

THE SURVIVAL OF PATHOGENIC BACTERIA AND
OTHER MICRO-ORGANISMS IN FRUIT JUICES

BY

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I. INTRODUCTION.

The survival of micro-organisms in fruit juices is of interest from several standpoints: (1) length of processing time required for preparation of a commercially sterile product, (2) relation of fresh fruits sold on the open market to potential disease-disseminating bacteria, and (3) prevention of spoilage of fresh fruits and their juices by fermentation and various types of decomposition.

Commercial packing houses have developed processes that deal adequately with canned juices. By using fresh, undamaged fruits, the third factor can also be eliminated from consideration, but disease producing bacteria and other micro-organisms still have easy access to these foods. Although numerous outbreaks of diseases due to organisms in or on fruits have been reported in the literature, an attitude of unconcern still prevails regarding proper sanitary precautions in the handling or storage of juices. Conversely, the problem of spreading sickness through fruits and their juices becomes larger with each succeeding year.

According to Tressler, Joslyn, and Marsh (1939), the only preserved fruit juice of any great commercial importance before 1925 was grape juice. In 1926 canned grapefruit juice was first produced on an important commercial scale, and by 1930 several hundred thousand cases of this beverage were packed annually. Tomato juice, introduced on a large scale in 1928, became leader among juices in 1930, and today over fifteen million cases are packed annually (reported from Agricultural Statistics 1941, U. S. D. A.). The rise in production among other fruit juices is correspondingly great, so

that at the present time, almost forty million cases are marketed yearly.

Development of the frozen food industry in the past twenty years has emphasized the importance of this method of preservation of fruits and vegetables. Whether lower temperatures are less bactericidal than conditions ordinarily present has not been determined definitely. Since large quantities of juices are consumed immediately upon thawing without further treatment, consideration of this method of storage is of importance.

Fortunately, the danger of diseases spreading by raw fruits is probably not as great in America as it is in countries where the great epidemic diseases run rampant. Mills, Bartlett, and Kessel (1925) account for some of the contamination of fruits as a result of the practice in the Old World of using human excreta as fertilizer. Among the more important factors in this country that may be responsible for outbreaks are (1) unsanitary methods of handling and storage, (2) hiring fruit pickers from different regions of the United States and Mexico and so increase the possibility for introducing a greater variety of organisms, (3) washing fruit with polluted water, and (4) insect vectors.

The common habit of serving citrus juices and tomato juice to school children and the equally prevalent practice of serving various fruit juices at road stands and markets may not be wise if fruit juices are not germicidal within very short periods of exposure.

The purpose of the following investigation will be to determine how bactericidal some of the more common fruit juices are to typical intestinal pathogens.

II. REVIEW OF LITERATURE.

The fact that foods vary greatly in their resistance to decay has been common knowledge for thousands of years. Primitive man knew that he could store some foods and they would "keep" while others would decompose so quickly that they were unfit for use within a short time. That living agents were responsible was not realized until much later, and it has been rather recent that man has been able to cope with various requirements necessary for preservation. Even today some of these problems remain unsolved. The manner in which organisms invade fruit, rate of reproduction or period of survival in or on fruit, period of survival at different temperatures, and tolerance toward various hydrogen ion concentrations must be considered.

Micro-organisms causing decomposition or contamination of fruit have two possible avenues of infection: (1) they may gain entrance through a break, or eruption in the skin, or (2) they may be present in the material initially. Coverings surrounding most fruits prevent the entrance of bacteria and other organisms as long as they remain intact. Bulloch (1937) stated that van den Broeck (1858, 1860), Pasteur, and Roberts were able to preserve fruit juices from healthy plants without the application of heat or chemicals. Van den Broeck is regarded as the pioneer in experimental work involving the sterility of the interior of fruit. That worker filled beakers with mercury, heated and evacuated by air pump until there was no trace of air, and then inverted the beakers over the mercury. Grapes, uninjured except for a small slice removed by sterile scalpel

were pressed so the juice within the fruit ascended into the inverted beaker. This liquid was found to remain sterile when oxygen was bubbled in, but when yeasts were also introduced, the juice fermented. Roberts (1874) expressed the juice of fruits and vegetables into a plugged pipette which was later sealed in a flame. Grape, orange, and tomato juice were successfully preserved by this method. Sterile grape juice was obtained by Pasteur (1876), who punctured the grape through a small cauterized area in the skin and collected the fluid by capillarity and suction. Experiments similar to these were reported by many other investigators. Mills, Bartlett, and Hessel (1935) state that normal uninjured fruits do not contain living bacteria within their tissues, nor do bacteria penetrate the unbroken skin in fruits kept under ordinary market conditions. They dusted finely divided particulate matter such as carbon - corresponding in size to bacteria, protozoan cysts, and helminth ova - over fruit and found that it did not penetrate to any appreciable extent. In direct contrast are the findings of Miethammer (1939) and of Romwalter and Kiraly (1939). The former found molds in many fruits and seeds, while the other two workers isolated bacteria from the inner structures of fruit, seeds, and plant tissues and from sap. Also, several varieties of yeasts were identified.

While molds, yeasts, and bacteria are all of importance where fruits and tomatoes are concerned, relatively few micro-organisms find these acid foods a favorable medium for growth. According to Tanner (1932), fruits rich in acids are more apt to be spoiled by molds than by bacteria. "On the other hand, fruits which are rich in carbohydrates and low in acids usually permit the bacteria to develop". Molds are important in the product before canning but

are seldom responsible for spoilage after canning. Bigelow and Cameron (1932) held excessive contamination of the raw material responsible for presence of flat-sour bacteria in tomato products. The same investigators found little reliable information on the extent of loss due to yeasts in canned fruits. However, many of these cases were not reported in the literature. One such instance was described by Ayers (1926) of a yeast forming gas in various catsups. The organism was destroyed in tomato juice heated to 60°C (140°F) for ten minutes but not for five.

Relationships among pH, time and temperature have been known for some time. In 1914, Clark remarked that temperature is an accelerating condition, and that concentration of hydrogen ions in a medium may be of great significance in cellular destruction. Bigelow and Esty applied this information to commercial processes. Using a known suspension of spores, they (1930) observed that time necessary for destruction decreased as temperature increased, and that initial concentration of spores per ml affects time necessary to sterilize a medium of known pH and temperature. In 1931, Bigelow and Cathcart discussed the relation of processing to the acidity of foods. Processes for canned tomatoes were published by Bigelow (1926). In the same year, Cameron and Esty made the statement that the lower limit for obligative thermophilic bacteria was pH 5.0 while at least one facultative thermophilic group was able to reduce the pH in most instances below pH 4.5. Bigelow and Cameron divided foods into three classes: (1) non-acid, (2) semi-acid, and (3) acid. Later Cameron and Esty split the last class. Foods having a pH of 4.5 to 3.7 were considered acid and included fruits such as tomatoes, pears, figs, pineapples, nectarines, etc.

while those of pH 3.7 or lower were called high acid foods. Braut, pickles, berries, grapefruit, citrus juices, rhubarb, and the like are examples of the latter division.

Bigelow and Cameron (1932) declared that "it is commercial experience that spore-forming bacteria will not cause spoilage in acid products". After the discovery of Bacillus thermoacidurans by Berry (1932) and the isolation of strains of Clostridium closely related to Clostridium pasteurianum Winogradsky by Spiegelberg (1938) and by Townsend (1939), Cameron and Eesty (1940) stated that the contents of pH 4.5 - 3.7 foods are subject to spoilage not only by non-sporing aciduric types, but also by spore-forming anaerobes allied in their characteristics to Clostridium pasteurianum Winogradsky. Berry's organism was found in tomatoes where it caused a medicinal taste without acid in the canned product. From bursting cans occurring in packs of canned pineapple, Spiegelberg recovered obligate anaerobes of the genus Clostridium, while Townsend identified the same or a similar organism from low-acid canned fruits such as pears, ripe apricots, ripe nectarines, and figs. In addition, the latter worker found the organism quite common in the soil of a number of districts in the West. Cameron (1938) believed that spoilage by spore-forming bacteria was very rare in acid foods and cited the abnormal reaction of Bacillus thermoacidurans as due to the acidity of the medium. In the high-acid group, freedom from spoilage caused by spore-forming bacteria is claimed. However, aciduric non-sporing bacteria are important.

Occurrence of spore-forming organisms in acid foods creates a special problem which apparently must be dealt with from the standpoint of contamination control rather than processing time increases.

A. Pathogenic Bacteria and Other Micro-organisms Found on Fruits.

Numerous writers have shown that fresh fruits may be constantly associated with bacteria and other micro-organisms transferred by soil, water, currents of air carrying dust particles, insects, man, and other agents with which they have come in contact. The skin or rind of fruit itself serves as a lodging place for thousands--and even millions--of saprophytic or parasitic fungi.

Among the earliest investigators to identify fungi residing on fruit were Anthon and Hoffman (1860) who, examining the scrapings of a gooseberry microscopically, found large numbers of spores of Hyphomycetes such as Stemphylium, Cladosporium, Oidium, Monilia, and Torula. Some of the spores had even produced germinating filaments. Pasteur and his students (1876) examined the washings from a bunch of grapes under the microscope. They observed many organized particles, some of which closely resembled fungoid growths, others "alcoholic ferments", and still others Mycoderma vini. In 1881, Hansen demonstrated yeasts on grapes similar to those found in the soil of vineyards where they were believed to have wintered. In periods of dryness the yeasts were carried by wind, and in wet weather by soil splatterings, to the grapes. Hansen also stated that insects and other small animals probably played an important role in transportation.

Time and again fresh fruits have been incriminated as the source of disease-disseminating bacteria. Many investigators have shown that pathogenic bacteria might be present on the surfaces of fruits. Among the earliest workers to investigate this subject were Schnirer (1892), Ehrlich (1901), Rommel (1902), Clauditz (1904), Ressel (1907), Sartory and Fillassier (1909, 1910), Neuman

(1910), Abbott (1910), and Garcia (1911).

As early as 1892, Schnitzer isolated mycobacterium tuberculosis from grapes bought in a market in the vicinity of a clinic for tuberculous patients. More recently (1938), Giuffrida examined many fresh fruits and vegetables from markets and stores, but was unable to find Mycobacterium tuberculosis in any of them although he used concentration methods in his search.

Fruits sold in open markets in Paris were found by Sartory and Fillassier (1909) to harbor many micro-organisms, chief among which were Penicillium glaucum, Rhizopus nigrificans, Staphylococcus aureus, Streptococci, Bacillus termo, Bacillus subtilis, and Micrococcus candidans. They found that one washing removed a large proportion of the bacteria. Second and third washes removed smaller numbers. A year later (1910) the same workers examined the wash water from grapes, strawberries, and currants and reported a mixture of bacteria. They advised the covering of fruits in the markets and suggested placing these foods under regulation.

