscopic estimate in microorganisms per gram." For reference to staining methods see Gray (1943) and North (1945).

In the direct method the following assumptions are made: all microorganisms are removed from the vegetable surface by washing; the sus-
pension of bacterial cells is uniform; and the drop of liquid is evenly spread over 1 sq. cm.

A convenient and frequently used method is Nickerson's (1943) modified small-plate count:

Ten grams of sample are placed in 90 ml of sterile water and thoroughly shaken. A sterile slide is placed on a warming plate (metal plate regulated to 45°C. [113°F.] by clamping on a ring stand above a hot plate) and 1 ml of the dilution water is delivered to the raised portion. The measuring pipette used (capacity 0.1 milliliter) is cleaned with water several times by filling and emptying the pipette, and the lower portion is wiped with sterile Kleenex® before the culture sample is delivered. Four drops of molten nutrient agar (35.5 grams Bacto-nutrient agar in 1000 ml of distilled water) are then placed on the raised portion of the slide and the material mixed thereon. Mixing is accomplished by running a sterile wire through the culture 15 times, first backwards and forwards, then from right to left, and left to right. The culture is spread to the edges of the raised portion by slanting the needle and following the edge of this area.

These slides are marked for identification and placed in a moist chamber for incubation [16 hr. at 25°C. (77°F.).). After this time the cultures are removed, heated on a hot plate at about 80°C. (176°F.) until dried, treated with 1% aqueous ferric sulphate solution for 20 sec., washed, and then treated with a 5% aqueous solution of hematoxylin for 15 to 30 sec. The agar film is then washed and dried. The prepared slides can then be examined microscopically to detect and count bacterial colonies.

Plate counts of more than 4,000,000 per gram of peas or direct microscopic counts of over 1,000,000 per gram may be considered indicative of poor sanitary conditions in the plant or poor handling in transit or in warehousing. In vegetable freezing plants, plate counts of over 500,000 per gram are generally not encountered in newly frozen products unless there is some degree of carelessness in the factory, such as faulty clean-up practice or prolonged holding of the material after blanching.

In the preparation of samples of frozen spinach it is important to obtain a certain degree of thawing to enable proper comminution:

Allow the package to thaw at room temperature for 1½ or 2 hr. Then the package is opened and 50 g. of the contents are weighed into a sterile, borosilicate glass, mechanical blender jar. The samples are then assembled from various portions of the package, taking care to select petiole and blade portions in about the same ratio as occurs in the whole package. Sterile water (450 ml.) is added and the mixture is blended for 2 min., then analyzed in the same way as samples of peas and beans, etc.

The procedure for broccoli and cauliflower is somewhat different:

Using a sterile scalpel, cut portions from the curd and stem of several representative pieces of the vegetable. This can be done after the broccoli has been allowed to thaw partially at room temperature. Aseptically transfer 50 g. of these portions into the sterile, borosilicate glass, mechanical blender jars, add 450 ml. sterile water and proceed as directed for frozen peas.

* (Kleenex is a trade-marked soft cleaning paper.)
The sampling method profoundly influences not only the amount of solids but also the bacterial count. This has been comprehensively studied by Kahlenberg et al. (1951). It was concluded that the common procedure for drilling frozen eggs to obtain shavings does not give representative samples. If satisfactory devices can be developed, drilling of plugs would be preferable, or an even better procedure is to allow a complete defrosting of the entire can before sampling. Freezing of small samples simultaneously with the large size cans for later use as samples also has been suggested.

3. Results of Examination of Frozen Foods

a. General. An extensive investigation on the occurrence of microorganisms in frozen berries and vegetables was made by Wallace and Tanner (1934, 1935). More than 2000 packages of cherries, strawberries, peaches, beans, peas, etc., were studied. The number of microorganisms continuously increased during pretreatment and packaging, but diminished substantially during freezing and subsequent storage. Yeasts decreased more rapidly than molds, and bacteria most slowly. After 1 year of storage, however, the microbial population did diminish any further. Even after 3 years of storage the proportion of microorganisms remained fairly unchanged. The longer the frozen foods are stored, the more rapidly, however, microorganisms develop during and after thawing. In other words, the longer the frozen products have been kept, the more important it is to consume them immediately after thawing, and not to keep them in an unfrozen state. As a rule, the microorganisms which develop are not dangerous to the health, but the frozen products spoil and become unpalatable. An equally extensive study was made by Smart (1934, 1935). He observed in strawberries a dying-off of 99.3% of the microorganisms after 1 year of storage; however, 7 varieties of fungi, 1 yeast, and about 30 species of bacteria were viable even after 3 years. In spite of the high mortality rate, no less than 1,000,000 bacteria per gram of frozen berries remained.

There is a preferential killing of more freeze-sensitive varieties and a survival of more resistant ones. Micrococcus and Flavobacterium belong to this last group (Lochhead and Jones, 1936) whilst Achromobacter steadily declines in the frozen pack. Coliforms also die off more rapidly (Larkin et al., 1955) whereas fecal streptococci remain constant.

b. Fruits and Fruit Juices. On arrival at the factory, fruit carries on its surface large numbers of microorganisms. Molds of the genera Aspergillus, Penicillium, Mucor, Rhizopus, and Sterigmatocystis, and yeasts such as Saccharomyces and Torula are the most common. Certain bacteria, such as Staphylococcus aureus, Bacillus subtilis, also occur in abundance.
In order to avoid unnecessary microbial development on berries, all present day textbooks recommend the greatest speed possible in preparation and quick chilling (preferably ice water) immediately after picking. Furthermore, as Magoun (1931) has already proved, half of the microorganisms on the surface of the berries can be removed by thorough washing.

Bactericidal and fungicidal substances may be added in washing. Direct investigations have shown the favorable influence of propionate (sodium and calcium salts) on berries and vegetables (Wolford and Andersen, 1945). These chemicals, however, exert their influence only for a short period of time. In freezing by immersion such protection is particularly important, since the freezing liquid is being continuously contaminated with spores and bacteria. The addition of benzoic acid (0.04%), acetic acid (0.07%), or propionic acid (0.06%) will also hold the bacteria in check. According to Lenhart and Cosens (1949), the use of ultraviolet radiation also has yielded good results.

Special importance is attached to the influence of sugar which, because of its enzyme-inhibiting effect, results in a better quality, but at the same time protects microorganisms against the killing effect of the freezing temperature (McFarlane, 1942). The acidity in fruits and berries also has some influence in maintaining good quality, due to its inhibitory effect on microorganisms.

Obold and Hutchings (1947) indicated that fruits and vegetables, after packing and before freezing, should not be stored for longer than 24 hr. at 4.5°C (40°F.), 5 hr. at 10°C. (50°F.) or 2 hr. at 27°C. (80°F.). In the case of berries, which are often frozen in large containers, there is danger of fermentation occurring in the innermost parts of the mass before the entire contents freeze. Ireland (1941) recommended on account of this that the fruit be precooled before harrowing for freezing and that the barrels be rolled frequently when in the freezer.

During slow freezing, the growth of certain fungi has been noted even in frozen tissues (Young, 1947). However, frozen fruits and berries, as well as frozen concentrates, all have such low pH values that the substrate is unfavorable for most organisms that might be factors in spoilage during the freezing. Several scientists have studied the behavior of microorganisms in frozen juices and have stated that such factors as acidity, temperature, and content of dry matter influence the number surviving (Irish and Joslyn, 1929; Tanner and Wallace, 1931; Beard and Cleary, 1932; Wallace and Park, 1933; Berry and Diehl, 1934; Shrader and Johnson, 1934; McFarlane, 1940).

During the first few weeks of storage after freezing a rapid decrease in the number of bacteria has been noted by Lochhead and Jones (1938).

After the eighth month, however, the rate of killing was remarkably slow. There is a notable reduction in the per cent of living microorganisms in the concentrate at all temperatures below -4°C. (25°F.) (Du Bois and Kew, 1951).

Depending on methods of handling and conditions of culture, strawberries in a fresh state carry comparatively large numbers of surface microorganisms, 7500 to 100,000 per gram of fruit, according to Berry (1934) and Gilbert and Wiegand (1950) and 19,000 to 800,000 per gram, according to Magoun (1931). The microorganisms were mainly molds (Penicillium, Rhizopus, Mucor, Botrytis, Stemphylium, and Fusarium), yeasts, and sporeforming bacteria. Recently it was reported an effective reduction of mold content was achieved with a synthetic detergent in the washing water (Haynes et al., 1953).

The number of microorganisms varies as well as the type of flora. Magoun (1931, 1932), observed on an average the following ratio of flora types: 65% bacteria, 23% molds, and 12% yeasts. The bacteria generally originate from the soil and are transferred to the product by the hands and containers. The yeasts are chiefly air-borne; mold spores are ubiquitous.

Mundt (1950) studied the microbiology of strawberries during harvest and handling and indicates that the following factors affect the number of microorganisms: climatic conditions, ripeness, temperature, and delay in preparation. By use of the Howard mold-count procedure and plating techniques it was proved that the coating on containers used for conveying fruit from the field to the plant favors the growth of yeasts, possibly by preventing the escape of juice.

It has recently been observed that the bacterial count in fruit juice is somehow causally related to the occurrence of fungi. Individual fruits contaminated by certain molds give an abnormally high bacterial count (Proctor and Nickerson, 1948a). This is probably due to the fact that a fruit damaged by fungi easily succumbs to bacterial infection. Remarkably high numbers of bacteria developed in these particular fruits.

The so-called soft rot of oranges, caused by bacteria, results in an abnormal increase in bacterial content (Wolford and Berry, 1948a, b; Beisel, 1951). The total count is often many thousand times higher than in sound fresh fruit. Even the count of coliform bacteria rises under these circumstances. Ordinarily coliforms are very rare in fresh, uncontaminated juice, and the death-rate is comparatively high. In citrus juice as in strawberries there is every evidence to indicate the presence of specific bactericidal substances (Jakovljev, 1948).

The decrease in the bacterial count which occurs in the juice of oranges contaminated by soft rot even during several months freezing storage is less important because the predominating kind of bacteria belongs to the
Aerobacter, rather than Escherichia, whereas fresh juice only under exceptional conditions contains Aerobacter. Because of these circumstances careful grading of the fruit is important. However, this cannot be done easily owing to the fact that the initial attacks of soft rot are very hard to detect. Only random bacteriological tests can be used for guidance in this connection (Wolford and Berry, 1948a). Beisel (1951) also recommends a thorough washing of the fruit surface with the use of detergents.

A few years ago a troublesome off-odor spoilage was encountered in several plants used for frozen concentrates. This was traced to lactic acid organisms in the genera Leuconostoc and Lactobacillus (Hays, 1951; Murdock et al., 1952; Hays and Riester, 1952). Rapid heating [1 sec. at 71°C (160°F.)] prior to freezing is gradually becoming a general procedure in order to eliminate these risks. This commercial pasteurization is more effectively applied to the single-strength juice than to the concentrate. The resistance of these microorganisms was greater in concentrates than in single-strength juice, possibly due to the increased sugar content (Murdock et al., 1953).