Abbott (1910), in the United States, found pathogenic bacteria and organisms of sanitary significance on grapes and berries from sidewalk stands. In another investigation of common fruits, Garcia (1911) found pathogenic organisms of the colon-typhoid-dysentery group on citrus fruits. Escherichia coli was always present on the surfaces of apples, lemons, oranges and bananas. This was confirmed by Herfarth (1921). Various fruits and vegetables purchased from dealers usually carried Escherichia coli and therefore must be assumed to be contaminated with fecal material.

Paquet (1923) reported twenty-four cases of typhoid fever resulting from the sweet cider of apples washed in polluted water.

The author cited a similar outbreak a few years before.

Guyot (1927) reported a viscous fermentation of lemonade.

The Medical Officers of Health of Cardiff, Wales (1927) found Bacillus anthracis wrapped around orange boxes in two separate shipments. Tanner (1932) says that "this organism might thus reach the fruit."

Several reports in the literature indicate possible development of Clostridium botulinum in fruits formerly thought to be safe because of their high hydrogen ion concentration. Meyer and Gunnison (1928) described an outbreak of botulism caused by Bartlett pears of pH 3.86. This was made possible by an accompanying development of a yeast and a lactic acid bacterium. Stone (1929) told of an outbreak of botulism caused by persimmons of pH 5.6. Toxin formation was observed by Wallace and Park (1933) when a yeast and an acid producing bacterium were growing in the medium. According to Tanner (1934) "development of sufficient toxin to kill guinea pigs in a few of the fruits has been explained by concomitant development of other organisms, especially molds, making it possible for Clostridium botulinum to grow."

Hoder (1928) carried out a series of examinations of different fruits obtained in the market and from street traders in Prague. Three cherries washed three times, gave plate counts of (1) 26,000, (2) 700, and (3) three, for the three respective washes. A single washing with water was shown to yield many different bacteria including: Bacillus proteus, Escherichia coli, Bacillus subtilis, Staphylococci, Streptococci, Sarcinae, yeast, moulds, and gram positive and gram negative bacilli. Hoder advocates washing all raw fruit at least once.

A severe case of colitis in a young child after eating packed dates prompted Hunwicks and Grinling (1930) to study the problem. "After obtaining a mucoid, Gram-negative bacillus in almost pure culture from the child's stool," they obtained a similar organism from six or seven of the boxes of dates. The authors remark that, when dates are packed in Europe, a solution of glucose is sprayed over them and it is possible that this is responsible for increased microbial viability on the fruit.

Of seventy-nine samples of dried fruits examined by Fellers (1930), eight contained lactose fermenting bacteria. Several of them closely resembled Aerobacter aerogenes. Anaerobes were found in over half of the samples but never in large numbers. Spore-formers were the most numerous while cocci--especially Sarcinae and Micrococci--were relatively abundant. Moulds were generally found, Aspergillus being the commonest form, followed in turn by Penicillium then Mucor. Yeasts were said to be uncommon. However, Tanner (1932) criticized the medium used and stated that dextrose agar would have permitted better development of yeasts. Smeall (1932) tested dates, grapes, and cherries to find the nature of organisms present on the fruits. The fruit was introduced into ordinary broth and incubated at 37°C (98.6°F). In sixteen examinations of Tunis dates the following organisms were isolated: Bacillus subtilis and allied organisms (16), Streptococci (16), yeasts (8), Escherichia coli (7), and Staphylococci (6). Grapes yielded a similar microscopic flora consisting of Bacillus subtilis and allied organisms (20), Streptococci (19), Staphylococci (6), and moulds (1). Cherries, from France (6) and from England (4), had: Bacillus subtilis (10), Streptococci (9), Staphylococci (6),

Diphtheroids (1), and moulds (1). On eleven occasions a "storm-clot" was noted in milk. Smell believed that this denoted Clostridium welchii but stated that he did not prove definitely whether such was the case. From these observations he concluded that gross contamination of fruit exists and, while the majority of surface contaminants are saprophytes, it is entirely possible that pathogens might also be found.

Johnston and Kaake (1935) washed eight lots of fruit from open markets in Toronto collected during the months of August and September 1933, with buffered saline. After centrifuging, part of the sediment was spread upon MacConkey- and blood-agar plates while the remainder was added to broth and incubated overnight. After incubation, a large selection of colonies which presented differences in appearance were identified by cultural tests. The bacteria on the skins of fresh fruit included staphylococci, proteolytic and alcaligenes bacilli, Bacillus dispar--one strain, Bacillus alkalescens--three strains, Aerobacter aerogenes--thirty-two, Bacterium coli communior--one, Bacterium coli communis--one, Shigella paradysenteriae Sonne-- (?) three.

Bharucha and Bharucha (1938) stated that cholera epidemics in India are said to be associated with watermelon season.

While other contributors have added to our general information regarding the contamination of fruit by various means, the examples given above should suffice in establishing the chance of disease being spread by unrecognized typhoid or dysentery carriers, by flies, or other insect vectors, by washing with polluted water, by unsanitary handling, or through the use of human excreta as fertilizer.

B. Survival of Pathogenic Bacteria and Other Micro-organisms Inoculated In or On Fruits

While many studies on the incidence of pollution indicators in fruits and fruit juices were made in the nineteenth century, little definite information on experimental inoculation of pathogenic forms previous to 1898 exists. In that year Bodin inoculated cider with one and one quarter million Eberthella typhosa per cubic centimeter. After twenty-four hours the number had dropped to 51,400. All of the organisms were destroyed in sixty hours. Bodin attributed this bactericidal property of cider to its acidity.

Dobroskowsky (1910) found that Vibrio comma lived three and one-half hours in the interior of the grape, but was not alive after twenty-four hours. The pathogen was not found alive after four days from the surface of the grape but was viable for twelve days on the stem.

Suspecting that fruits might harbor disease-disseminating bacteria and working with cucumbers and papaya fruit, Barber (1914) found in one experiment that cholera vibrios survived overnight on the cut surfaces. In a second he found that the vibrios survived twenty hours but failed to live forty-four hours. From a study made in Mesopotamia, Mackie and Trasler (1922) stated that slices of melon exposed for sale were "fraught with danger and should be avoided." They further remarked that in the East the idea is prevalent that outbreaks of cholera are related to consumption of certain fruits--particularly melons. That this idea might be well founded was shown by recovery of Vibrio comma for seven days and from cucumbers and melons three days after inoculation. From these results it seems that "melon pulp appears to be a particularly suitable medium

for the growth of cholera germs." Mackie and Trasler's experiments were paralleled by those of Bharucha and Bharucha (1938) in studies made in India. The latter workers found that the maximum period of survival of Vibrio comma when inoculated into a melon was four days. They gave evidence that the organism in question grew in watermelon at a pH 4.7 although the statement that it could not grow in nutrient broth at a pH lower than 5.6 exists.

In 1910, Thoni^H investigated sixty-five different lemonades and concluded that the bacterial content of fresh lemonade progressively decreased. Ko (1918) found lemonades to be decidedly germicidal.

The outside of various fruits and vegetables were studied by Russ (1916), Bindseil (1917), and Murillo (1919). The second investigator examined the inside of fruits as well. Russ sprayed cultures of Eberthella typhosa, Vibrio comma, Salmonella schottmulleri, and Shigella paradysenteriae (Flexner) on plums, peaches, lemons, apricots, and vegetables and examined them at intervals. S. schottmulleri was found to last the longest. It was followed in turn by Vibrio comma, Shigella paradysenteriae (Flexner), and lastly Eberthella typhosa. Lemon was said to have a bactericidal oil in its rind. The organisms were found to remain alive on the other fruits for several days. When other variables were controlled, Eberthella typhosa remained alive longer on apples than on plums and oranges. Salmonella schottmulleri preferred apples and peaches to plums and lemons. Russ claimed further that these pathogenic forms survived even longer on fruits mechanically cleaned because removal of saprophytic bacteria reduced competition. Other protective substances provided by nature may also have been removed. Murillo, in a somewhat similar study, sprayed fruits with cultures of Eberthella

typhosa and Vibrio comma. They remained alive for a considerable time. Dindseil, in an extensive study of the viability of Eberthella typhosa in foods and condiments, included a number of common fruits. In his inoculations a fresh culture of Eberthella typhosa was washed from nutrient agar with 10 ml of physiological salt solution and a loopful (5 mg) added to the food tested. That part of his findings dealing with fruits has been extracted from Tables V and VI

Period of Observation (in Days)

		2	4	6	8	10	12	14	16	18	20	25	30	35	40	45	50	55	60	65	70
Tomato	*o.s.	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	-		
	*c.s.	x	x	x	x	x	x	x	-	x	-	-	-	-							
Apple	o.s.	x	x	x	x	-	-	x	x	x	x	-	-	-	-	-	-	-	-	-	-
	c.s.	x	x	x	x	x	x	-	x	-	-	-	-	-	-	-	-	-	-	-	-
Pear	o.s.	x	x	x	x	x	x	x	x	x	x	-	x	-	-	-	-	-	-	-	-
	c.s.	x	x	x	x	x	x	x	x	x	-	-	-	-	-	-	-	-	-	-	-
Grape (white)	o.s.	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	-	-	-
	c.s.	x	x	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Grape (blue)	o.s.	x	x	x	x	x	x	x	x	x	x	x	x	$\frac{x}{-}$	x	x	-	-	-	-	-
	c.s.	x	x	x	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cherry (sweet)	o.s.	x	x	x	x	x	x	-	x	-	-	-	-	-	-	-	-	-	-	-	-
	c.s.	x	x	x	-	-	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cherry (sour)	o.s.	x	x	x	x	x	x	-	x	-	-	-	-	-	-	-	-	-	-	-	-
	c.s.	x	x	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Orange	o.s.	x	x	x	x	x	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-
	c.s.	x	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Straw- berry	o.s.	x	x	x	-	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	c.s.	x	x	x	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lemon	o.s.	x	x	x	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	c.s.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Plum	o.s.	x	x	x	x	x	x	x	x	x	-	-	-	-	-	-	-	-	-	-	-
	c.s.	x	x	x	x	x	x	-	-	-	-	-	-	-	-	-	-	-	-	-	-

*o.s. signifies outer surface; c.s. stands for cut surface.

Generally it was found that the survival of Eberthella typhosa was more prolonged on the outer surface than on the cut surface. Birdsall stated that the acidity of the fruit juice was distinctly bactericidal. It may be seen from the above table that Eberthella typhosa could be recovered from the cut surfaces of tomatoes and pears for eighteen days, on apples for sixteen, on sweet cherries and plums for twelve days, on strawberries and blue grapes for ten, on white grapes and sour cherries for eight, and on oranges for six days. Eberthella typhosa was killed in less than forty-eight hours on lemons. These same organisms remained alive for forty days on the outer surface of figs and raisins, and for thirty-five days on the skin of prunes.