Lactose-fermenting yeasts occurring in orange juice cause false positive results with standard (American) lactose broth and brilliant green bile and lauryl sulphate-tryptose broth when large inocula are used. Colonies of some of these yeasts cultured on eosin-methylene blue (EMB) agar closely resemble colonies of E. coli. In 62 samples of commercially packed orange juice showing presumptive positive tests in one of the above media no true coliforms were found by Martinez and Appleman (1949).

It has been shown in the case of frozen juices or berries and other fruits that some of the most important sources of infection occur along the production line. Bacteria carried on the raw material are, as a rule, of less importance than coliforms which are common and occur abundantly in slime, on conveyer belts, elevators, washing tubs, waste, etc. More than 3,000,000 bacteria per gram have been recorded frequently (Teunisson and Hall, 1947; Wolford and Berry, 1948b). Coliforms and above all Aerobacter, easily survive in orange juice according to Wolford (1950). Shrader and Johnson (1934) obtained results at variance with those reported above, claiming that three organisms (E. coli, Lactobacillus acidophilus, and B. subtilis) failed to multiply in orange juice at temperatures ranging from 37 to 10°C. (99 to 50°F.). It was even concluded that organisms of this coli type fail to survive longer than 2 weeks in frozen orange juice but that spore-forming bacteria would probably survive for a considerable period of time. There are also strains of yeasts that grow slowly at −18°C. (0°F.) and rapidly at 0°C. (32°F.).

Recent reports from Florida reveal that extensive sanitation pro-

cedures, have been introduced in citrus juice factories. Fruit surfaces are washed to reduce the bacterial load (80 to 95%) and all preparation equipment is regularly cleaned during operation (Brokaw, 1952). Continuous plate counts and microscopic examinations of the juice are used to check the bacterial levels and determine the time schedule for regular clean ups.

The number of bacteria originally present in fruit juices vary in extremely wide ranges from 0 to more than 1000 colonies per milliliter (Nolte and Loesecke, 1940). In concentrated frozen juices bacteria are killed considerably more efficiently (Faville and Hill, 1952; Faville et al., 1951; Hahn and Appleman, 1952a,b). This is remarkable, since most of this juice is not pasteurized. With longer storage periods there is a notable reduction in the percentage of living microorganisms in the concentrate at all temperatures below −5°C. (23°F.) (Miller and Marsteller, 1952). In concentrates, however, yeasts seem to have the standpoint of survival (Patrick, 1949). This is of great importance from the standpoint of the storage of thawed concentrates. Coliforms and other nonspore-forming bacteria generally do not survive long range storage in frozen juice or concentrates (Patrick, 1953).

The recent observation that enterococci frequently appear in citrus juice is somewhat of a riddle. The organisms have been identified as Streptococcus fecalis and S. liquefaciens. Whether they originate from the soil, are conveyed by water or birds, or are carried inside the fruit tissue during growth and development is yet to be determined (Kaplan and Appleman, 1952). These research workers even suggest that enterococci showing greater survival capacity might serve better than E. coli, which is rapidly eliminated, as an indicator of pollution in frozen food. This has been confirmed by Larkin et al. (1955).

c. Vegetables. The bacterial flora in frozen vegetables has proved mainly to include the following genera: Achromobacter, Aerobacter, Alcaligenes, Bacillus, Cellulomonas, Chromobacterium, Erwinia, Flavobacterium, Lactobacillus, Leuconostoc, Micrococcus, Mycobacterium, Neisseria, Phytophomonas, Pseudomonas, Sarcina, Serratia, Staphylococcus, Streptococcus (fecalis), and Vibrio (Sanderson and Fitzgerald, 1940; Nickerson, 1943; Hucker et al., 1952; Hucker, 1954). Many of these bacteria are typical soil organisms (Smart, 1937b). They have been isolated from vegetables in the frozen state or immediately after thawing and consequently they are not present as a result of infection. In peas, species of Lactobacillus dominate (Berry, 1933b) but spores of yeasts and molds are also common (Nickerson, 1943). Hucker et al. (1952) state that there are indications of a basic cold-resistant flora of bacteria in frozen peas, beans, and corn.

Coliform bacteria in vegetables have been studied by Burton (1949a)
who found by use of the IMVIC tests that 53% were \textit{Aerobacter aerogenes} and 25% intermediate types. The ratio of the different bacterial species did not change after a year of storage at \(-20^\circ\text{C.} (-4^\circ\text{F.})\) and the enterococci were still dominant. Elrod (1942) showed that \textit{Erwinia}-bacteria may give reactions similar to coliform bacteria, which means that a fecal contamination may be erroneously indicated.

The numbers and types of organisms found in frozen vegetables stored over long periods and the presence of types which grow at 0°C. (32°F.) were recently studied by Hucker (1954). Snap beans and Lima beans were found to contain a significant freeze-resistant flora which remained viable after storage at \(-17.5^\circ\text{C.} (0^\circ\text{F.})\) for 2–10 years. Facultative psychrophilic types were found in asparagus, brussels sprouts, broccoli, and cauliflower but were not generally present in peas, squash, corn, and snap beans. Obligate psychrophilic types were not encountered in any of the samples. In frozen vegetables held at \(-17.5^\circ\text{C.} (0^\circ\text{F.})\) for 2 years or longer, the predominating organism was a large, paired biscuit-shaped coccus when the isolations were made from plates incubated at 32°C. (90°F.). When the isolations were made from plates incubated at 0°C. (32°F.) for 30 days, the predominating type was a gram-negative rod, resembling the \textit{Flavobacterium esteromoramicum} type.

In blanching vegetables the bacterial content may decrease as much as 90% and in some cases as much as 99% (see Table IV) (Smart and decrease occurs only within the last 1%. Properly speaking, the killing of the bacteria should always be recorded exponentially. Rather few such determinations have been made, however. Besides other important technical effects, blanching nevertheless has a hygienic implication.

Accordingly it seems, as a rule, justified to conclude that a high bacterial count in frozen vegetables is due to contamination during the time between blanching and freezing (Vaughn et al., 1946; Hucker et al., 1952). Proper sanitary procedure subsequent to blanching is an important factor in determining the number of organisms in the final package. This was recently confirmed by Larkin et al. (1954) tracing the occurrence of fecal bacteria.

The flora which is built up prior to blanching is predominantly mesophilic. Small pieces of vegetable material which become lodged in the conveyer belt, and cell sap which exudes from the cut tissue provide a better medium for the growth of microorganisms than the raw product itself (Pederson, 1947). For this reason the regular cleaning of conveyer belts and other equipment is so very important (Hucker and Robinson, 1950; Hucker et al., 1952).

Pederson (1947) made a study of the extent of contamination encountered in frozen vegetables and on the effect of various methods of handling on the bacterial count. The greatest danger is when vegetables get contaminated by organisms in the active growth phase. Counts of 10,000 to 100,000 per gram may be expected in frozen vegetables, but when they exceed 1,000,000 this is a clear indication of careless handling.

During the freezing storage \([-20^\circ\text{C.} (-4^\circ\text{F.})]\) of vegetables the microbial flora gradually diminishes as various organisms are destroyed. This process slows down with time, but even after 8 months sufficient bacteria may remain so the vegetables may spoil after thawing (Lochhead and Jones, 1936). Greater reduction in counts were recorded at \(-12^\circ\text{C.} (10^\circ\text{F.})\) than at \(-18^\circ\text{C.} (0^\circ\text{F.})\) and \(-23^\circ\text{C.} (-9^\circ\text{F.})\) and was most pronounced in samples with higher initial counts (Hucker et al., 1952). Above all, coliforms and \textit{Lactobacillus} are most apt to survive (Weiser, 1951).

Actually, with respect to frozen vegetables, attention has been primarily directed to the occurrence and danger of botulism. The possibility that there might be sufficient time for the botulinus toxin to develop during preparation before freezing and thus be left in the product even after long-term freezing storage has been considered. There is of course also the possibility that this organism might develop during transport and distribution of frozen vegetables, particularly if some thawing has been permitted to occur. Since vegetables usually have pH values which are quite favorable to bacterial development, it is fortunate that most frozen

<table>
<thead>
<tr>
<th>Vegetables</th>
<th>Total microorganisms per gram before blanching</th>
<th>Total microorganisms per gram after blanching</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potatoes</td>
<td>7,050,000</td>
<td>170</td>
</tr>
<tr>
<td>Carrots</td>
<td>435,000</td>
<td>60</td>
</tr>
<tr>
<td>Cabbage</td>
<td>50,000</td>
<td>25</td>
</tr>
<tr>
<td>Beets</td>
<td>3,150,000</td>
<td>100</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>150,000</td>
<td>80</td>
</tr>
<tr>
<td>Broccoli</td>
<td>450,000</td>
<td>150</td>
</tr>
<tr>
<td>Peas</td>
<td>1,250,000</td>
<td>175</td>
</tr>
<tr>
<td>Beans (Lima)</td>
<td>2,500,000</td>
<td>50</td>
</tr>
<tr>
<td>Corn</td>
<td>495,000</td>
<td>75</td>
</tr>
</tbody>
</table>

* From Koelenmid (1950).
vegetables are cooked before serving. If they are heated sufficiently, the destruction of viable organisms and their toxins is insured, thereby rendering the cooked product safe for consumption.

According to experience in the United States, it is usually possible to produce frozen peas with bacterial counts less than 100,000 per gram. On the other hand, neither frozen corn nor spinach can be prepared with such a low count. Humphrey (1950) and Hucker (1950) arrived at the conclusion that there is no direct relation between quality and bacterial counts. He is, however, of the opinion that bacterial counts do reflect the sanitary conditions under which the products have been prepared. In other words, the hygienic condition of the factories are important with respect to quality and certain maximum values for bacterial counts should be stipulated.

Peas should contain less than 50,000 per gram, cut green beans less than 100,000, and corn less than 60,000 organisms per gram on entering the final freezer. Samplings made in a number of factories showed the very wide range of counts from 1300 to 870,000 organisms per gram.

In the United States most vegetables have low bacterial counts upon arrival at the freezing plant because of the methods of handling and transportation used. The methods of handling various products during the different steps in preparation and the plant is the factor determining the final load of bacteria. On conveyer belts for beans and for cauliflower respectively, as many as 1,000,000 and 15,000,000 organisms per sq. cm. have been found after a few hours (Koelemsmid, 1950).

In this connection it is of interest to note some figures collected by workers of the Western Regional Laboratory in California (1944) at 13 pea freezing plants. The counts, given below, represent averages of results obtained in all plants:

<table>
<thead>
<tr>
<th>Time of Sampling</th>
<th>Bacteria per Gram of Peas (X1000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>On arriving at the factory</td>
<td>11,346</td>
</tr>
<tr>
<td>After washing</td>
<td>1,090</td>
</tr>
<tr>
<td>After blanching</td>
<td>10</td>
</tr>
<tr>
<td>After rinsing and transport</td>
<td>239</td>
</tr>
<tr>
<td>From inspection belt before packaging</td>
<td>410</td>
</tr>
<tr>
<td>When entering the freezer</td>
<td>736</td>
</tr>
<tr>
<td>After freezing</td>
<td>560</td>
</tr>
</tbody>
</table>

Further information in this area may also be found in another paper (Anonymous, 1945b).

Hucker et al. (1952) were unable to observe any correlation between bacterial content and the nutritive value of peas or beans.

Spinach must be chilled immediately after harvest; otherwise the bacterial count will increase rapidly and washing becomes relatively ineffective. If large quantities are blanched at one time the hot water fails to penetrate properly, and conglomerates are not removed. These experiences have been reported from California by Weiser (1951).

In spite of what has been said above on the possibility of drawing conclusions with respect to the quality of frozen foods, it is in order to point out that, according to Tressler and Evers (1947), low values are indicative of good handling practices. The latter are of the opinion that if counts as low as 10,000 per gram are found in a frozen vegetable the following conclusions are possible:

1. The raw product has been adequately blanched.
2. The product has been cooled rapidly and effectively after blanching.
3. The factory and particularly the production line are in a good sanitary condition.
4. The packaging material was in excellent condition from the standpoint of bacterial contamination.
5. Freezing took place rapidly.
6. The product has not had an opportunity to thaw.

A study of the bacterial content of frozen foods has been carried out in Canada (Anonymous, 1950a). A large number of commercial samples of vegetables (376) were investigated for fecal bacteria, primarily from the standpoint of coli-types and enterococci. Coli analyses proved to be more effective for the detection of infection in raw material, whereas the determination of fecal Streptococci was better for frozen products since coli-bacteria are less liable to survive freezing. Hajna-Perry's SP-substrate for the diagnosis of enterococci was used (Burton, 1949b).

According to Diehl (1945) practical experience in the United States has shown that peas and spinach in particular offer difficulties since damage usually appears in the stored product only after a considerable period of storage. Recently Larkin et al. (1955) found both coliform bacteria and fecal Streptococci in many commercial samples of frozen vegetables. If not rightly handled, they constitute a possible health menace. 

d. Summary of Discussion on Fruit and Vegetables. The number of microorganisms in frozen fruits and vegetables may reflect the quality of the raw material, act as a measure of the extent of bacterial contamination of the raw product, and reflect the sanitary condition in the processing plant and the speed with which the product was processed. Bacterial infection takes place principally during transportation and storage of the raw material under unfavorable conditions and along the production line during preparation. All raw products including fruits, berries, and vegetables should be properly washed in potable water in order to remove soil bacteria as thoroughly as possible.
e. Meat. The presence of microorganisms in frozen meat may not be important from a pathogenic viewpoint, but they are of primary influence to the storage life of these products. The occurrence of large numbers of bacteria in meat may be related to the method of handling prior to freezing, such as careless management or improper ageing. Blood is particularly sensitive and easily shows high bacteria counts if strict hygienic controls are not maintained in the slaughter houses. The chilled storage of meat for ripening or other purposes is definitely detrimental to the frozen product in so far as bacterial growth is concerned. Psychrophilic bacteria propagate well on the surface during storage so it is most advisable to freeze rapidly immediately after slaughtering. At temperatures just below 0° C. (32° F.), molds may develop but their growth rapidly ceases at lower ranges of temperature (Brooks, 1924; Wright, 1923).

Geer et al. (1933) have observed an 80% decrease in the bacterial count of meat during freezing and a slight further decrease (84%) after 1 month of freezing storage. In a number of cases an increase immediately after freezing has been observed (Anonymous, 1946a). It is not yet possible to give a satisfactory explanation to this phenomenon. There have been suggestions to the effect that it might be due to bacterial populations (conglomerates) breaking up during the freezing process.

In studies on frozen pork, Sulzbacher (1950) observed a decrease in the microbial count in all samples regardless of the storage temperature or the protective procedures used. One requirement is said to be that the storage temperature be kept below -20° C. (-4° F.).

The freezing of pork is naturally of special importance in destroying Trichinella spiralis, but freezing has to take place at extremely low temperatures (Anonymous, 1953).

Observations by Hendrickson and Miller (1950) in Kansas indicated that nearly 75% of the organisms naturally present died during the first two months of storage of pork sausage but that the death-rate for the remaining 25% was much slower. The vegetative and nonsporeforming bacteria were most susceptible to low temperatures and therefore died early in the storage period. The spore formers, being more resistant to freezing, were still present in large numbers after 310 days in storage.

The results of Sulzbacher (1950), on the other hand, show that several bacteria manage to multiply even at freezing temperatures. Several low temperature strains of Pseudomonas and Acetobacter were isolated from both frozen lamb and pork. All of them developed well at temperatures as low as -4 to -6° C. (25 to 21° F.). Perhaps the protective effect of fat may account for the survival of so many coliforms for long periods of storage at -18° C. (0° F.). Even a few molds succeed in growing on meat at -5 to -6° C. (23 to 21° F.) according to Brooks (1924) and Griffiths et al. (1932). When temperatures decrease to about -10° C. (14° F.), microbial development is inhibited provided freezing has taken place. Some bacteria may grow under supercooled conditions at temperatures as low as -20° C. (-4° F.).

Bacterial studies on a variety of frozen foods containing chopped meat have been made in military laboratories in the United States. The average colony counts were 16,000 per gram on pork, 263,000 per gram on beef and 63,000 per gram on lamb. After cooking, these counts decreased considerably, to between 30 and 720 per gram. Various thawing and cooking procedures had no effect on the counts (Anonymous, 1950b). Bacteria in hamburgers showed a remarkably low rate of mortality, even when stored at -10° C. (14° F.). On an average the bacterial count decreased from 200,000,000 to 10,000,000 per gram in one year (Weinzirl and Newton, 1915). On pork sausage; large numbers of bacteria are conducive to the development of off-flavors (particularly rancidity) according to Hendrickson (1949).

Microbiological problems have become increasingly relevant as a result of the development of self-service of meat in retail stores. Contamination during packing, microbial flora of the packing material, and quality of the primary products are factors of great importance. Unfortunately, no investigations of significance have as yet been reported in this field.

Various prepared meat products such as sausages should, in fact, not be stored in frozen condition since deterioration in quality proceeds too rapidly when compared to the fresh material. In the United States substantial amounts of sausage are held in locker plants, but few bacterial studies were made. Special attention has been given to the contaminating influence of seasoning ingredients. The work of Ysair and Williams (1942, 1945) showed that nearly all the common spices contain large numbers of bacteria. When these ingredients were used in the ratio of 1 g. of seasoning to 1 lb. in pork sausage, pepper was found to contribute 30,000 bacteria per gram of meat, sage 60 per gram, and salt fewer than 1 per gram (Hendrickson and Miller, 1950).

j. Fish. Fish, unlike most other foods, carries from the start a markedly psychrophilic flora. As to its composition reference is made to Griffiths (1937), Zobell (1946), Aschenhoug and Vesterhus (1946) and Castell and Anderson (1948). Anaerobic sporeformers are encountered less frequently (Castell, 1947; Castell and Anderson, 1947).

In order to effectively check the psychrophilic flora, freezing and freezer storage must take place at particularly low temperatures. These temperatures must be -12° C. (10° F.) according to Stewart (1934). The familiar fish contaminating organism, Pseudomonas fluorescens, retains its motility and even grows at -7° C. (19° F.) (Bedford, 1931, 1933) and
also retains its chemical activity at still lower temperatures (Hess, 1934a,b). Slow freezing, which is quite often used for fish, is therefore an evident inconvenience, if only from a qualitative point of view. Several bacteria may then develop in freezing, and to a much greater extent than in other frozen products. According to Birdseye (1929) the death of bacteria on fish during freezing is as a rule unimportant. During freezing, Fellers (1932) has indicated that growth ceases completely. The bacterial flora of mackerel is greatly reduced by rapid freezing (Kiser and Beckett, 1942). In spite of the considerable movement of frozen fish in commerce, only one case of poisoning from such products has been reported (Fitzgerald, 1947a).

The death rate of Pseudomonas fluorescens held at low temperature has been studied by Hess (1934a,b). When subjected to various temperatures for 30 min., the count of viable bacteria decreased as follows: 0°C. (32°F.), 26%; -3°C. (27°F.), 27%; -6.5°C. (20°F.), 35%; -10°C. (14°F.), 95%; and -16°C. (3°F.), 90%. Refreezing at -16°C. (3°F.) reduced the bacterial content still further. The experimental time range was presumably too short to give a clear picture of the killing effect of various temperature levels. The high figure at -16°C. (14°F.) may indicate the more disastrous influence of the temperature range between zero and minus ten below. Due to growth concentrating psychrophilic bacteria slow freezing counterbalanced destruction and resulted in a lower rate of mortality than did rapid freezing. The pH has a marked effect on the survival of halophilic bacteria. Hess (1934a,b) observed a minimum effect in the pH range 6.0–6.3.

More bacteria survive on halibut fillets in freezing storage at -20°C. (-4°F.) than at -15°C. (5°F.) or -10°C. (14°F.). This corresponds to the observations made by Haines and is related to the fact that the effect of low temperature on proteins is far less pronounced at -30°C. (-22°F.) than at -10 or -15°C. (14 or 5°F.). Handling of the raw product decides the bacterial load according to Proctor and Nickerson (1943a,b). Studies undertaken in various countries, therefore, are aimed at reducing the risk of bacterial contamination. With this object in view the merits of thorough disinfection on board ship, by use of new storage procedures and the addition of bactericidal substances to ice are being considered. The improvement of methods for storage of the raw material in harbor is also under consideration. Investigations on the effect of an initial and immediate freezing at sea should be regarded as part of this work. This procedure definitely gives a superior product (Fieger, 1950).

Fish should be thoroughly washed before filleting in order to avoid an infection by bacteria in slime-coatings. Good results are achieved by this procedure with cod (Dyer and Dyer, 1949).

Escherichia coli is the most common bacterial species in Caribbean shrimps according to Holmes and McCleskey (1949). The number of viable bacteria decreased more rapidly in peeled shrimps than in unpeeled ones. Generally, freezing storage at -12°C. (10°F.) effected more killing than at -40°C. (-40°F.). Shrimp frozen immediately after catching even after one year of freezing storage retained considerably fewer bacteria (22,000 per gram) than those stored in ice for several days before freezing (>1,000,000 per gram) (Green, 1949).

Bedford (1934) studied organisms on frozen fish and found at the higher temperatures of storage the decrease was more rapid than at the lower temperatures.

In fish a preferential killing effect of thawing was observed on certain bacteria e.g. Flavobacterium in comparison to other varieties more resistant on Achromobacter (Castell and Mapplebeck, 1952). According to these authors Pivnick studied the composition of the flora of cod when freezing and storing in frozen condition and found the percentage of Flavobacterium remarkably constant.

g. Whalemeat. Whalemeat has to an increasing degree been processed as food, particularly as frozen products. Chiefly British studies have been published as to the bacteriological and hygienic problems related to such products. The bacterial flora found in whalemeat is of either an intrinsic or extrinsic origin.