A year later Ko (1918) stated that most fruit juices, which are acid, exert a bactericidal action on Eberthella typhosa and that the juice of half-ripe fruit usually is more efficient than that from fully ripe fruit. Ko ranked the organic acids in order as to bactericidal properties, tannic acid being the strongest, followed in turn by citric, then tartaric, and finally, mallic. His list of fruits whose juices did not favor the viability of Eberthella typhosa included: Ananas sativus, Pisidium gayava, Eugenia javanica, Diospyros kaki (persimmon), Musa sapientum, Nephelium litchii (litchii nut), Vitis vinifera (grape), Prunus persica (peach), Prunus communis, Citrus decumana, Myrica rubra, Averrhoa carambola. Fruits whose juices did not kill typhoid organisms were: Nephelium longana (longan fruit), Citrus nobilis (navel orange), Eryobotrya japonica (Biwa), Mangifera indica (mango), Citrullus vulgaris (watermelon), Eigen cariae, Pyrus sinensis (pear). The above statements seem rather meaningless unless definite temperatures and periods of

incubation are considered.

Alliot (1919) added five cc of twenty-four hour cultures of Eberthella typhosa, Salmonella paratyphi and Salmonella schottmuelleri to a liter of sterile lemon juice and made transfers to nutrient broth at five minute intervals. He found that it took more than fifteen minutes to destroy Eberthella typhosa and Salmonella paratyphi, and more than twenty minutes to kill all Salmonella schottmuelleri.

Koch's silk thread method was employed by Morel and Rochaix (1922) for studying the effect of certain essential plant oils on the meningococcus, Eberthella typhosa, staphylococcus (golden), Corynebacterium diphtheriae, and Bacillus anthracis spores. The oils used included: lemon, thyme, orange, bergamot, juniper, clove, citron (southernwood), lavender, gun, mint, rosemary, eucalyptus, sandalwood and star anise. Threads impregnated with pure cultures of one of the organisms tested were suspended in test tubes containing three ml of one of the essences. The meningococcus was the most sensitive. It lasted less than fifteen minutes in the first four oils. Eberthella typhosa did not live more than one hour in lemon, thyme, or orange. Staphylococcus lasted two hours in lemon, five in thyme, and seven in orange oil. Corynebacterium diphtheriae was viable for 7, 24, and 24 hours respectively, while the anthrax spores were not destroyed at all. The vapors from lemon, thyme, and orange essences exhibited the most bactericidal action.

Faquet (1923) carried out experiments to determine how long members of the typhoid group survived in cider. He found that Eberthella typhosa and Salmonella paratyphi were destroyed within a week but that Salmonella schottmuelleri could live longer.

Three of the most important intestinal pathogens, Vibrio comma, Shigella dysenteriae, and Escherichia typhosa, were tested by Vasquez-Colet (1934) in fruits from the public markets of Manila. The raw fruits used included banana, lanzones, apple, guavo, mango, and chico. Pure cultures of the bacteria were emulsified in water and introduced into the wide-mouthed bottles containing the fruit by pipette. The bacteria were also smeared on the outside of some of the samples by means of sterile swabs. Bananas, chicos, and lanzones still harbored Vibrio comma in their juices at the end of one day. On the cut surface of guavas they survived at least five hours, while infected apples were still positive for Vibrio comma after six days. Results of examinations of material from the outside of the fruits showed the cholera organisms to last for two days on bananas, for one day on lanzones, and for four days on apples. Escherichia typhosa lived on the cut surface of mangoes, bananas and guavas at least one day, on chicos two days, and on lanzones seven hours; on the cut surface of apples it remains alive for at least three days. The organism survived on the outside of apples and lanzones for seven hours and on bananas for six hours. Experiments with Shigella dysenteriae (Shiga and Flexner) showed this group to be easily destroyed by the fruit juices. The Flexner strain lasted four hours on the cut surface of mangoes, one hour on bananas, three hours on chicos, less than an hour on lanzones, three days on apples, and on guavas one day. On the outsides of these same fruits Shigella dysenteriae lasted six or seven hours. The Shiga strain did not thrive either on the cut surface or the outside of apples, bananas, guavas, or lanzones. No bacilli could be recovered one hour after inoculation.

Douglas, in 1929, reported that Gram-negative intestinal bacteria produce acid until a maximum hydrogen ion concentration has been reached. The pH was constant regardless of the number of cells introduced initially. According to Douglas and Edin (1930), Escherichia coli was killed in fluids having an acidity greater than pH 4.2. Eberthella typhosa and Salmonella paratyphi died on the second day in peptone broth of pH 4.3 but grew in tubes with pH 4.5 and 4.7. The bactericidal action of various fruit juices was tested by placing about 2,000,000,000 cells of each organism in two teaspoonfuls of the different fluids with the following results:

	<u>Eberthella</u> <u>typhosa</u>	<u>Salmonella</u> <u>paratyphi</u>	<u>Salmonella</u> <u>schottmuelleri</u>	<u>Escherichia</u> <u>coli</u>
Orange juice (pH 3.2-3.4)	9-22 hrs*	9-30 hrs	48-120 hrs	48-120 hrs
Peach Juice (tinned) pH 3.6	30 hrs	30 hrs	72-120 hrs	72-120 hrs
Lemon Juice-ph 2.0	5-9½ min	3 hours	3 hours	6 hours
Apple Juice-ph 3.5	48 hrs	24-30 hrs	not in 48	not in 48

(*figures given represent time necessary to kill all cells).

The authors state that, while it took six hours to kill Escherichia coli, there was very great diminution of the number after three hours. Undiluted lemon juice destroyed Eberthella typhosa in from 5 to 9½ minutes while dilutions of 1 in 2 and 1 in 4 were sterile after fifteen minutes. Lemonade made from the juice of one lemon, two teaspoonfulls of sugar, and ten ounces of water (pH 2.8) killed E. typhosa, S. paratyphi, S. schottmuelleri, Shigella dysenteriae Shiga, and S. paradysenteriae Flexner in ten hours. E. coli was not completely destroyed. Staphylococci were not viable after five hours. Several experiments were conducted using apple pulp boiled with sugar and without sugar. There was very little difference in results. Shigella dysenteriae Shiga was dead in ten hours. Shigella paradysenteriae Flexner and Eberthella typhosa did not last forty

eight hours. Also, subcultures showed only slight growth after ten hours. While S. paratyphi was quite sensitive to the bactericidal action of apple pulp, Escherichia coli and S. schottmuelleri were able to live longer than forty eight hours.

Sixteen fruit juices and nineteen condiments were studied by Tetramoto in 1934. Juices having strong sterilizing power were "Yuzu", lemon, madarin orange and strawberry. Of these, "Yuzu" was the most bactericidal. The sterilizing power of the fruit juices was said to be due to the action of fatty acids such as citric, l-malic, and d-tartaric. Essential oils and a few glucosides were likewise considered as possible germicides.

In a study of the growth necessities of Clostridium botulinum Cameron and Lesty (1940) found that the dividing line of acidity between nutrients in which the bacterium would grow and those in which it failed to reproduce was about pH 4.5. Also, when there was insufficient nutriment, growth was inhibited regardless of hydrogen ion concentration. Spiegelberg (1940) conducted similar studies on Clostridium pasteurianum. A pH tolerance of 4.4 was found to be the minimum with no butyric spoilage below that point. He states that "less than pH 4.5 is the point below which spore-forming bacteria cease to be factors in spoilage and it becomes unnecessary to use a process sufficiently severe to destroy them". Verification of these results would be of great importance in industry since products under pH 4.5 could then be safely processed in the open bath (100°C or 212°F) without pressure. It must be borne in mind, however, that spores may remain viable. Bigelow and Cameron (1932) considered destruction of the vegetative cells as "effective" because of the inability of the spores to germinate.

Tests were performed by Rochaix and Jacqueson (1938) to show the bactericidal power of grape juice upon Eberthella typhosa, Salmonella paratyphi, Salmonella schottmuelleri, Escherichia coli, Vibrio comma, and Shigella dysenteriae (Shiga). The time required for each juice to kill is recorded in the following table:

Organism	in red grape juice	in white grape juice
<u>Eberthella typhosa</u>	45 - 75 minutes	15 - 75 minutes
<u>Salmonella paratyphi</u>	15 - 60 minutes	15 - 45 minutes
<u>Salmonella schottmuelleri</u>	1 hr-24 hours	15 - 90 minutes
<u>Vibrio comma</u>	1 hr-2½ hours	1 hr-2½ hours
<u>Escherichia coli</u>	1 hr-28 hours	1 hr-2½ hours
<u>Shigella dysenteriae</u> (Shiga)	5 hr- 7 hours	5 hr- 7 hours

According to Rochaix and Jacqueson the juice of white grapes is slightly more bactericidal than red grapes but the difference is not marked. In one experiment on red grape juice a long interval elapsed between tests. Shigella dysenteriae (Shiga) was found the most resistant to the action of grape juice.

Vendramini (1939) artificially infected strawberries with Eberthella typhosa and found that the fruit was not rendered sterile by short contacts with wine or with lemon juice. According to tables included in the report, less than ten colonies were present after one hour and none were found two hours after inoculation.

From these reports one can see that there is ample reason to confirm the belief that raw fruits may convey potentially dangerous organisms unless proper precautions are taken. While the juices of some fruits are germicidal, reliance cannot be placed entirely on them to keep a fruit product safe.

C. The Effect of Temperature on Bacteria

Literature relating to the effect of temperature on bacteria is quite exhaustive. Many of the reports do not agree. The great difference of opinion among earlier investigators may be attributed in part to variations in environmental factors such as minerals and foreign matters in the crystallized water, dissolved oxygen, pressure, pH, size of crystals, temperature of the frozen suspension, time required to change from fluid to solid state, and cultural medium used to test viability following incubation. Quantitative tests were not commonly used to test survivals. Hence the few survivors making living cultures possible after a frozen suspension had been thawed were the sole criterion used for measuring resistance to temperatures below 0°C (32°F). The size of the inoculum was often disregarded as was variation among strains and species of bacteria of the same kind. More recently, investigators have taken these factors into consideration with more favorable agreement.

Survival of a living organism is influenced by the temperature at which the experiment was performed. This fact was realized by H. Marshall Ward (1895) in his study of Bacillus ramosus, an organism which he had isolated from the Thames River. At the optimum temperature growth was very rapid and lasted for a long time while at a higher temperature growth, "though at first as rapid as at the optimum temperature, lasts for a shorter and shorter time, according as the temperature is further and further removed from the optimum." Ward inferred from experiments at higher temperatures that growth occurs at a very slow rate at some minimum temperature near 5°C (41°F) or 6°C (42.8°F) and the germination time rapidly shortens for each degree rise to 12° to 15°C (53.6° to 59°F) and then falling

more slowly at each step as the temperature became more favorable. Barber (1903), using Escherichia coli in a similar study, obtained similar results. Lane-Clayton (1909) noticed the Escherichia coli maintained a maximum rate of reproduction of $7\frac{1}{2}$ hours at 37°C (98.6°F), for 8 to $9\frac{1}{2}$ hours at 35°C (86°F), for 12 to 15 hours at 25°C (75.2°F), and for 20 to 24 hours at 20°C (68°F). However, the temperature coefficient for every rise of ten degrees centigrade was found to be 2.2. The work of Chick (1912), using the same organism (E. coli) at temperatures between 20° and 40°C , supports Lane-Clayton's findings. Chick found that between these two limits, each rise of 10° Centigrade increased the rate of growth by 2.01.