The Streptococi and Clostridia probably originate in the intestine of the whale, and they are distributed throughout the tissues at the time of death, constituting most of the intrinsic flora of whalemeat. These bacteria originating from the body of the whale are largely mesophilic. The source of the aerobic sporing bacilli is unknown. The evidence available at present indicates that the remaining aerobic species, Micrococi and gram-negative bacilli, are probably mostly contaminants from the extrinsic flora of which they form the predominant part (Shar and Marsh, 1953). These are mostly psychrophilic and are picked up in the freezing plant. The number of bacteria added here depends almost entirely on the conditions of hygiene observed in working the plant, but, even with regular and efficient cleaning, about 100 times more bacteria are added, in practice, at this stage than were present in the meat beforehand. As a result, the proportion of psychrophilic bacteria is at the same time increased.

The over-all result of processing whalemeat, from the carcass to the frozen block, is to increase its bacterial load 10 to 100 times. In effect, this is comparable with normal slaughterhouse practice, where even with the best technique a 100-fold increase in the numbers of bacteria on exposed surfaces may be expected. Special measures can greatly diminish the
amount of bacterial contamination, but they do not eliminate it entirely.

These extrinsic contributions of bacteria thus increase the proportion of bacteria in the meat able to grow at temperatures below 15°C (59°F), an important factor in relation to the effects of freezing. Freezing kills many bacteria, and after frozen storage the number of bacteria in whalemeat was found to be relatively small again, being of the order of 10⁶ to 10⁸ per gram. Freezing made no great difference, however, to the proportions of the various types of bacteria; hence the variety of psychrophilic types was greater than that found among comparable bacterial counts in fresh meat.

The bacterial count is in every instance lower in the post-rigor than the pre-rigor meat, although there is no appreciable difference between them at the time of removal from the carcass. The meat becomes contaminated, presumably, during preparation for freezing (Robinson et al., 1953). This contamination is in the end (i.e., after freezing) less in post-rigor meat, possibly either because pre-rigor meat is more sticky or because some of the contaminating bacteria drain away in the "weep" from post-rigor meat while it is being cut up and frozen (Robinson et al., 1953).

Taken over-all, however, there is little bacteriological difference in the frozen and stored products prepared from pre- and post-rigor whalemeat (Robinson et al., 1953).

When storing frozen whalemeat for a period of 2 to 3 months at −10°C (14°F), there was a decrease in the number of aerobic bacteria and Clostridium in every case. In the pre-rigor meat, the number of aerobes with a 37°C (99°F) optimum decreased 12-fold, while those with a 20°C (68°F) optimum decreased 63-fold; whereas in the post-rigor meat, the numbers decreased 11½- and 3-fold, respectively. The proportion of Clostridium killed on storage was not influenced by whether the meat was pre- or post-rigor, the numbers decreasing 6-fold in both cases.

The rate of increase of aerobic bacteria in defrosted whalemeat at room temperatures of 15°C (59°F) to 20°C (68°F) is rapid, largely because of the amount of free fluid available in the form of drip. Spoilage shows up within 24 to 48 hr. after thawing (Robinson et al., 1953). For this reason whalemeat is not suitable for the ordinary process of retail meat distribution but it can be kept in the frozen state, or for a week or two at temperatures not exceeding 0°C (32°F) to −3°C (27°F) until a few hours before use; however, it is no more perishable than some other foodstuffs, such as milk or egg pulp.

b. Poultry. There have been several investigations relating to the bacterial flora on poultry and its behavior in the production of frozen foods (Sair and Cook, 1938; Gunderson et al., 1947). Bacterial growth totally ceases at −10°C (14°F.) (Pennington, 1945). It has been impossible to enlist here the many species of bacteria found in poultry. Even in the bone marrow several have been observed, esp. Micrococcus. It has not yet been completely clarified whether the discoloration of bones cannot have a bacteriological cause. Coliforms are extremely common in freshly drawn poultry. For the rest, the flora is dominated by Acetobacter, Alcaligenes, Flavobacterium, and Micrococcus, which are all fairly common in processing plants. Their number decreases in freezing and subsequent storage, but all types of bacteria survive and are thus represented in the frozen product after thawing. Sair and Cook (1938) studied the effect of freezing rate using temperatures between −5 and −70°C. (23 and −94°F.) in freezing poultry, and found that the number of surviving bacteria does not depend on the freezing rate. Nevertheless, Heitz and Swenson (1933) have been able to show that the number of bacteria on ducks which are frozen slowly turns out to be 1000 times greater than in a rapidly frozen product. According to the results obtained by Sair and Cook, it seems justified to presume that this latter result is to be attributed to the fact that the products have been frozen so slowly that they have been allowed to keep above the limit of bacterial growth temperature during a long period of time. In the tests performed by these two investigators, it was not possible to state any effect of the freezing rate, or at the most a very slight one. In practice, the commodities are frozen as soon as possible after slaughtering or the product is stored in a cold-storage warehouse or refrigerator at about 0°C. (32°F.). As bacteria grow very slowly at this temperature, there is no reason for supposing that this might have essentially changed the results. If handling practices are satisfactory, the bacterial content can be kept at a reasonably low level.

Above all, removing the meat from the carcasses is a procedure which may involve heavy bacterial contamination. By boiling the eviscerated carcasses before the bones are removed (the most common practice) the bacterial flora is, of course, essentially reduced, although it always increases in subsequent handling (Gunderson et al., 1947).

The original bacterial load of the poultry carcasses, when being frozen, depends on the cooling procedure, the use of a sterilizing wash prior to packing, and the time lag from slaughter to freezing. Of great interest are the distinct differences in composition of the flora and the number of bacteria connected with the diet and the microbiology of the intestines (Sisler et al., 1940).

Schneider and Gunderson (1949) concluded that freezing only partly eliminated the surface bacteria, chiefly Salmonella, on eviscerated chicken even after long storage. Various Salmonellae have been recovered from
the skin of frozen turkey even after long time storage (Cherry et al., 1946; Browne, 1940).

Considerable numbers of bacteria remain in poultry meat even after 3 years of freezing storage (Harshaw et al., 1941). The various bacteria in the dressing of frozen stuffed chicken showed no significant changes during storage for one year at -23.3°C. (-10°F.) (Esselen and Levine, 1954).

By these investigations it seems to have been proved that even for poultry meat the number of bacteria constitutes a good indicator of the hygienic conditions in the processing plants. For judging the risk of bacterial contamination in poultry meat it may be pointed out that research workers have traced organisms of the paratyphoid type in drawn poultry (Gunderson and Schneider; unpublished data). Typhus bacilli can survive for a considerable period of time in frozen products (Gunderson et al., 1947; Gunderson and Rose, 1948a, b; McCleskey and Christopher, 1941). Owing to these circumstances it is necessary to maintain strict bacteriological control both in evicerating and freezing plants.

i. Eggs. The bacterial flora of frozen egg products is important because it influences the wholesomeness of end products, their keeping quality, and functional properties in the preparation of food. Haines (1939), Gibbons and Moore (1944), and Winter et al. (1946) have presented data which indicate that more than 80% of freshly laid eggs are bacteriologically sterile. However, nearly all commercial liquid, frozen, and dried egg products contain several hundred to several million bacteria per gram, according to reports by Redfield, 1920; Johns, 1948; McFarlane et al., 1945; and Winter and Wrinkle, 1949a. This stresses the importance of a regular sanitary control of frozen egg products. Special methods for bacterial counts in frozen eggs and egg products have been developed (Schneider, 1940; Schneider et al., 1943). Hartsell (1949) is of the opinion that better plating media need to be developed for testing frozen eggs for pathogenic bacteria.

That Escherichia coli and the coliform group can survive in frozen eggs has been shown by many investigators (Brown and James, 1939; Colien, 1942; Holtman, 1943; Johns and Berard, 1946; Nielsen and Garnatz, 1940; Pennington, 1948; Quinn and Garnatz, 1943; Schneider et al., 1943; Sherman and Naylor, 1942; and Wallace and Park, 1933). In previous studies it has been observed that a rapid reduction in viable cells occurs in the early periods of exposure and storage at subfreezing temperatures. However, viable bacteria were present in some samples even after 18 months. Enterococci, invariably present in chicken feces, also survive and are considered a more reliable index of fecal contamination (Brown and Gibbons, 1950).

Of special significance in freezing plants are the sanitary conditions prevailing in areas intended for breaking the eggs. The bacterial flora of the egg shell influences the count of the frozen product (Winter and Wrinkle, 1949a; Winter, 1952). This is why washing with germicides generally is applied before breaking, at least for soiled eggs (Penniston and Hedrick, 1944, 1947). Close control is necessary. A single bad egg may contain billions of bacteria and spoil a whole batch of liquid egg.

Frozen yolks as a rule contain more bacteria than frozen egg whites (Winter and Wrinkle, 1949a). In freezing, the bacterial content in liquid egg immediately decreases. Coliforms generally die within 3 months in subsequent freezing-storage, but a further destruction of bacteria generally does not take place, even during a fairly long time. The egg evidently possesses a protective substance of some kind (Johns and Berard, 1946). Many of the species of bacteria found in frozen eggs multiply even at the low temperature found in freezing and defrosting processes. Even rapid freezing gives a certain concentration of bacteria to the central part of 30 lb. packages (Anonymous, 1953).

By pasteurizing liquid egg at 61 to 62°C. (143 to 144°F.) for 3 to 4 min., more than 99% of the bacteria (appearing on standard plates) are killed, including nearly all coliforms and gram-positive cocci as well as the pathogenic bacteria (Winter et al., 1948; Winter and Wrinkle, 1949a; Wrinkle et al., 1950; Winter, 1952). Particularly valuable is the complete elimination of Salmonella through this procedure. It has also been suggested (van Oijen, 1940) that liquid egg can be pasteurized by heating at 65°C. (149°F.) for 20 min. if 1 to 2% trisodium citrate were added as anticoagulant. This method is practiced commercially (Ingram and Brooks, 1952).

The egg white cannot be pasteurized at a higher temperature than 56 to 57°C. (133 to 135°F.) without damage taking place. Pasteurizing at this temperature for 4 min. results in destroying 92% of the bacteria. As a whole, bacteria are killed more readily in egg white than in yolk or whole egg. Freezing and storing both nonpasteurized and pasteurized samples of egg gave rise to some destruction of bacteria. The number of bacteria killed in freezing and storing at -22 to -23°C. (-8 to -9°F.) for 2 to 3 weeks, however, was by far not as great as pasteurizing for 3 to 7 min. at 61 to 62°C. (142 to 144°F.). Freezing and storage of liquid egg products at -18 to -28°C. (0 to -18°F.) resulted in an average destruction of 55% in 12 days, 62% in 30 days, 87% in 60 days, and 90% in 100 days (based on plate count). Therefore, the destruction is most rapid in the first four weeks (Holtman, 1943; Lepper et al., 1944; Winter and Wrinkle, 1949b; Winter et al., 1951; Wrinkle et al., 1950).