Graham-Smith (1920), using Staphylococcus aureus at 17° , 27° , and 37°C (62.6° , 80.6° , 98.6°F), confirmed Ward's findings and, in addition, found that the largest "crop" was secured at a temperature lower than the one at which more luxuriant growth was obtained in shorter time. The organism grew faster but soon exhausted itself.

While there is a limit in the temperature scale upward wherein the protein in the cell coagulates or at which metabolism proceeds at an irreplaceable rate, it is not known whether a critical temperature exists in the scale downward at or near the freezing point. If the same temperature coefficient holds at 0°C (32°F) (just above the freezing point) as at the range from 20° to 40°C (68° to 104°F), then Escherichia coli should be able to reproduce sixteen times more rapidly at 40°C (104°F) than at 0°C (32°F). Or, if reproduction requires one-half hour at 40°C (104°F), the same cycle would need eight hours for completion at 0°C . Also, if the maximum rate of reproduction at 40°C is maintained for 7 hours, the period of logarithmic increase would be lengthened to 112 hours at 0°C .

Lane-Guyon (1900) found that Escherichia herbacea grew more slowly than Escherichia coli and that the period of logarithmic increase persisted longer at all temperatures.

The comparatively rapid death of bacteria in non-nutritive materials at higher temperatures agrees well with the theory of simple starvation or destructive metabolism according to Keith (1913). At the higher temperatures they perish quickly because they burn themselves out quickly; at lower temperatures metabolism is retarded, and at temperatures where metabolism ceases altogether they continue to exist in a state of suspended vitality similar to that of higher plants in the far north to temperatures much below 0°C (32°F).

Inasmuch as many of the frozen fruit juices, especially apple juice, grape juice, lemon juice, and grapefruit juice, are quite watery, it will be well to review some of the pertinent findings on the survival of bacteria in water.

Studies on resistance of bacteria to low temperatures can be divided into two groups according to Wallace and Tanner (1933) who state that the first group of studies demonstrated that bacteria were quite resistant to low temperatures while reports starting in about 1900 showed that bacteria were very easily destroyed by low temperatures. Present-day observations indicate that the earlier reports were probably the more accurate.

In 1870 Madsen believed that bacteria were not destroyed by low temperatures. Cohn (1872), Göppert (1875), Pictet (1893), and Meyer (1900) found that neither spores nor vegetative cells of yeasts or bacteria were killed by several hours of exposure to temperatures ranging from -17.5° to -200°C (0° to -328°F). In

1884, Pictet and Joung exposed Micrococcus lutea and vegetative cells of Bacillus anthracis to temperatures of -70°C (-94°F) for 100 hours and -130°C (-202°F) for twenty hours. These tests resulted in the death of vegetative cells but spores of Bacillus subtilis, Bacillus anthracis, and Bacillus ulna exposed to the same conditions were not affected.

In an Annual Report of the Secretary of the State Board of Health of Michigan (1884), Pengra claimed that 90 per cent of the bacterial population were destroyed by freezing. However, he based his claims on observations that were subject to many variables. Fränkel (1886) likewise observed a great reduction in number of bacteria in ice during the first few days of freezing, but, after that time, reduction proceeded much more slowly. He remarked that ice from badly polluted water would probably contain living bacteria.

Prudden (1887) froze pure cultures of Eberthella typhosa, Serratia marcescens, an organism like Proteus vulgaris, Staphylococcus aureus, and a fluorescent bacillus. After exposure to temperatures ranging from -10°C (14°F) to -1.1°C (30°F) for fifty-one days, the Proteus vulgaris like organism and Serratia marcescens could no longer be recovered. Staphylococcus aureus, Eberthella typhosa, and the fluorescent bacillus were quite resistant. Large numbers remained viable after 66, 77, and 103 days respectively. In a comparative study of cells kept constantly frozen for twenty-four hours to the same number (40,896/cc) alternately thawed and frozen three times during the same period, Prudden found over 29,780 cells per cubic centimeter remaining in the first suspension while only 90 per cubic centimeter were still alive in the second. By oiling the sides of the tube to prevent crystallization, the same

investigator was able to compare moist cold with dry cold. He counted 81,940 cells per cubic centimeter in the frozen tube and 16,400 in the unfrozen tube. From this data he concluded that moist cold was more detrimental. Keith (1913) and Hilliard and Davis (1918) could not confirm this work.

From a study of the bacterial flora of snow, Janowski (1888) noted that continued low temperatures had little effect on bacteria. Forster (1892) stated that the kinds of bacteria able to grow at 0°C (32°F) were widely distributed though not very numerous.

Klepzoff (1895) believed that long exposure of Bacillus anthracis to low temperatures reduced its virulence. While bacteria in emulsions of blood or various organs were killed in twelve days at -24°C (11.2°F), colonies on agar plates were viable until the twenty-fifth day. Kasansky (1899) said that cultures of Corynebacterium diphtheriae and the "pest bacillus" exposed for five months to temperatures ranging from -10°C (14°F) to -30°C (-22°F) were not completely destroyed. Working with pure cultures of Eberthella typhosa, Sedgwick and Winslow (1900) found that thirty to sixty per cent of the bacteria were destroyed by one hour of freezing and that after two weeks of continuous exposure to sub-freezing temperature, 99 per cent were killed. Alternate freezing and thawing were little more harmful than continuous freezing. Park (1901), found 14 per cent alive at the end of one week in ice at -5°C (23°F) and none after twenty-two weeks. Using liquid air temperatures, he found that his cultures were alive and virulent after an exposure of over two hours.

According to MacFadyen (1900) cultures of some of the common parasitic and saprophytic bacteria were unharmed by hours and even

by months of exposure to -190°C (-310°F) or -252°C (-421.6°F).

Staphylococcus aureus retained its fermentive and pigment-producing power as well as its pathogenicity after an exposure of six months. MacFadyen believed that cells exposed to extreme cold were unharmed because they were in a state of suspended animation. Smith and Swingle (1905) stated that some cells in a culture survived -190°C (-310°F), but they represented only a small proportion of the number originally present. They considered 0°C (32°F) as the critical temperature and thought that an organism able to resist this temperature was also able to withstand absolute zero.

Jordan, Russel and Leit (1904) suspended Eberthella typhosa in river water and allowed it to freeze at average winter temperature. After one day only two bacteria were found in a five milliliter sample and none were recovered after two days.

Spark's (1908) results were determined quantitatively using cultures of Escherichia coli, Eberthella typhosa, a sewage streptococcus, and an anaerobic spore-former embedded in ice. The non-spore-formers were reduced by 99 per cent within two or three weeks, but some of the suspensions still gave growth until the twentieth week. The spore-forming anaerobe was found in over 1 per cent until the seventh week but was sterile after twenty weeks. Sparks noted that some strains of Eberthella typhosa died out in five weeks while others required sixteen weeks. Reudiger's findings (1911) using cultures of Escherichia coli and Eberthella typhosa showed the coliform organism was destroyed by eight weeks storage in ice while Eberthella typhosa could not be recovered after one month and only one in a thousand lived longer than eight days.

The results of Winslow and Sedgwich (1900), Sparks (1908), and Keith (1913) are in close agreement regarding the longevity of Escherichia coli and Shorthella typhosa. Keith held cultures of Escherichia coli frozen in Boston tap water as solid ice at -20°C (-4°F). After five days only a fraction of one per cent remained alive and after a few weeks of storage the bacteria were completely destroyed. When E. coli was not solidly frozen but was frozen as a water-ice and held in this condition at -20°C (-4°F), a large proportion remained alive for months. Keith also froze E. coli in milk and in milk diluted with varying amounts of water. The death rate increased with the dilution. From this Keith concluded that "the more watery food materials, when frozen, contain the fewest, and the least watery the most, living bacteria." Keith discussed the probability that water-bearing food materials as well as sugar solutions and the like freeze in such a manner that most of the bacteria and other non-watery matters are extruded from the water crystals and "lie in or among these matters without being crushed or injured." In pure water suspensions, the whole mass becomes solidly crystalline and the particles and bacteria are caught and are "ultimately mechanically destroyed between the growing crystals."

In a comprehensive study of the effect of freezing on bacteria, Hilliard, Terossian, and Stone (1915) discussed various factors which influence the longevity of bacterial suspensions: (1) species and strain of bacteria used, (2) history and cultural manipulation prior to freezing, (3) chemical and physical composition of the medium in which the organism is frozen, (4) temperature of the frozen suspensions, (5) duration of freezing, (6) abruptness of

temperature change, and (7) the cultivation of the organism subsequent to freezing.

Hilliard, Torossian, and Stone found that 93 per cent of the total number of Escherichia coli cells were killed within three hours as compared to a maximum reduction of 80 per cent of Bacillus subtilis. Intermittent freezing was only slightly more injurious than extended freezing for the same length of time, but a temperature of -15°C (5°F) proved more fatal than -2°C (28.4°F). Boston tap water and distilled water gave strikingly similar results, but cream gave a great deal of protection. In the latter medium alternate freezing and thawing was found to be much more destructive than continuous freezing. They stated that the ways in which bacteria may be killed by freezing are: (1) low temperatures interfering with metabolism, (2) freezing of the cell contents and rupturing of the cell by internal pressure, (3) by grinding and external pressure resulting from crystallization, and (4) by death of old age or starvation. Later Hilliard, working with Davis (1918), reversed some of his previous findings. They noted that intermittent freezing was much more injurious than continuous freezing and that the degree of cold was not very important. There was no critical temperature below which death followed rapidly. The death rate of Escherichia coli was much higher in solidly frozen media than in the same material not solid and at a lower temperature. They concluded that destruction is caused by mechanical crushing since the greatest decrease occurs promptly upon freezing and refreezing.

Vass (1919) accounted for increases in numbers after freezing of soil bacteria as due to the breaking up of clumps rather than to any ability to reproduce in temperatures below 0°C (32°F).

D. Survival of Bacteria and Yeasts Frozen in Fruit and in Fruit Juices

While papers dealing with the survival of organisms at low temperatures can be found in great abundance, references pertaining to the microbial content of frozen fruits are not so easy to find. In 1910, Gore studied the growth of micro-organisms in fresh cider held at 0°C (32°F). No signs of fermentation were noticed for fifty days and one hundred twenty-five days elapsed before the apple-juice fermented sufficiently to be considered "hard" or "sour".