The ratio of direct plate count to standard plate count averaged 2:1.
but was much higher in frozen samples than in unfrozen (Winter et al., 1951). Possibly a methodological difficulty might have given rise to an erroneous picture, as the frozen product could not be dissolved in water as easily when sampling for bacteriological tests (formation of clots was noticed).

*Alcaligenes, Flavobacterium, Proteus,* and *Pseudomonas* predominated among the 13 species found in nonpasteurized eggs. Freezing reduced the number of bacteria, but not the number of species, which afterwards remained unchanged (Wrinkle et al., 1950). On thawing there was always a considerable increase in the number of coliform bacteria or Gram-positive cocci, but seldom a great increase of both types in the same sample. (A determination on agar plates from pasteurized samples showed that *Alcaligenes, Escherichia, Flavobacterium,* and *Proteus* were predominant among the 13 strains from nonpasteurized eggs.) Pasteurization on the other hand always reduced the number of genera present, in this case from 13 to 6 (Wrinkle et al., 1950). The viscosity is, however, reduced (about 17%) through pasteurization in the subsequently defrosted whole egg (Miller and Winter, 1951).

*Alcaligenes* and *Escherichia,* the two most frequent species in liquid egg, gave a sour smell within 60 hr. after incubation on a bacteriological medium. *Aerobacter* also gave a faint odor and often showed coagulation; *Proteus* gave souring and occasionally coagulation, and *Pseudomonas* only souring (Wrinkle et al., 1950).

In earlier investigations a correlation had been stated between the number of bacteria and the amount of reducing sugar in liquid egg (Pearce and Reid, 1940). The higher the bacterial count, the lower the residual reducing-sugar content. This was, however, not confirmed in later studies (Johns, 1948) and may in fact not be expected, as a high bacterial count may be attributed to two different causes: either it is due to the bacteria having developed in the liquid egg mass and consequently contributed to a high degree of breakdown and a corresponding consumption of sugar, or the product may have been exposed to a heavy bacterial infection, which need not necessarily have developed through growth prior to freezing in the egg mass. In this latter case there is no direct relation to the amount of sugar but rather to the degree of sanitation in the handling of the eggs and their content.

The bacteriological count of commercial frozen whole egg varies widely. A standard plate count of less than 50,000 per gram is unusual, a figure below 500,000 per gram is probably better than the average and counts of 1,000,000 to 5,000,000 per gram are often encountered (Ingram and Brooks, 1952).

Recently analyses have been made of commercial frozen whole egg in Canadian laboratories. More than 10% had a bacterial count of over 10,000,000. During 3 years (1944-47) bacterial counts were reduced by using precooled eggs, rapid freezing of the melange, breaking shell egg stock immediately, and improvement in equipment and plant sanitation (Fletcher and Johns, 1951). A new technique similar to that employed by the British Ministry of Food was tested. A plug was drawn and used for measuring texture emulsification and color. It was found that the bacterial counts from the plug were approximately twice as high as the sample taken by the electric drill method. The original Canadian frozen egg standards were rather lenient from the standpoint of bacterial counts. These have been reduced to 2,500,000 for Grade A; a count of 500,000 is considered to be practical. During 1949 the Burri slant technique was introduced to check the plate counts on all samples analyzed and any sample which exceeded 500,000 was then directed to an official laboratory, where the count was determined by the official plate method.

j. Dairy Products and Miscellaneous. In butter, the bacterial count decreases by ten or twenty times in freezing storage in spite of the protection given by fats. On the whole the number of bacteria shows a slow decrease in dairy products (Wilster, 1946).

Ice cream, as pointed out in the introduction, has not been included in this survey. A few words should, however, be said about cream (Fabian and Trout, 1943). Pasteurization at 85° C. (185° F.) for 5 min. most effectively brought down the bacterial content of the original product and subsequent freezing storage for 1 year resulted in a continuous decrease in the number of viable organisms. A more effective homogenization multiplied the bacterial counts obtained.

Frozen milk also shows a decrease in bacterial content during storage (Babocek et al., 1947). Both *Lactobacillus casei* and *Escherichia coli* die off more rapidly in milk at pH 7.0, when the storage temperature is 2°C. (28°F.) than when it is 21°C. (-6°F.) (Ulrich and Halvorson, 1947).

Hiseox (private communication) found viable cells of *Streptococcus fecalis* and of aerobic sporeformers in Cheddar cheese after 7 years of storage at -25° C. (-13° F.). No other organisms survived.

Butter appears to protect effectively microorganisms. Even after 9 to 12 months at -26° C. (-15° F.) it is not uncommon to find appreciable numbers of certain cocci, *Pseudomonas* sp., and aerogenes bacteria. Salt does not always destroy these bacteria, chiefly because of its uneven distribution. The concentration of salt in the water phase may reach 15% but there may still be droplets almost free of salt (Mattick, 1952).

k. Bread Dough. Freezing storage of dough raises a great many special problems owing to the particular nature of this product. A com-
plete account of the special reactions in the dough-mass at low temperatures will not be given here. Briefly, it may be stated that investigations have proved that freezing storage of the unfermented dough is more favorable. The fermenting ability of the yeast will be better retained thereby, compared to storing semifermented products. On the other hand freezing rate is also of importance. When a dough containing yeast is frozen slowly, the dying-off will be considerably higher than when it is frozen rapidly. The biological activity of yeast is retained during 1 to 3 weeks of storage at \(-18\) to \(-23^\circ\) C. (0 to \(-9^\circ\) F.). Even if yeasts lose their ability to grow, their enzyme systems may still be active. This is why a count of the number of surviving cells does not constitute a reliable measure of the fermenting power of yeast (Godkin and Catheart, 1949).

1. Precooked Frozen Foods. A special problem is offered by these products. They are very easily contaminated and invaded by microorganisms because the structure of the tissues is soft as a result of cooking when compared with those of frozen raw products. Cooking may destroy some of the bacteria but there are numerous chances for contamination during subsequent handling. Precooked frozen foods in many cases also offer a better medium for the development of microorganisms. Cleanliness and sanitation are imperative, since freezing does not sterilize the food. Rapid cooling is less likely to result in spoilage, since the foods remain for a shorter time within the optimal temperature range for growth of bacteria. When leftovers are frozen it is particularly important to be careful since they may have become contaminated during holding.

Changes in bacterial count should be observed continuously during manufacture in order to locate the most important sources of infection. This is said to have been done in the United States after it was observed that precooked frozen foods contained more bacteria than similar preparations made in the home. If there exists a risk of infection with pathogenic organisms during preparation it may lead to disastrous consequences. In direct incubation tests with foods consisting of fish or shellfish it was possible to establish that several such bacteria were not killed by freezing or during subsequent storage (Hutchings and Evers, 1946). Furthermore precooked frozen foods are cooked and prepared for eating in a much shorter time than is customary for similar foods prepared entirely in the home so an effective sterilization is much more difficult to achieve.

Most of the reports in the field of frozen cooked foods have been directed toward problems concerned with home or commercial production. There is, however, little if any information on the freezing of cooked foods for institutional use, where freezer space may be available for storing precooked foods prepared in advance for peak load periods, or as a method of storing leftovers until they can be served. Information is needed about the

wholesomeness of these foods after freezing, storage, thawing, and reheating. A recent study of meat loaves stresses the importance of avoiding long keeping periods after preparing the food (Iee et al., 1952).

How long the precooked frozen foods will keep after thawing depends on the ingredients contained in the products. Control of raw products is fundamental in preparing frozen foods. In order to ensure a low bacterial count in desserts, special standard methods have been developed for different ingredients (such as evaporated milk, dry milk, sugar, eggs, and egg products) which may be used for this purpose (Anonymous, 1948). The microbiological standard of meat loaves also may be determined by the bacteriological count of the constituents (Iee et al., 1952). Poultry meat intended for the manufacture of chicken chow-mein, according to investigations by Gunderson and Rose (1948a) contained 9,000,000 bacteria per gram, which was reflected in the bacteriological count of the frozen product. The effect of prefreezing delay at 25° C. (77° F.) on survival and multiplication of a Micrococcus sp. in creamed chicken was detrimental when exceeding 2 hr. (Strakos and Combes, 1952).

Tests at the Western Regional Research Laboratory in the United States on samples of pork and beans failed to show bacterial growth in 18 days at 4.5° C. (40° F.) but after 21 days, growth could be noted. On the other hand, at room temperature the bacterial count rose considerably on the second day (Hutchings and Evers, 1946). Apparently this product contained no psychrophilic types of bacteria. In samples of the same food from commercial packages, more than 500,000 bacteria per gram were found. Whether this implies risks is, of course, totally dependent on the kinds of bacteria developing. It is also evident that similar phenomena may be observed in all precooked foods.

In spite of the fact that precooked frozen foods have been on the market in the United States for more than 10 years no case of food poisoning has been attributed to this category of foods. Investigations on precooked frozen foods of this kind have been performed by Proctor and Philips (1947) and Hasseman (1951). They determined both the total bacteria count, using the plate count method, and the frequency of coliforms. More than 100 such frozen products were examined. The flora of surviving bacteria varied considerably. Certain products contained more than 1,000,000 colonies per gram. Among products examined were fish stews, meat, poultry, and soups. The average number of colonies in the different products of each category as a rule constituted less than 50,000 per gram. In only 2 products was the average number of colonies above 100,000. As for the separate samples, it was found that 21 samples of fish stews (9.5% of the total number) contained more than 100,000 colonies. The highest figure obtained was 154,000. In 23 samples of frozen
meat products (17.4% of the total) this count was surpassed, but the highest figure was only 250,000. Five hundred samples were examined for coliforms; and in most samples the number of colonies was below 50,000.

Fitzgerald (1947a,b) arrived at the conclusion that for most precooked frozen foods there should be fixed an upper limit of about 100,000 colonies per gram. The total permissible number of colonies, including coliforms, should be at most 1,000,000 per gram. Furthermore, it should be established that no lots be rejected, unless the number of colonies exceeds 500,000 per gram. This means that a distinction is made between a qualitatively desirable figure, or in other words, from a sanitary point of view, a preferable figure, and a dangerous limit.

In extensive investigations (Proctor and Phillips, 1947; Proctor and Nickerson, 1948a,b; Buchbinder et al., 1949; Hussemann, 1951) the total bacterial count in precooked frozen products was taken into consideration as was the taxonomic composition of the bacterial flora. Great variations in the number of colonies on plates from different samples of the same food were noted. In some cases more than 1,000,000 bacteria per gram were found, although the average count was as low as 50,000. Proctor and co-workers examined 64 different foods; fish foods showed a tendency for higher counts, but these frozen products were in most instances good from a bacteriological point of view.

Marked exceptions were chicken stews, such as “chicken a la king,” the number of bacteria here was essentially higher than in other dishes. The same results were obtained at the University of Wisconsin by Hussemmann (1951) and Logan et al. (1951). Of 39 samples examined more than half had a bacterial count exceeding 1,000,000 per gram, 7.7% had more than 100,000, but in all cases less than 500,000 coliforms. This stands in marked contrast to the results of Proctor and Phillips (1947) who showed that in 68 samples of creamed meat and poultry dishes, only 14.7% contained more than 100,000 organisms per gram. Buchbinder et al. (1949) stated that toxic Staphylococci were present in 12 out of 39 samples studied. Eight of these gave Staphylococcus counts between 1,000 and 100,000 per gram, 2 gave 200,000 to 400,000 per gram, and in 1 case, more than 2,000,000. In 9 of the samples, Streptococcus was absent; in 3, the number of colonies was below 20,000; in 1, it was 90,000 and in another 300,000. Four samples showed a count of more than 1,000,000. The dressing of frozen stuffed poultry is an important source of contamination (Esselen and Levine, 1954).

In order to obtain a comparison for the evaluation of these counts Buchbinder and co-workers undertook an investigation in which samples were taken from restaurants in New York. In no case were more than 100,000 bacteria obtained. Neither coliforms nor enterococci were found in any of the twenty samples checked. In this connection, the effect of heating frozen chicken stew (“chicken a la king”) was also studied. It was found that heating for 8 min. to about 66° C. (151° F.) was sufficient to kill most bacteria. This temperature is evidently sufficient to destroy enteric pathogenic bacteria, but would of course only exert slight effect on the Staphylococcus toxin already formed.

The great difference in the bacterial counts for frozen “chicken a la king” and a recently prepared dish of the same kind as served in restaurants is surprising. The infection probably takes place when the poultry meat is removed by hand from the boiled chicken for preparation of the stew. Such meat is not sterilized again so enterococci may be transmitted easily. When the meat is later combined with a special sauce consisting of starch, fat, milk, etc. possibilities for the development of bacteria are increased. It is therefore likely that the infection occurs before freezing. The heating of the frozen product before it is consumed will, of course, destroy the majority of the microorganisms, but offers little protection against the heat-stable toxins formed by Staphylococcus. Due to this risk, very strict requirements with respect to the bacterial content of frozen precooked foods should be established.

The very high figures for counted colonies are due primarily to the high degree of infection, chiefly during the preparation. This clearly proves the importance of good sanitation in the factories and during processing and handling of the products. These conditions are decisive with respect to the bacteriological condition of the product. The number of bacteria, and consequently the number of colonies obtained in a plate count, decreases steadily during freezing. In spite of this there are high counts. This makes the indicated causal relationship with hygienic conditions very obvious.

Fitzgerald (1947b) stated that frozen cooked foods should be regarded as a potential health hazard. Sanitation controls should be aimed at two objectives: first, to keep contamination to a minimum and to avoid the possibility of the development of off quality during processing, storage, distribution, and consumption; second, to keep the “most probable number” (M.P.N.) value for coliform organisms low to diminish the possibility of including pathogenic organisms and hence prevent the possibility of illness in cases where subsequent reheating might not kill all such organisms.

VI. Pathogenic Bacteria in Frozen Foods

Hartsell (1951) prepared packs of beef and peas inoculated with various strains of Salmonella. He showed that these pathogens would survive for many months at −9° C. and −17° C. (16° F. and 1° F.). The cells
seem to have their metabolic requirements markedly altered, since with a highly nutritive medium much larger numbers were recovered than on selective media. A greater destruction of cells on frozen beef and on peas was observed at 9°C (16°F.) than at 18°C (65°F.). The order of resistance, from greatest to least, of cultures on frozen beef or peas, was Staphylococcus aureus, Salmonella oranienburg, S. typhosa, and S. dysenteriae.

Wallace and Park (1933) determined the survival rate for pathogenic bacteria inoculated into frozen cherries and cherry juices. Similar studies were performed later for strawberries by McCleskey and Christopher (1941) concerning various intestinal active species of Staphylococcus, Eberthella, and Salmonella. A certain per cent of the inoculated bacteria survived even after 8 months of storage (see Table II). See also p. 167.

In connection with the remarkable observation was made that the destruction of these bacteria was far more effective at room temperature and even at 6°C (32°F.) than in the frozen product. It was not indicated whether this was dependent on cell structure or on definite bactericidal effects of the strawberry juice. Similar destruction of bacteria has been observed in apple juice.

A strong germicidal effect has also been demonstrated in orange juice concentrates. Even stock cultures of Escherichia coli, Salmonella typhosa, and Shigella paradysenteriae inoculated into the concentrate, which was subsequently frozen, could not be recovered after 24 hr. of storage at 1°C (34°F.). This effect has been attributed to pH and the organic acid molecule. To a lesser degree, small amounts of the orange peel oil exert a toxic effect on these pathogenic bacteria (Hahn and Appleman, 1952a,b). Fecal coliforms are particularly sensitive and do not survive.

Of special interest is the botulinum problem. Studies on the behavior of the Clostridium botulinum organism, when inoculated into frozen vegetables, yielded extremely contradictory results. The fears uttered by experts concerning the risk of the formation of dangerous toxins by this particular bacteria was finally dispelled by an investigation published in the late thirties (Prescott and Geer, 1936). It was shown that Clostridium botulinum endured freezing well, but did not grow. In a few specific cases the formation of toxins was noticed when the thawed product was stored at room temperature (Tanner and Wallace, 1931; Straka and James, 1932, 1933; Wallace and Park, 1933). Other investigators could not find toxin (Berry, 1938; Edmondson et al., 1942; Prescott and Geer, 1936). Defrosted frozen foods, inoculated with Clostridium botulinum proved to contain toxins, often after 4 days at 20°C (68°F.) (Tanner and Oglesby, 1936; Tanner et al., 1940). At 15°C (59°F.) the formation of toxins took place at an appreciably slower rate, e.g., spinach and lean beef developed toxins in certain cases in 4 days at temperatures as low as 10°C (50°F.). The competition with other bacteria, however, generally is responsible for the fact that Clostridium botulinum does not have sufficiently favorable conditions for development. An extensive investigation has recently been reported in this field in which several important vegetables were examined (Perry et al., 1948). In no cases were toxins found, not even after storing the thawed product for 10 days at 21°C (70°F.). Although the commercial freezing of vegetables in the United States has increased from 4994 tons a year in 1930 to 454,000 tons in 1952, and although considerable quantities of vegetables have been frozen in home freezers and locker plants, no case of botulism caused by frozen foods has as yet been recorded. This is even more remarkable when one considers that industrial, as well as home freezing and storing was, in many cases, done in an unsatisfactory way. One case of botulism is reported from locker frozen fish kept at home 24 hr. prior to freezing (Dolman et al., 1950).

Many investigators have stated that other bacteria inhibit the development of Clostridium botulinum or possibly destroy any toxin that may form. Among such antagonistic forms are: Aerobacter aerogenes, Bacillus subtilis, Clostridium sporogenes, Escherichia coli, Lactobacillus casei, Proteus vulgaris, Streptococcus lactis, and Streptococcus thermophilus (Hall and Peterson, 1923; Jordan and Dack, 1924; Dack, 1926; Sherman et al., 1927; Stark et al., 1928; Kanyukova and Kremer, 1940; Ramon et al., 1944, 1945). When thawed vegetables were stored at room temperature by Berry (1933b), acid-forming bacteria developed most rapidly.

Liquid eggs were experimentally inoculated with pathogenic bacteria, then frozen at -25°C (3°F.), and stored at -1°C (30°F.) and at -18°C (0°F.). The following organisms survived storage for periods up to 10 months at -18°C (0°F.): Salmonella typhosa, Salmonella oranienburg, Escherichia coli, Salmonella enteritidis, and Staphylococcus aureus. Sensitivity of the organisms increased in the order given above. Destruction was greater at -1°C (30°F.) than at -18°C (0°F.) (Hartell, 1949).

Schneider and Gunderson (1949) concluded that freezing only partly eliminated the surface bacteria, chiefly Salmonella, on eviscerated chicken even after long storage. Schoening et al. (1949) showed that lyophilizing S. cholera-suis had no effect on virulence. Cherry et al. (1946) report the recovery of a nonmobile Salmonella from the skin of frozen turkeys and Brown (1949) S. typhi-murium after 13 months of frozen storage.

Six strains of Salmonella were studied and inoculated into frozen chicken stew by Gunderson and Rose (1948a). The death rate was directly proportional to the concentration of organisms, which emphasizes the great importance of plant sanitation. The mortality of Escherichia coli
and *Aerobacter aerogenes* also has been studied under similar conditions. In neither case was a full pasteurizing effect reached. In 2 or 3 cases the bacterial content remained high, even after 5½ months of storage at −25 to −30°C (−13 to −22°F). Similar observations were made by Proctor and Phillips (1947) in studies on cooked foods, experimentally inoculated with pathogenic strains of *Staphylococcus*, *Streptococcus*, and *Salmonella*. These foods (which were chicken a la king, creamed salmon, beef stew, and cooked shrimp) were frozen and stored at −18°C (0°F). In a few cases 10% or more of the organisms survived storage for 6 months.

**VII. Defrosting Problems**

When food is defrosted, the bacteria are liberated and immediately begin multiplication and hence decomposition i.e. chemical breakdown of the food. Under industrial and institutional conditions spoilage may be considerable. It is thus essential to maintain control of the microorganisms after thawing. The problem of how bacteria grow and multiply after defrosting a frozen food apparently has not been investigated extensively. A voluminous literature is available with reference to the total number of viable cells in frozen foods immediately after defrosting, but little attention has been given to later intervals and to the growth requirements of the surviving cells.

Even during the very thawing of the frozen foods, bacteria start growing within the temperature limits characteristic for the development of respective strains. The higher the external temperature is kept, the more favorable are the growth conditions for most bacteria. Thus defrosting at a low temperature is the best method for keeping the bacterial count at a low level (Winter and Wrinkle, 1949e).

Rapid thawing can be readily accomplished by the use of high-frequency heating (Gilb, 1943; Sherman, 1946; Cathcart and Parker, 1946; Cathcart, 1946; Bartholomew, 1948). It must, however, be borne in mind that there is no evidence that dielectric defrosting or heating has a specific "bactericidal" effect even though this has been often claimed. Of this type of radiation, only the ultraviolet has any specific bactericidal effect. The earlier assumption of the existence of a point-heating effect sufficient to kill microorganisms has been disproved by Higasi according to Asami (1950). High-frequency energy has no effect on a material other than that of inducing heat in it. It is, therefore, necessary to use another medium than high-frequency energy for bactericidal effects. But the short-time defrosting obtained with this method does indirectly imply that few microorganisms manage to develop.