Joslyn and Irish (1929) suspended Burgundy wine yeast and a California wine yeast in grape juice sugar solutions and froze them at from -8°C (17.6°F) to -25°C (9.4°F). Samplings after eighteen weeks showed the organisms to be as active as they were at the start. They state that Creuss was able to demonstrate yeast survivors over a period of four and one half years in a fermented fruit juice.

Tohyana (1930) suspended Vibrio comma in different fluids and froze at -70°C (-94°F). He found that the resistance of the organism varied as the substrate was changed. Eberthella typhosa was mixed with various substances including fruits and vegetables and then exposed to outdoor temperatures (-3.5°C or 25.7°F). Eberthella typhosa remained alive in strawberries only four days, in bananas eight days, and in oranges ten days.

From a study of commercially packed frozen strawberries, Magoon (1930) observed that 19,000--800,000 micro-organisms per gram of material entered the pack. Two-thirds of these were bacteria. In a study made in 1932, he found that exposure of foods to freezing temperatures resulted in heavy mortality during the earlier period of the treatment but that some species were able to survive in tens,

hundreds, and even thousands over long periods. Magoon stated that "freezing, like cooking, brings about certain physical changes in the tissues that permit and favor microbial development."

Tanner and Wallace (1931) determined the survival times of eighteen pure cultures of micro-organisms frozen in a number of menstrua including several fruit juices. A low pH was shown to be detrimental to the survival of bacteria especially. There was complete destruction of non-spore forming bacteria held at -16°C (3.2°F) in acid media within five to ten months. Alkaline media were not found to be so highly unfavorable. In 1933, Wallace and Tanner used pure cultures of a number of organisms isolated from fruits and other sources as well as several common forms whose etiology had been determined. Each of these forms was inoculated into sixteen different substrates. The suspensions were sealed in 2 cc sterile ampoules and stored at -23.3°C (-10°F). Each month an ampoule was thawed and plated on agar. In general, the acidity of the medium influenced the rate of decrease. Cherry and strawberry juice had a greater detrimental effect than did nutrient broth of pH 4.0. The writers suggested that this difference in effect may have been due to "some inhibitive property other than that due to acidity." Escherichia coli was found to survive three weeks but not four in cherry juice when held at -16°C (3.2°F), but it remained viable for at least six weeks at -40°C (-40°F). Alternate freezing and thawing were not found to be any more destructive than continuous freezing, but freezing in cherry juice was found more harmful to the bacteria than was freezing in water. Molds and mold-like organisms were more resistant to an acid-cold combination than were the bacteria. Temperatures of -40°C (-40°F) and -80°C (-112°F) were less

destructive on microbial life than was -16°C (3.2°F).

Fellers (1932) froze various foods at -23.33°C (-10°F) in sealed tin cans and stored for twelve months at -12.22°C (10°F). Fruits packed with sugar had the fewest organisms of all. Upon thawing, the greatest increase occurred in the fruit frozen without sugar. No bacteria were found in the frozen sour cherries after one year in frozen storage.

Certain foods, such as frozen orange juice, stored at temperatures of -6.6°C , -12°C , and -18°C (20°F , 10.4°F , and -0.4°F), were found by Prescott, Bates and Highlands (1932) to decrease more rapidly and to a greater extent at the higher temperatures. They believed that this may have been due to the lack of protective action which extreme cold gave to those at lower temperature against the unfavorable environment provided the organisms by the foods.

Beard and Cleary (1932) think that a hydrogen ion concentration rapidly bacteriocidal at ordinary temperatures might allow the survival of organisms over appreciable periods at low temperatures. For their experiment, they selected strains of Eberthella typhosa, Shigella paradysenteriae (Hiss and Flexner), Shigella dysenteriae (Shiga), Salmonella schotmuelleri, Salmonella paratyphi, Staphylococcus aureus, and Staphylococcus albus. In a preliminary experiment, Eberthella typhosa strains were more resistant to nutrient broth at a pH 3.5 than were the Salmonella, Shigella, or Staphylococcus groups, but all strains were killed within twenty-four hours. Eberthella typhosa and Shigella dysenteriae survived for one hundred seventy hours in orange juice held at -4°C (24.8°F). Salmonella paratyphi was viable for three days and Salmonella schotmuelleri strains lasted four days. The staphylococci survived fifty hours.

In general, the survival time in orange juice was of longer duration than in broth of the same pH--a fact attributed by Beard and Cleary to differences in the viscosity of the menstruum. No data was given concerning the period of viability of organisms in orange juice at room temperatures or so-called "ordinary temperatures."

James (1932) found that pathogens contaminating frozen foods, i.e., Eberthella typhosa, Salmonella paratyphi, etc., were mostly destroyed by freezing and were all killed if frozen for a sufficient length of time. Toxin-producing anaerobes still carried the spores after defrosting but, under usual conditions of handling, neither vegetative cells or spores developed nor were toxins produced. The toxin produced by Clostridium botulinum was not readily destroyed by freezing. Wallace and Park (1933) were of the opinion that fruits properly canned and used immediately after defrosting were not dangerous, but if allowed to thaw, this condition might be reversed. However, Berry (1932) considered the possible danger of botulism from thawed frozen foods as exaggerated since: (1) the food would show visible decomposition and (2) frozen foods are usually cooked before eaten. A year later (1934) Tanner argued against these two points on the basis that they were not commonly practiced.

Berry (1932) found that the chief organisms in a large number of canned cherries, strawberries, and raspberries were Penicillium, Fusarium, yeasts, and spore-forming bacteria in varying percentages numbering between 7,500 to 100,000 per gram. After freezing for a year, the number of viable cells was reduced by 90--99 per cent. In most cases only a few hundred Penicillia and spore-forming bacteria--including Bacillus mycoides--were yielded per gram. Berry (1932), in comparing berries frozen at -9.44°C and -15°C (15°F and

-5°F), found that the higher temperature was more bactericidal.

Scott and Le (1935) to determine whether there is more rapid destruction in frozen pack berries at 15°F than at -5°F confirmed this observation. Both carbonated cans and uncarbonated cans contained more organisms when stored at -5°F. Cans held at 15°F for three months had comparable flora with those kept at -5°F for twelve months.

Haines (1933) believed that the lower limit for bacterial growth is about -3°C (26.6°F), and for molds about -7°C (19.4°F). He suggested -10°C (14°F) as a safe storage temperature. Bacteria with an optimum temperature of 37°C (98.6°F) cease to grow or grow very slowly within the range 5°C to 0°C (41°F to 32°F). Bacteria were more rapidly destroyed a few degrees below the freezing point than at a lower temperature. Magoon found (1932) that exposure of food to freezing temperatures resulted in a heavy mortality during the earlier period of the treatment, but that some species were able to survive in tens, hundreds, and even thousands, over long periods of time. Berry (1934) described the development of a cold tolerant yeast at -2.2°C (28°F). In the same year Berry and Diehl noticed a rapid increase in numbers of organisms in frozen cider (-5°C or 23°F) at the end of one month which amounted to ninety percent or more. After this period the rate of destruction proceeded at a progressively slower rate. They did not find that cider became sterile from prolonged freezing.

Smart (1934) found that yeasts, molds, and bacteria could survive at -9.44°C (15°F) for from one to three years or longer in frozen foods. She stored twenty-six species for one year and reported (1935) that eight had produced growth at -8.9°C (16°F). In all probability Fruit Products Journal was referring to this work in a

citation (1936) that "Bacteriologists of the Bureau of Plant Industry found several species of bacteria, yeasts, and molds still alive after three years" at 15°F in frozen strawberries, raspberries, and cherries. To study the behavior of these microscopic fungi at low temperatures, they placed twenty-six cultures in storage at -3.9°C (16°F) for one year. Eight of the twenty-six were able to grow, thirteen, while showing no reproduction during refrigeration, produced abundant growth when removed to room temperature for twenty-four hours, and only five failed to last the year.

Wallace and Park (1933) used cultures of Escherichia coli, Proteus vulgaris, Salmonella aertrycke, Salmonella schottmuelleri, and Lberthella typhosa frozen in cherries and cherry juice at -17.8° and -40°C (0° and -40°F). In cherry juice, Lberthella typhosa did not last two weeks. The others, with the exception of Escherichia coli, were destroyed before three weeks. E. coli died after a storage of three weeks. Wallace and Tanner (1934, 1935) conducted similar experiments with approximately the same results. Juices were found to become sterile much more rapidly than fruits plus juice. Tanner (1934) summarized the findings of a number of microbiological examinations of fresh and frozen fruits and vegetables.

A histological study of growing stems, mature fruits, immature seeds, and young leaves of a number of fruits and vegetables was made by Woodroof (1938) in order to account for the effect of freezing. He found that by slowing down the rate of freezing, ice crystals were increased by as much as five hundred times. Presence of large ice crystals within tissue was said to result in crushing, rupturing, and distortion of cells. When crystals were small enough to be contained within plant cells, destruction was at a minimum.

That few micro-organisms were to be found in frozen fruits was attributed by Smart (1939) to high acidity and to sugar in which they were packed acting as inhibiting substances to the growth of bacteria.

McFarlane (1940) showed the similarity in redistribution patterns of yeast cells, sucrose, and hydrogen ions in water frozen at -20°C (-4°F). When approximately neutral solutions or suspensions were frozen, yeast cells survived in greatest numbers in those areas highest in sucrose. This was not true in a frozen acidified medium in which the area of high sucrose concentration was also the area of high hydrogen ion concentration. In "Malt quarts" of frozen cider microbial forms, soluble solids, and total (titratable) acids were concentrated in central cone-shaped areas and in horizontal surface areas. No marked freezing redistribution was observed in the case of frozen syrup-packed raspberries or brine-packed peas. Hydrogen ions were not appreciably redistributed in the three foods. In frozen cider at -20°C , viable micro-organisms decreased most rapidly in sections high in soluble solid content, while in samples at -10°C (14°F) the greatest number of viable cells after thirty-one weeks was found in the unfrozen concentrate. This concentrate had approximately six times greater soluble solid and total acid than normal cider.

In a study of the effect of freezing on the viability of pathogenic organisms, McCleskey and Christopher (1941) found that commercial pack, quick-frozen, sliced and sweetened strawberries did not destroy either Eberthella typhosa or Staphylococcus aureus in five months storage at -18°C (-0.4°F), but Salmonella paratyphi was not recovered two hours after freezing. When strawberries were packed

whole with addition of twenty percent sugar and placed in storage before all sugar was dissolved without mixing, the test organisms were recovered over a period of fourteen months in small numbers. The writers account for greater longevity of this pack over sliced berries since organisms are not immediately subjected to acid juice and sugar. When organisms of the intestinal group were added to berries at room temperature, more than ninety-nine per cent died within five hours. Inoculated berries held at 4° to 5°C (39.2° to 41°F) showed rapid destruction, but viability was longer than at room temperature. McClesky and Christopher advocated the use of fifty percent sucrose diluting fluid since the high concentration of sugar contained in the berries may have caused plasmoptosis of cells when a hypotonic medium - such as water - was used in dilutions to give quantitative estimates of the numbers of cells of Eberthella typhosa or other pathogenic organisms present. They report much higher survivals and more accurate checks with sucrose diluting fluid than with water blanks. This study was confirmed by McFarlane (1942) insofar as the preservative action of sucrose was concerned. Under identical storage conditions, destruction was found to be much greater in unsweetened than in sweetened frozen berries and berry juices. At -17.8°C (0°F), McFarlane states that there may be quantities of unfrozen highly concentrated fruit juice-sucrose sirup and that, when the mass thaws, moisture is made available which may dilute the sucrose and make growth of micro-organisms possible. The sucrose concentration was thought to be high enough in some sections to offset any germicidal action of the fruit acids and, when the temperature was favorable, allowed rapid growth of the micro-organisms.