Actually, frozen foods deteriorate principally in the same manner as fresh products; however, they decompose more rapidly. Consequently thawed frozen products may be regarded as slightly more perishable than fresh ones. Particularly this refers to fruits and vegetables, where the tissue disintegrates and the open and shattered cells permit a rapid growth of microorganisms. The bacteria invade the partly softened tissue earlier than fresh intact tissue. Whether or not freezing storage influences the subsequent growth rate of the bacteria physiologically is still an open question. Hartsell (1951) noted that *E. coli* was stimulated by being held in freezer storage and showed a greatly shortened lag phase after freezing and thawing. Sulzbacher (1952), however, found no evidence for the belief that frozen meats are more perishable after thawing than fresh meat. Studies on viability and the effect of longer freezer storage remain to be made.

A group of bacteria of great hygienic importance are the species of *Staphylococcus*. Canadian research workers have shown that if frozen foods are stored after thawing at temperatures below 10°C (50°F.), bacteria belonging to this group do not develop. Frozen vegetables sometimes get sour. This is particularly the case with asparagus, peas, and yellow and green beans. The souring agent is a special bacteria (*Streptococcus fecalis*) which develops luxuriantly immediately when defrosted or prior to freezing (Sanderson and Fitzgerald, 1940). This species has been found on peas in the pod. It is a strongly acid-forming species, rendering the product indelible long before other microorganisms have had time to form toxins. It has been proposed that frozen foods be inoculated with this or some other acid-forming bacteria as a safety factor, by means of which the frozen foods would be given a sour taste before toxigenic forms could produce any injurious enterotoxins (Prescott and Geer, 1936; Berry, 1936c; Prescott et al. 1932; Wallace and Park, 1933; Smart, 1934, 1939a,b; Wallace and Tanner, 1935). Since poisoning by frozen foods occurs so rarely, such an inoculation with spoilage organisms may, however, be considered a very doubtful procedure. The rich bacterial flora which is generally found in frozen products offers the best protection against infections. If stored for a long time at a temperature favorable for bacterial development, these bacteria are most likely to develop taint or odor-producing substances long before toxins may possibly appear. In other words, there is a warning long before there is danger.

The risk of a dangerous bacterial development in fruits and vegetables in connection with thawing may as a rule have been overestimated. In samples containing one of the acid-forming bacteria from peas, the number of bacteria grew in 24 hr. from 1600 to 5000 per g. at 4.5°C (40°F.), whereas at 21°C. (70°F.) the increase was to 2,000,000 (Berry, 1946c). After 6 days the number of bacteria at 4.5°C (40°F.) was still less than
100,000. If the thawed foods are stored in a refrigerator the risks of both bacterial development and impaired quality are slight, if the storage period is not extended for more than a week. Refreezing is, however, as a rule not advisable as the number of bacteria has increased during thawing, and the structure and nutritive value are also adversely affected by enzymes contained within the plant cells themselves. There is also the risk that the thawing or storage may be carried out in a careless manner and at a higher temperature than in a refrigerator [about 2 to 4° C. (36 to 39° F.)]. Often thawed packages are stored in the kitchen or in a shop. According to the investigations made by Sanderson (1941) storing for 7 days at zero centigrade implies no risk, not even for vegetables, but at 30° C. (86° F.) the product spoiled in 12 hr.

Fruit thawed at room temperature keeps for 24 hr.; however, vegetables deteriorate under the same conditions (Fellers, 1932). It is well known that bacteria do not grow nearly as rapidly in the acid media of fruits as under the more neutral conditions prevailing in vegetables. Recently figures were published as to the keeping quality of frozen orange concentrate after defrosting. It was preferable to hold the concentrate below 5° C. (41° F.) (Miller and Marsteller, 1952). Yeasts, which constitute the principal surviving organisms, will grow in concentrated orange juice stored at 6° C. (43° F.) and will cause spoilage (Patrick, 1949).

With regard to frozen eggs, an important practical point is that they cannot be thawed rapidly by methods normally available in bakeries. At the same time the length of the defrosting period is a most decisive factor in determining the number of bacteria developing. Plate counts often increase 100% during the thawing of large size cans (30 lbs.) of frozen whole eggs.

Egg yolks thaw more rapidly than both egg white and mixtures of the two. The white thaws most slowly because heat conduction is lowest for the whites. A repeated shaking of the containers accelerates thawing and hence the bacterial content may be kept low during defrosting (Winter and Wrinkle, 1949c).

In thawing egg at 15° C. (59° F.) bacteria grow more slowly than at 20° C. (68° F.). Remarkably enough, it has been stated that, if liquid egg is kept at temperatures lower than 13° C. (55° F.), so few bacteria on the whole develop that it is of no practical importance (Winter and Wrinkle, 1949c). Hence is derived the assumption that psychrophilic types are almost absent. This implies the practical advantage that liquid egg can without any inconvenience be kept in ordinary cold storage for a couple of days. Nor does the transport of frozen egg require a strict maintenance of fixed limits of temperatures, as the product can without risk be allowed to thaw and the temperature raised to about 12° C. (54° F.) for as long as 3 days.

On an average, yolk contains more bacteria than whites whereas a mixture of both yields medium values. This is in accordance with the fact that initially they show similar differences in bacterial contents. As thawing proceeds from the surface inward, a higher bacterial count is found in the exterior areas than in the interior areas (Winter and Wrinkle, 1949c).

Liquid egg can be refrozen after thawing without risk if the initial count of bacteria is sufficiently low (Stiles and Bates, 1912). Based on the results of investigations, United States authorities have issued special but fairly mild regulations for the handling of frozen eggs to be used as raw material for subsequent manufacture into egg powder (Pennington et al., 1914; McFarlane et al., 1945). They imply that thawing in transit is permissible under the following conditions: The maximum is 4.5° C. (40° F.) for 3 days or 10 to 25° C. (50 to 77° F.) for at most 24 hr. If thawed, they must be held below a maximum of +8° C. (46° F.) for no longer than 16 hr. These regulations may eventually be accepted for the handling of frozen eggs in maritime and railway transport.

Esselen and Levine (1954) established a marked increase in aerobic and anaerobic bacteria counts in stuffed chicken after 20 hr. during thawing and holding at room temperature. This was accompanied by an increase in acidity and development of off-odor.

Causey and Fenton (1951) studied the bacterial flora of home-frozen creamed chicken and rice, chicken paprika with gravy, spaghetti and meat balls, and ham patties, before and after 5 methods of heating. The initial counts were low, less than 2000 per gram in all products. Thawing at room temperature in most cases resulted in an increase in counts. Organisms occurring were found to be strains of Bacillus and Staphylococcus.

Bacterial pathogens such as Staphylococcus, Streptococcus, and Salmonella in frozen cooked foods increased in number after storage at defrosting temperatures of 30 to 37° C. (86 to 99° F.) for 6 to 8 hr. At lower thawing temperatures the number of these bacteria increased less rapidly (Proctor and Phillips, 1947). Some increase did occur at 10° C. (50° F.) storage. All these observations lend emphasis to the need for proper storage and thawing of all such foods. Straka and Combes (1952) claim to have demonstrated that creamed chicken containing a pathogen (Micrococcus pyogenes var. aureus) could be defrosted at 25° C. (77° F.) over a 10 to 11-hr. period without encountering excessive counts.

It has often been reiterated that the number of microorganisms surviving is primarily influenced by the freezing rate. But it is equally true that the length of time for defrosting has a profound influence. Slow
freezing and thawing give the lowest counts for surviving organisms, although as a general rule, rapid freezing and defrosting result in less destruction. There are, however, certain aspects of the defrosting which require further studies. Thus far we have too little information to indicate what thawing might do toward diminishing the number of organisms (Stille, 1942). As temperatures between $-4$ and $-10^\circ$ C. (25 and $14^\circ$ F.) destroy bacteria and spores rather quickly (as has been well established when studying counts during the freezing process) it would be worth while to elucidate what actually happens when thawing products pass this temperature zone considered so dangerous to microorganisms in the freezing stage (p. 165). It is possible, although not likely, that in passing through this zone from a lower to a higher temperature there is a corresponding effect on the protein structure to what occurs during freezing. It is most likely that the process of denaturation is continued as thawing generally takes place less rapidly than the freezing. Perhaps prolonged freezing storage also may affect the physiological reactivity in some way or other, similar to what often takes place in higher plants when cold treatment stimulates subsequent growth. Such destruction during defrosting along with a subsequent growth stimulation has now (1954) been experimentally established at SIK (in press).

In order to be certain that frozen foods do not thaw during distribution a number of indicators have been invented (mostly in the United States) which show by coloring of strips, or other reactions, whether or not the product in question has at some time undergone thawing (Andersen, 1949; Ramstad and Volz, 1950). The earlier indicators were based mainly on the principle that a printed statement of some kind was eradicated by coming into contact with a thawing liquid. These could not give sufficient indication of for how long a period the product had been in a thawed state; this is the reason why new types have been developed. The most reliable one consists of a gelatin gel, enclosed in an oxygen-permeable transparent envelope. A gel containing an enzyme system (an oxidase), a colorless phenolic compound, and a suitable amount of ascorbic acid is packed in a plastic envelope. Intensity of the color which develops upon thawing by darkening resulting from oxidation of the phenolic compound measures the time during which liquid from the thawed product has been in contact with the indicator (Ramstad and Volz, 1950). The ascorbic acid regulates the rate of the color development. The color developed is formed by reactions similar to those taking place in enzymatic browning of fruit tissue. By means of these indicators we are able to tell whether or not a package has been kept frozen during the entire period of storage and distribution. Furthermore, we may also estimate the length of time a package may have been thawed.

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Frozen foods should always be stored on a dunnage and not adjacent to exterior walls, due to the risk of heat penetration causing local defrosting. According to experiences in the United States, the danger of packages thawing is greatest during transit and in retail shops.

**VIII. Packaging Problems**

On purely theoretical grounds it might be inferred that, from a hygienic point of view, paper is safer than a tin can for frozen foods. Such a package cannot be sealed hermetically. It is generally not possible for anaerobic bacteria to develop even in the interior of food not exposed to air in paper packages. More important, however, is the fact that the consumer need not risk mistaking frozen foods for canned ones. This eliminates the danger of storing unsterile frozen food for a long period of time at temperatures which permit bacterial development.

The use of tin cans, however, offers great advantages; among others, more rapid freezing, more efficient filling, and easier handling. In addition, the effective method of immersion freezing may be practiced. This is certainly the reason why the United States frozen food market now offers several products packed in cans, particularly juice concentrates and berries. But in order to avoid any error some frozen fish products in cans are overwrapped with an extra paper package. As mentioned above, tests with several vegetables have shown that there is very little danger of the development of *Clostridium botulinum* in sealed cans, even after several days of storage in a thawed state at room temperature (Perry *et al.*, 1948).

It is well known that frozen foods are packed in a great many different packaging materials. From a microbiological point of view, it is of course desirable that any material used should be free from microorganisms and their spores. In other sectors of the food industry the microbiology of the package has received much attention. This has been the case only to a limited scale in the frozen foods industry. In the United States packaging materials specially treated against bacteria have been designed for frozen foods (White, 1951). Such packaging materials are used primarily for frozen fish fillets in order to destroy surface bacteria. It is highly probable that in the future greater attention will be given to the microbiological problems concerned with the packaging of frozen foods.