E. Effect of Hydrogen Ion Concentration and Titratable Acidity on Micro-organisms.

Knowledge of the effect of acidity, both total and dissociated, is requisite for an ultimate understanding of microbial longevity in fruit juices. Early workers reported all of their findings in terms of per cent acid. Then followed a period in which investigators confined their attention to the amount of initial free hydrogen ions in the solutions without considering the buffering action of neutral salts in the solutions. Within the last twenty years the undissociated molecule, or the anion part of the electrolyte, as well as available hydrogen ion concentration has been considered. Neither the determination of pH or titratable acidity, when used alone, adequately explain the difference between a heavily buffered juice of high pH and one of a lower pH but only slightly buffered. Also, the organic radical or anion forming the remainder of the total molecule should be determined before definite evidence is possible on the effect of any menstruum in which the organism is grown.

For one of the first references to the effect of acidity on micro-organisms, the classic work of Pasteur (1876) must be cited. Pasteur noted that the relatively high acidity of "must" favors a natural alcoholic fermentation in wine, while the low acidity of wort made brewing of beer difficult. Study of the disinfecting power of the hydrogen ion may be said to have begun with the work of Pick, who, in 1893, determined the influence of a few organic acids on Eberthella typhosa and Vibrio comma. Two, three, and five per cent concentrations of formic acid, acetic acid, tartaric acid, and citric acid were employed. Their bactericidal properties were in the order named. Solutions of citric acid in concentrations of

1-1000 to 1-100 were ineffective as disinfectants for drinking water. Kahlenberg and True (1896) found that the toxic action of the highly dissociated acids for the plant Lupinus albus was due to the hydrogen ion, but that, in weakly dissociated acids, the undissociated part of the electrolyte played a role. Formic acid proved to be the most lethal in their experiments on the fatty acid series. It was also the most highly dissociated. According to Paul and Kronig (1896) and Kronig and Paul (1897), the more an acid was dissociated the greater germicidal action it exhibited against spores of Bacillus anthracis. Undissociated molecules and anions affected bacteria. Various micro-organisms were shown by Fermi (1897) to differ in their resistance toward ten per cent solutions of hydrochloric, lactic, citric, tartaric, and oxalic acids. Clark (1899) observed that acetic acid at a dilution only two per cent ionized inhibited the germination of spores of filamentous fungi, while highly dissociated mineral acids failed to show so high a retarding effect. Again, the activity of weakly dissociated acids was attributed to the undissociated molecule. Bial (1902) was of the opinion that the degree of acidity rather than the nature of the acid was the controlling factor in the germicidal and antiseptic action of acids on yeasts. The less an acid was dissociated, the higher was the concentration necessary for it to exhibit killing power toward yeast. A study made by Winslow and Lochridge (1906) showed that mineral acids exerted an effect commensurate to the hydrogen ion concentration. Toxicity of the organic acids was due mainly to action of the undissociated molecule and varied widely with the acid employed. Acetic acid and benzoic acid were fatal to Eberthella typhosa and Escherichia coli at a strength at which these

acids were but slightly dissociated. Paus (1903), working with fatty acids, concluded that there was little relation between the hydrogen ion concentration and growth, but that the kind of acid as well as the acidity was responsible for the germicidal value. Both Escherichia coli and Eberthella typhosa grow in fairly strong acid media. As a rule, the colon bacillus grew more rapidly and in a more acid medium than did Eberthella typhosa, but the latter developed faster in the lower concentrations of oxyacids and fruit acids.

Lazarus (1908) adjusted media with various indicators and determined roughly the hydrogen ion concentration which limited bacterial growth. Using more accurate methods, together with a clearer concept of the problem, Michaelis and Marcora (1912) demonstrated that Escherichia coli is limited in its acid fermentation of sugars upon reaching a rather definite hydrogen ion concentration. The year before (1911) Michaelis found that Eberthella typhosa responds in the same manner as do certain proteins and that it agglutinates within certain zones of hydrogen ion concentration with characteristic optima.

In accordance with the idea of Overton and Lubs, Burri (1912) stated that rate of inhibition and hydrogen ion concentrations were not parallel but that the ability of the acid to penetrate the cell determines its capacity to inhibit bacterial development. From his observations, he concluded that weak organic acids were as detrimental as strong mineral acids in suppressing growth of harmful micro-organisms. Burri assumed an action of one hundred for hydrochloric acid for Aerobacter aerogenes and by the same measure nitric, acetic, propionic, formic, and lactic were as harmful. By comparison, sulfuric acid was eighty, phosphoric acid sixty, citric acid

forty, and tartaric acid twenty. Fiftieth normal lactic acid did not retard Aerobacter aerogenes while seventy-fifth normal inhibited Escherichia coli.

Johannessohn (1912) noted that yeast fermentation increased in the presence of acetic acid and its higher homologs when in sufficiently dilute solution. Slightly more concentrated solutions inhibited but did not destroy the organisms. Prevention of fermentation did not depend on absolute amount of acid but on quantities of yeast and the necessary concentrations of acids were not directly proportional but stood in the same ratio as the abscissas and ordinates of a parabola. Responsibility for action of the acid was placed chiefly on the undissociated molecule and not on ions. No perceptible adsorption of acid by yeast was said to have taken place. According to Bruenn (1915), disinfecting power of lactic and acetic acids for Escherichia coli and Eberthella typhosa was due to activity of hydrogen ions.

Credit must be given to Clark (1914, 1915, 1917) for clarifying the picture insofar as titratable acidity and hydrogen ion are considered. In 1914, he remarked that temperature is an accelerating condition, and that the concentration of hydrogen ions in a medium may be of great significance. The next year (1915) Clark showed that two media adjusted to the same "degree of titratable acidity" may have widely divergent hydrogen ion concentrations. In another publication during that year Clark confirmed Michaelis' deduction regarding agglutination of bacteria at definite concentrations of hydrogen ions. This view was amplified in 1917 when Clark and Lubs inferred that stability of the bacterial cell or its membrane is

somewhat dependent upon the pH about it if that protoplasmic structure responds to any extent like protein solutions.

Horton and Hsu (1916) thought that acids act as disinfectants through the agency of hydrogen ions produced by electrolytic dissociation and that the disinfecting power of an acid is approximately proportional to the hydrogen ion concentration. According to them, addition to an acid of a salt containing an anion common to this acid diminishes its disinfecting power as the result of a decrease in the hydrogen ion concentration and an increase in the concentration of the undissociated acid molecule. On the other hand, salts which do not appreciably affect the dissociation of an acid, greatly increase the disinfecting properties of the acid. Acid anions are positive catalyzers and undissociated acid molecules are negative catalyzers in acid disinfection.

Clark and Lubs (1917) objected to some of the work done previously in trying to discover effects of acids or of hydrogen ions because no account was taken of "buffer action" in the media used. In the presence of proper buffers, acids may not furnish hydrogen ion concentrations which can be judged merely from an inspection of their dissociation constants. In many instances a weak acid may alter the proportion of dissociated hydrogen ions to the same extent as the same quantity of a much stronger acid. Using the same reasoning, one may see that unbuffered solutions are susceptible to enormous changes in pH under the action of so-called neutral salts. Taylor (1917), and Wolf and Harris (1917) ascribed auto-intoxication to simple acid poisoning of the organism. Each organism would appear to have a definite hydrogen ion concentration in a given medium at

which development is impossible. The first worker used two mineral acids and six organic acids against different bacteria. The quantity of hydrochloric and nitric acids were of importance and not their different quality. By contrast, organic acids varied greatly among themselves in their effect on growth of specific organisms. Thus, acetic acid was quite effective against Bacillus pyocyaneus but much less damaging against cocci.

Wyeth (1918) reported that a greater hydrogen ion concentration was necessary to inhibit growth of Escherichia coli using strongly dissociated acids such as hydrochloric and lactic acids, than was needed with a weak acid. However, when equivalent amounts of acids were used, the more highly dissociated ones were most inhibiting.

Vegetable juices tested by Sarti (1919) showed various degrees of bactericidal power, being very marked with garlic and lemon, medium with onion and orange, and least with tangerine. He attributed bactericidal ability of lemon and orange to amount of free acid that they contained while in garlic and onion "this power is due to the peculiar chemical constitution of the essence."

In studying resistance of Clostridium botulinum to heat, Weiss (1921) noted that the greatest change in survivals occurred just beyond the neutral point. As the hydrogen ion concentration increased, the decrease in resistance for a similar change in hydrogen ion concentration was not as marked or, in other words, the rate of loss of resistance decreased as the pH decreased. The hydroxyl ion was said to have nearly, if not exactly, the same effectiveness as the hydrogen ion. Weiss correlated the rate of change in pH with temperature and secured essentially the same results as those of

Bigelow and Eddy (1923). Optimal, minimal, and maximal hydrogen ion concentrations necessary for growth of twenty pathogenic bacteria were plotted graphically by Bernby (1921). Minimum pH for any of the bacteria was pH 4.5. Both Salmonella paratyphi and Salmonella schottmulleri were able to reproduce slowly at that acidity.

According to Wolf and Shunk (1921) different acids at the same pH were reported to inhibit certain plant pathogens unequally. Acetic and formic acids were more efficient antibiotic agents than hydrochloric or sulfuric acids. Traube (1921) criticized Michaelis' methods and in the same report concluded that acid action must be reduced rather to anion and undissociated molecules.

Somogyi (1921), Traube (1921), and others compared relationship between acid action and bacteria with surface tension, absorption, swelling, and flocking character, osmotic action, and electric potential. In Yaoi's opinion (1925), these phenomena throw light on certain properties but give no satisfactory explanation of the complicated processes involved in the influence of acids upon viability of bacteria. Yaoi found that some acids vary considerably in their effect upon bacterial growth when applied to the medium in low concentrations. Inorganic acids such as hydrochloric and nitric stimulated growth while organic acids other than acetic and oxalic had no stimulating effect. Sulfuric and phosphoric acids delayed growth. Pederson and Breed (1926) found that one per cent acetic acid stopped growth in tomato catsup of all micro-organisms studied. Combinations of salt and sugar with the acid did not make much difference in amount of acetic acid required. Rather, acetic acid inhibited growth in almost direct proportion to the amount present and at

equivalent pH values greater toxicity was usually observed in those tubes containing the greater amount of acid.

In 1929, Douglas studied the effect of hydrogen ion concentration on Escherichia coli and Sherrhella typhosa and found that fluids with an acidity below 4.2 caused death of the organisms rapidly, the rapidity depending largely on degree of acidity.

Facultative anaerobes and obligate anaerobes isolated from swelled and from flat-sour cans of tomatoes, pears, pineapple, pimientos and chiles by Townsend (1939) were divided into four groups on the basis of their morphological and cultural characteristics. Some of the strains isolated were sufficiently acid tolerant to grow in almost any canned food if the medium was found to be suitable otherwise. The first group was the most acid tolerant, being able to grow in acetic acid of pH 3.96, citric acid of pH 3.21, and hydrochloric acid of pH 3.41. In addition, all strains in groups one and three reduce pH value of the medium still further after growth. Members of the second group produced alkaline waste products from peptone.

Bach (1932) stated that at an interval where the pH has no importance, the undissociated part of lactic acid is the active factor although generally hydrogen ions control the antiseptic effect. In another paper (1932) he suggested that the antiseptic action of formic, acetic, propionic, and butyric acids was connected with their influence on surface tension. Reid observed (1932) that resistance of Bacillus pyocyaneus is not constant, but varies with the kind of acid. Mono-basic acids, the least dissociated of the acids used, inhibited growth at a much lower hydrogen ion concentration than did highly dissociated acids. Wide difference existed

between ability of an acid to exert a bactericidal effect and to inhibit growth. Acids which were strongly bactericidal frequently exhibited weak inhibiting powers in liquid media. Acetic, propionic, and butyric acids, which were weakly bactericidal, were among the most strongly inhibiting of all the acids used. It appeared that the bactericidal action of the weaker organic acids was dependent not only upon cations but undissociated molecules were also active in this respect. Levine and Fellers (1939) showed acetic acid more toxic than either lactic or hydrochloric to Salmonella aertrycke, Saccharomyces cerevisiae, and Aspergillus niger. These organisms were destroyed at a higher pH value with acetic than with lactic or hydrochloric acids. Certain acids appeared to have a toxicity in excess of that which could be due to pH alone. In 1940 the same workers investigated the inhibiting effect of acetic acid with salt and sugar added. Apart from the indirect effect of altering the hydrogen ions, salt and sugar aided little to acetic acid's toxic effect on bacteria and yeast. Levine and Fellers concluded that all lethal effects of vinegar are due to the acetic acid content and that the acid inhibited growth in almost direct proportion to amount present. At equivalent pH values greater toxicity was observed in those tubes containing the greater amount of acid.

Munheimer and Fabian (1940) used acetic, citric, lactic, malic, and tartaric acids and compared their bactericidal action with benzoic and hydrochloric acids. Acetic acid was germicidal at a higher pH than citric, lactic, malic, tartaric, or hydrochloric acids. In antiseptic action acetic acid was again the most inhibiting, but the order differed in that lactic acid arrested growth at

a lower hydrogen ion concentration than did citric acid. The other acids followed in the same order as in bactericidal value. Difference in action of various organic acids upon the same strain of staphylococcus was believed due to a specificity of reaction likewise brought about by either anion or undissociated molecule, or both. Erickson and Fabian (1942) found that acid preserving and acid bactericidal values of lactic acid was greatest according to grams required. The term, preserving value, was used to explain failure of growth in the original broth tube inoculated but growth in broth upon subculturing from this tube. Acetic acid was second and citric third. If preserving or bactericidal values of acids were based on pH, they proceeded in the order acetic, then citric, and then lactic. Streptococci were more resistant than thermophiles, and yeasts were more tolerant than bacteria to the action of acids. A .5 to 5 N solution was required for yeasts as compared to a .1 to .3 N solution for bacteria. For yeasts the order and effectiveness of acids in both preserving and germicidal ranges was acetic, then lactic, and finally citric.

General consensus is that organic acids have a toxicity in excess of that due to dissociated hydrogen ions alone. One possibility for increased activity is that, as hydrogen ions are absorbed by bacteria and any other hydrogen receivers in the medium, more hydrogen ions are dissociated and the accumulation gradually coagulates the protein of the cell or denatures certain functional groups or enzymes. From recent observations acids of the same pH with large parts of the molecule un-ionized have been shown to be the most effective killing agents. Functional groups of some electrolytes may react specifically with certain strains of bacteria or

with particular enzymes in cells. One possible way for explaining this action is that the positive ion is able to penetrate the cell or become adsorbed on the wall but does not have the ability to completely alter or denature the cell so that growth is no longer possible. This ability may be resident in the anion or un-ionized molecule.

III. MATERIAL

A. Procedure.

In the following investigation, an attempt has been made to determine quantitatively the period of survival of the more important aerobic non-sporing pathogens of the gastro-intestinal tract together with several of the most commonly used bacterial indicators of pollution in a number of fresh and canned fruit juices. Vibrio comma was not included in the study since Cholera is a disease rarely encountered in North America.

Pure cultures of all organisms with the exception of Staphylococcus aureus and the Army strain of Eberthella typhosa were originally received by the University of Illinois Bacteriology Department in 1934 from the Illinois Medical College. The Staphylococcus aureus strain was part of the Jefferson County Board of Health Collection and is marked "C₂ from cream puffs", while the Army strain of Eberthella typhosa has been in the University of Illinois Collection since March, 1939 and is labelled "Army Medical School Number 58". Gladys C. Gallagher isolated Serratia marcescens (American Type Culture Number 960) and Escherichia coli (American Type Culture Number 4348) strains. Aerobacter aerogenes, Salmonella paratyphi (40), Salmonella schottmuelleri strains were not further identified. Salmonella aertrycke came from the American Type Culture Collection Number 884. Pearl Kendrick, Michigan State Department, isolated the Salmonella suispestifer strain employed. E. O. Jordan's Number 206 was the strain of Salmonella enteritidis used. Beside the Army strain, two other Eberthella typhosa types were utilized, i.e., the Rawling's strain and the organism isolated by Ruth Gilbert, New York State Department of Health. Shigella paradysenteriae Sonne was from

the original Lister Institute Collection. Tokushima (in Japan) isolated Shigella dysenteriae. Hartman, in 1934, isolated the strain of Staphylococcus albus used from a case of Osteomyelitis.

Purity of each culture was determined by streaking on tryptone-dextrose agar and then making microscopic and cultural examination of a representative colony. Stock cultures were held on nutrient agar and subcultured every two weeks to keep their resistance uniform. Organisms used for inoculation were allowed to multiply on tryptone-dextrose agar for sixteen to twenty hours at 37°C (98.6°F). The following medium was shown to give better growth than either nutrient agar or dextrose agar:

distilled water	- - -	1000 ml
shredded agar	- - -	15 grams
meat extract	- - -	3 grams
sodium chloride	- - -	5 grams
tryptone	- - -	5 grams
dextrose	- - -	10 grams

Cans containing fruit juices were wrapped in a clean towel soaked with 1:1000 mercuric chloride solution and shaken thoroughly on the automatic shaking machine. Then the can was placed at an angle on a wooden trough and the amount of vacuum determined by a sterile vacuum gauge. After this determination, a sterile special type can opener was employed to open the can. Both the vacuum gauge and can opener were devised by American Can Company and were ideally suited for the above purpose. (See Plate I). The juice was removed by using the large end of a sterile ten ml pipette and transferred to a sterile 200 ml tube or to a sterile square dilution bottle. At the same time a one ml and a five ml sample were plated directly to check condition of the original contents. In one can of pineapple and one can of lemon juice organisms were isolated. These may have

been contaminative forms. The samples were discarded.

Sterile fresh fruit juices were obtained by soaking the fruit in a 1:100 mercuric solution for thirty minutes and thoroughly rinsing several times in sterile distilled water. The fruit was cut in half with a sterile knife under cover of large outline dishes and the juice expressed aseptically through the aid of sterile forceps and a sterile mechanical squeezer-type juice extractor. For the most part, fresh juices were shown to be sterile. In a few instances there was subsequent growth by Aspergillus niger and other types of molds but this was, in all probability, due to contamination during extraction or transfer of the juice to storage bottles. (See Plate II).

A sixteen to twenty hour incubation period was chosen in order that the cells would be reproducing rapidly and that their normal reproduction rate would not be greatly impeded in a new medium favorable for growth. At the end of this time ten ml of sterile distilled water was added and a suspension made with the aid of a platinum loop. Cultures were then allowed to stand at room temperature for a short time before being placed in ice-water. Fruit juice was likewise chilled in order that there would not be any shock to the organisms upon transfer. In earlier experiments, i.e., lemon juice, lime juice, grapefruit juice, and orange-grapefruit juice, the fluid was placed in a sterile 200 ml deep tube (pyrex). A suspension of the organism tested was added, the mixture thoroughly shaken, and one ml removed at definite time intervals. Later this procedure was modified. The fruit juice was placed in a sterile dilution bottle, the test organism added and two ml of the infected

juice transferred by means of a sterile syringe to each of fifteen sterile two ml vaccine ampoules. After filling, the ampoules were sealed with a Bunsen flame and stored at room temperature or placed in the ice pans of an electric refrigerator where the temperature varied from -5° to -10°C (17.0° to 14°F). In addition, one uninoculated series of samples were placed at both temperatures. First sampling of the juice was made immediately after the fluid had been inoculated and shaken. For the highly bactericidal liquids, shaking was effected by hand since the time interval was too brief to allow agitation in the shaking machine. Juices whose germicidal action was less severe were allowed to shake for ten minutes before sampling.

At regular time intervals, frozen ampoules were removed from the refrigerator and melted at room temperature in five per cent phenol solution. Samples held at room temperature received similar treatment. In either case, one ml of the contents was placed directly into sterile petri dishes or in dilutions of sterile distilled water or sterile water containing calcium carbonate. Dilutions varied with time intervals and organisms tested, ranging from one to ten to one to ten million. The final dilution was usually high enough to give a count of from thirty to three hundred colonies when one ml or one-tenth ml of the proper dilution was placed in a petri dish and covered with tryptone-dextrose agar. Introduction of calcium carbonate into blank water tubes was found necessary in lower dilutions of lemon, lime, grapefruit, and blended orange-grapefruit juice. Higher survival rates were obtained with apples and grape juice as well. Orange-, pineapple-, and tomato-juice, and apricot nectar did not show as great variation.

PLATE I.