Of great interest, in point of principle, are the observations reported by Weiser (1951) to the effect that microorganisms in frozen berries, which are not hermetically packed, rapidly increase at $-2$ to $-4^\circ$ C. (28 to $25^\circ$ F.) whereas those properly sealed show a heavy decrease. This attributes greater significance to the use of air-tight packages. This observation becomes particularly important when one considers that, during the period when freezing heat is released and the temperature is
stabilized at a level immediately below 0° C. (32° F.), aerobic bacteria may grow freely if air is admitted. No significant effect due to packaging material was revealed through bacterial studies on dressings of frozen stuffed poultry (Esselen and Levine, 1954).

IX. COOKING

As already pointed out, the preparation of several frozen foods before they are eaten also has a prophylactic significance. All preserved vegetables are thoroughly heated in boiling water (spinach, cauliflower, peas, etc.). Bakery products, stews, and ready-to-serve dishes of fish and chicken are not always effectively heated through, yet the temperature is insufficient to call this procedure pasteurization (even if it does not always have a completely sterilizing effect) greatly reducing the number of bacteria.

Observations have been made on the bacterial flora of commercially frozen precooked chicken a la king, beef stew, and creamed fish as they reached the kitchen and at different stages during subsequent kitchen procedures, such as cookery and refrigeration. Although cooking reduced the number of all kinds of microorganisms, it did not eradicate any type. Continued multiplication of bacterial cells was observed in chicken a la king and beef stew under conditions of household refrigeration (Hussemann, 1951).

X. HYGIENIC ASPECTS

The reason for this survey is, of course, to collect as much material as possible for estimating the hygienic dangers connected with frozen foods, and, if possible, to specify under what conditions safety may be considered to the same degree as with other foods in the market. This problem received early attention in the United States (Fellers, 1932; James, 1932), and the progress of frozen foods in the thirties and forties was watched with some uneasiness, particularly since adequate quality control did not exist. Especially disturbing were the obvious risks of toxin formation by Clostridium botulinum and species of Staphylococcus. But nothing happened despite the fact that the raw products originated from all parts of the continent and preparation methods varied considerably (Tessler, 1946; Fitzgerald, 1947a). During the 6 to 7 years that ready-to-serve foods have been in the United States market, not a single case of food poisoning caused by frozen products has been recorded or established with certainty. As for other frozen foods, only one case of poisoning (from fish) has been reported (White, 1951).

The bacterial count, therefore, is more of an indication of the standard of factory sanitation rather than of the unsanitary condition of the product. As in other foods, the important aspect is concerned with the kinds of bacteria which develop and to what extent these cause intestinal infections or affect consumers in other ways.

Water bacteriology is of prime importance according to experiences in the United States. Frozen food factories, therefore, have been installing chlorination systems for water used in plants. This has resulted in a considerable reduction of bacterial counts in the frozen foods (Scarlett and Martin, 1948). In the United States, 90% of the water supplied by the cities is chlorinated.

As is the case in certain sectors of the canned food industry, there has been a tendency within the frozen food industry to underestimate the importance of strict sanitary handling of the products during preparation for preservation. The subsequent heating or freezing was supposed to kill the bacteria rather effectively and the risks were regarded as very slight. This is not recommendable for either hermetically sealed products or frozen foods. The statements made above show clearly how important it is to keep the bacterial content at as low a level as is possible. This is particularly true for frozen foods which will never become sterile, not even after prolonged freezing storage. From the point of view of quality, it is consequently desirable to make rapid estimations of bacterial counts regularly during manufacture, using the methods described earlier in the review. The bacteria occurring in frozen foods are, as a rule, harmless to man. From a public health point of view, however, it is important to test the products from time to time for the presence of fecal bacteria (above all the Escherichia group) so that the possibility of such contamination may be eliminated. The simplest preventive measure in factories is, however, to undertake regularly a thorough cleaning of all equipment with bactericidal substances. The sanitary problems in the processing and distribution of frozen foods have been well reviewed by Evers (1950). A definite health hazard is the one of frozen fruits and vegetables in raw salads which could easily, through carelessness, be a source of infection.

XI. PRACTICAL ASPECTS

The practical consequences of investigations on the bacteriology of frozen foods may be summarized briefly in the following way:

Most frozen products are not sterile, i.e. they contain natural forms of bacteria (above all spores) which may develop as soon as the external conditions are favorable. The same is also true of spores of certain molds or yeasts. Cleanliness and good hygiene, consequently, must be maintained in all aspects of the handling of foods intended for freezing. The handling of raw products in factories must, therefore, be done carefully, and, in reality, the requirements in this respect are greater than for other methods
of preservation. A regular cleaning of plant and equipment should be considered as routine in a frozen food plant. Furthermore, preparation of products should be done as rapidly as possible. The longer it requires to get the products ready for freezing, the more bacteria will develop to impair quality. The demand for cleanliness, hence, is ultimately a prerequisite for good quality. The time between harvest of fruits and vegetables, catching of fish, slaughtering of meat and poultry, and freezing must be as short as possible, and the product should be kept as near 0° C. (32° F.) as possible in order to assure a minimum rate of bacterial growth.

If a reassuring sanitary control of plants is provided for, the risk of infection with pathogenic bacteria may be of minor importance. Sanitary measures, in this case, must be extended even to the personnel. Strict demands concerning personal hygiene will have to be maintained. All water used for cleaning containers, preparation, and rinsing must be clean and, as far as possible, free from bacteria. All equipment which is used regularly should be washed. A strict schedule should hereby be observed.

In this connection it must be remembered that there is an essential difference between freezing and such preservation where most of the bacteria are killed by heating. Only a fraction is rendered harmless in freezing as is to be seen from this review. In accordance with the discussion given in the section on sanitation, every freezing plant should have regular bacteriological controls run on the finished products. Raw products also should be subjected to microbiological examination.

The most crucial risk occurs during thawing, when conditions are such that the chances for the development of surviving bacteria and their spores are great. In the preparation of vegetables for freezing, the tissues are so affected that bacteria, yeasts, and molds can develop considerably faster than is possible in canned products. As has already been pointed out, vegetables are exposed to a far-reaching sterilization in blanching. Consequently, the bacterial contamination takes place between blanching and freezing. It is, therefore, particularly important to avoid any delay and to freeze the products as rapidly as possible after blanching.

Precooked frozen foods are subject to similar risks. In connection with the cooking and preparation of the raw products, large numbers of bacteria may be destroyed, but subsequent contamination may take place. Even though dangerous bacteria might not be involved in this case, generally there are bacteria that may cause a deterioration in quality.

It is entirely justified to conclude that most spoilage encountered in the frozen food field is due to improper handling of the product either prior to freezing or after it is thawed. Freezing and storage conditions play a secondary role.

Due to a break in the electric current or to pure neglect, it may happen that frozen foods are unintentionally allowed to thaw. This risk is also present during transit and in retail shops. If the thawing has not proceeded sufficiently to be complete, the package may in exceptional cases be refrozen, but such packages should be used as soon as possible and previous to other unhawed ones. The keeping quality is no longer the same. If the thawing has been complete and the temperature of the products has risen above 0° C. (32° F.), the measures to be taken depend on the type of food in question. Meat, poultry, and fish which still have an unchanged fresh odor (determined by opening some packages) may be refrozen if the product appears fresh upon examination and if a rapid estimation of bacterial load gives a reassuring result. But even in this case it is essential that such a product be consumed within a short time. It must, however, be taken into consideration that enzymic activity is stimulated by such a procedure and this inevitably leads to an inferior product. Fruit can also be refrozen, but as a rule it is better to make jam, compote, jelly, etc. of such lots. Shellfish and vegetables should never be refrozen. They should be boiled without delay or used in cooking. If they have been standing at temperatures higher than 10 to 15° C. (50 to 59° F.), they should not be used at all for dangerous bacteria may have had a chance to develop, and no such risks should be taken. Finally, consumer information on the correct procedure for handling frozen foods is of great importance for eliminating risks. The experiences gained up till now in all countries of the world show that this seems to have met with considerable success.

References

Anonymous. 1938. Microbiology of frozen foods. *Ice and Refrig.* 85, 75-76.


Bequerel, P. 1950. La suspension de la vie au-dessous de 0°C absolue par déséquilibre thermique à partir de l’alum de fer dans le vide le plus vide. Compt. rend. 231, 261–63.


Berry, J. A. 1932a. Bacteria question in cold packing. Western Canner and Packer 27(10), 17–18.


Berry, J. A. 1932e. Microbiology of the frozen pack. Canning Age 13, 251–54.


Berry, J. A. 1933c. Microbiology of frozen pack berries and vegetables. Ice and Refrig. 84, 201–05.

Berry, J. A. 1933d. Some findings on microbiology of the cold pack. Canning Age 14, 445–46, 463.


Berry, J. A. 1938. No danger from botulinus in frozen fruits or vegetables. Western Canner and Packer 30(4), 32.

Berry, J. A. 1941. The fewer the bacteria, the better the frozen pack. Canner 94(4), 13–14.


Microbiological Problems of Frozen Food Products 219

Haines, R. B. 1934. The minimum temperatures of growth of some bacteria. J. Hyg. 34, 277–82.

Hays, G. L., and Riester, D. W. 1952. The control of "off-odor" spoilage in frozen concentrated orange juice. *Food Technol.*, 6, 386–89.


Kaplan, M. T., and Appleman, M. D. 1952. Microbiology of frozen orange concentrate. III. Studies of Enterococci in frozen concentrated orange juice. *Food Technol.*, 6, 167–70.


Luyet, B. J., and Gelenio, P. M. 1940. Life and Death at Low Temperatures. *Biodynamica*, Normandy, Mo.


Owen, R. E., and Van Duyne, F. O. 1950. Comparison of the quality of freshly baked cakes, thawed frozen baked cakes, and cakes prepared from batters which had been frozen. Food Research 16, 169–78.


MICROBIOLOGICAL PROBLEMS OF FROZEN FOOD PRODUCTS


Rahn, O., and Bigwood, F. M. 1938. The effect of subminimal temperatures upon Streptococcus lactis. J. Bacteriol. 35, 64.


Smart, H. F. 1939b. Microbiological studies on commercial packs of frozen fruits and vegetables. "Food Research" 4, 293–98.


Tanner, F. W., Benner, P. H., and Rickner, C. J. 1940. Further studies on development of *Clostridium botulinum* in refrigerated foods. *Food Research* 5, 323-33.


Weisier, H. H. 1946. Some fundamental observations on the activities of microorganisms in frozen foods. *Frozen Food Ind.* 2(8), 8-9, 36.


Wolfford, E. R. 1943. A direct microscopic method to estimate the sanitary history of frozen pack peas. Western Canner and Packer 35(13), 58; Food Research 8, 163.