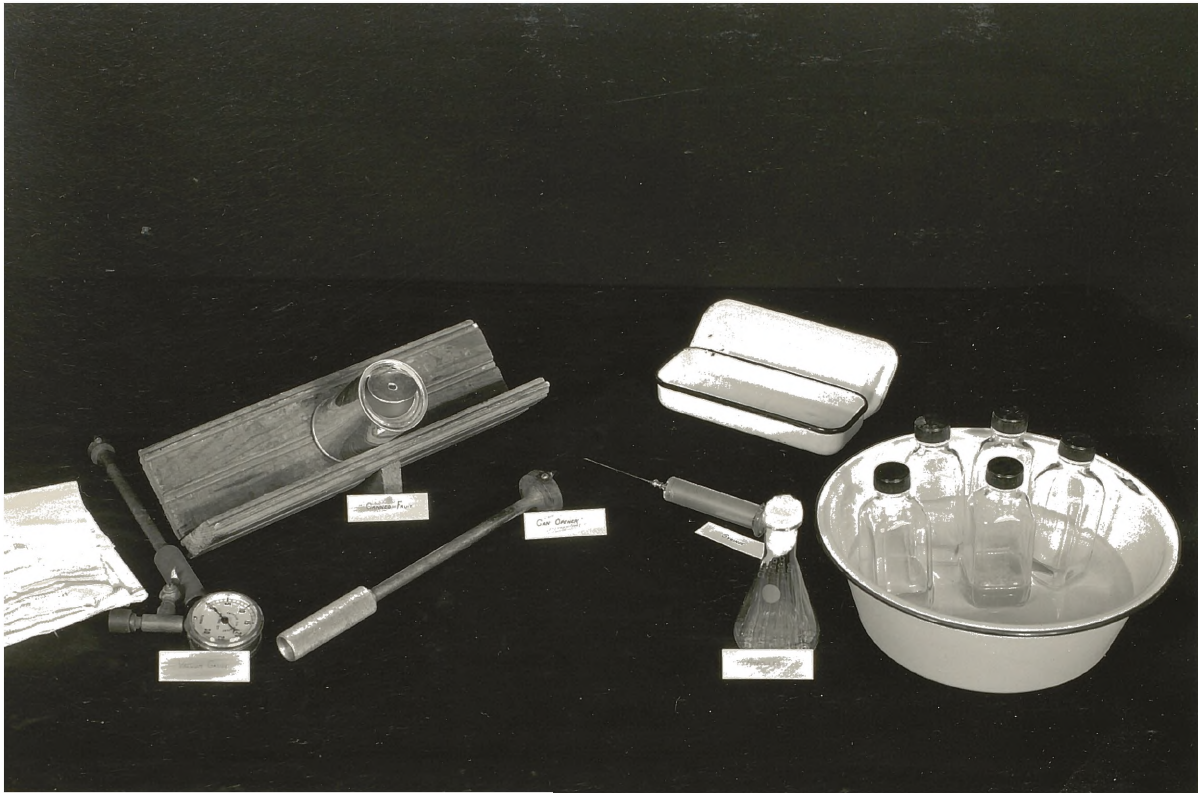
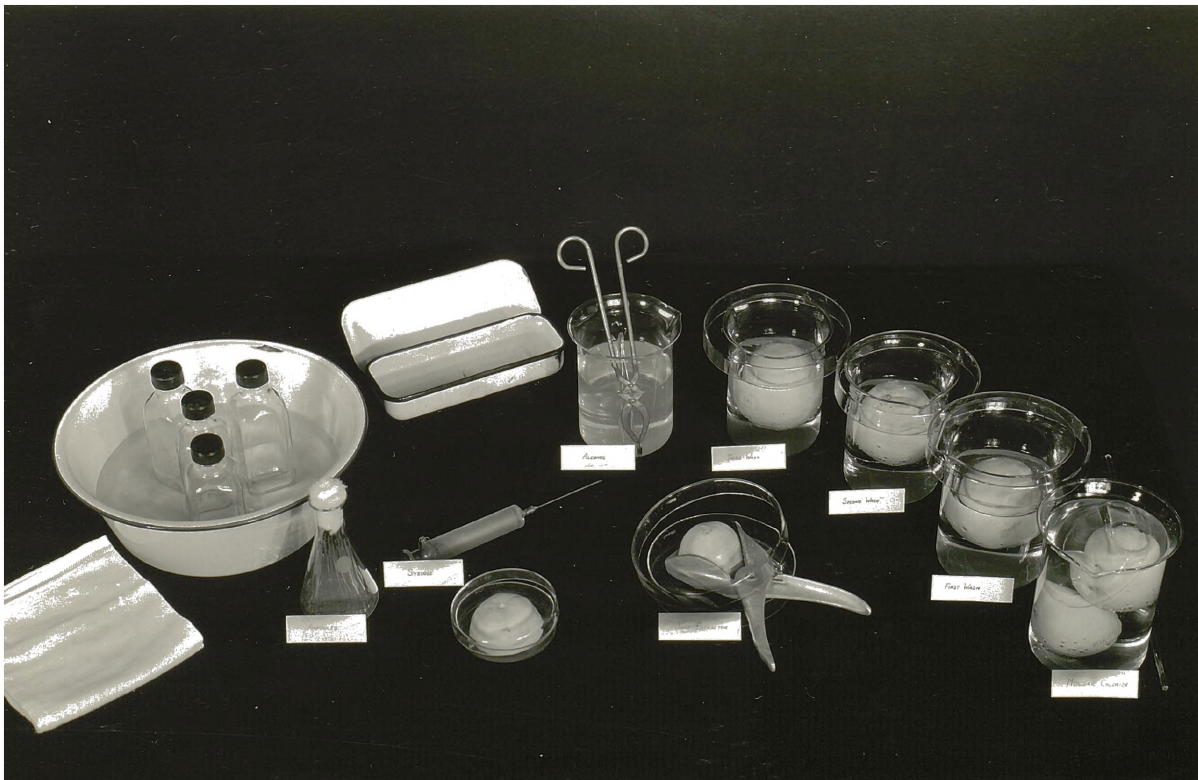


PLATE II.



In addition, canned pineapple-, and canned grapefruit-juice were inoculated with the various organisms and, upon sampling, were placed in sterile water blanks containing ten per cent added sucrose to determine whether that diluting medium was a more favorable one than sterile distilled water.

Another experiment was conducted at -10°C (3.2°F) as well as at -3°C (17.6°F) to test the effect of different sub-freezing temperatures on organisms placed in the same substrate but at different temperatures. A preliminary study undertaken to show the difference in survival time at -3° , 20° , 37° , and 55°C (17.6° , 33° , 93.6° , and 131°F) is also reported.

While results of this study depend entirely upon plate counts, an attempt was made to standardize the method of sampling so that any errors would be made constant and interfere less with results. Tryptone-dextrose agar was selected as a suitable substrate because it gave good growth with all of the organisms. Express juice of fresh fruit was the blend of the fluid of at least three fruits for each organism tested. In canned juices, sampling was made by alternate removal of contents from the lower and upper regions of the can. One ml of bacterial suspension was injected by beginning at the lower limit of juice and gradually withdrawing the pipette at the top when one ml had been delivered. After inoculation, the glass container was placed on the shaking machine for five to ten minutes and immediately after mixing a determined amount was removed to a petri dish, blended with warm tryptone-dextrose agar and incubated for one day at 37°C (93.6°F). Dilutions were made as quickly as possible to prevent plasmolysis or diffusion of salts. After

counting, the plates were returned to the incubator for another two days and the counts checked. Ampoules, after rinsing in five per cent phenol, were opened at regular time intervals and most of the content tested for viable organisms. The same method of incubation and determination of counts was employed as for the initial sample. In doubtful cases, incubation was continued for one week at room temperature.

Two separate series of ampoules were maintained at room temperature and in the refrigerator with each organism stored in dextrose-tryptone broth (i.e., dextrose broth to which three grams tryptone and five grams sodium chloride had been added). The broth was adjusted to pH 6.8 in order to compare the effect of a favorable medium with that of the various fruit juices on the viability of the organisms.

Determination of hydrogen ion concentration was made colorimetrically by Hellige comparator and electrometrically by Coleman potentiometer (glass electrode). Total acidity was determined by titrating against .1071 Normal NaOH, using phenolphthalein as the indicator. The results for citrus fruits were reported as per cent citric acid or grams citric acid per hundred milliliters since that acid makes up the great bulk of acid content and by this method no distinction can be made between the various acids present. In the other juices, the composition is not so uniform and for that reason the number of milliliters of tenth normal sodium hydroxide necessary to neutralize ten ml of tenth normal sodium hydroxide has been reported.

Survivors were determined morphologically and culturally. Staphylococci, Serratia marcescens, and Aerobacter aerogenes produced

characteristic colonies. Escherichia coli was identified by acid and gas in lactose broth. Eberthella typhosa, Salmonella paratyphi, Salmonella schottmuelleri, Salmonella aortrycke, Salmonella suis-pestifer, and Salmonella enteritidis were confirmed as characteristic colonies on Bismuth sulfite (Wilson and Blair's) agar or MacConkey's agar. Doubtful Shigella species were streaked on MacConkey's agar and examined microscopically. These methods, while not exact, are sufficiently accurate to eliminate most contaminative forms.

B. Data.

Counts of surviving cells for each species of organism studied have been plotted semi-logarithmically. Dotted- or broken-lines represent bactericidal action or inhibiting action in frozen (-8°C or 17.6°F) samples, while solid lines signify behavior at room temperature (20°C . or 68°F).

The following tables and figures are, for the most part, self explanatory. When unusually heavy inoculums were used, or when reproduction was effected, the number of cells were plotted in terms of thousands rather than singly. Thus, in tomato and pineapple juice, survivals of Escherichia coli and Aerobacter aerogenes, as shown, have a lower limit of one thousand rather than one cell on the vertical axis.

Table I.

Survival in canned lemon juice.
Vacuum 9 inches, pH 2.4, acidity
as citric acid 7.6.

Serratia marcescens

	Frozen	room temp.
Count per ml	4,400,000	4,400,000
One minute	316,000	-
Two minutes	200	0
Four minutes	0	0
Six minutes	0	0
Eight minutes	0	0

Staphylococcus albus

	Frozen	room temp.
Count per ml	15,800,000	15,800,000
Fifteen min.	38,000	116,000
Thirty minutes	1,800	28,000
Sixty minutes	0	400
Two hours	0	0
Four hours	0	0

Survival rate shown in figure 1.

Staphylococcus aureus

	frozen	room temp.
Count per ml	17,100,000	17,100,000
Thirty minutes	-	7,900,000
Sixty minutes	169,000	2,200,000
Two hours	308,000	630,000
Four hours	65,000	21,000
Eight hours	5,700	370
One day	430	0
Two days	0	0

sin 1-10 dil'n.

Survival rate shown in figure 2.

Escherichia coli

	Frozen	room temp.
Count per ml	19,000,000	19,000,000
Thirty min.	6,300,000	4,120,000
Sixty minutes	2,600,000	1,770,000
Two hours	960,000	530,000
Five hours	13,700	38,000
Ten hours	470	130
One day	0	0
Two days	0	0

Figure 1.

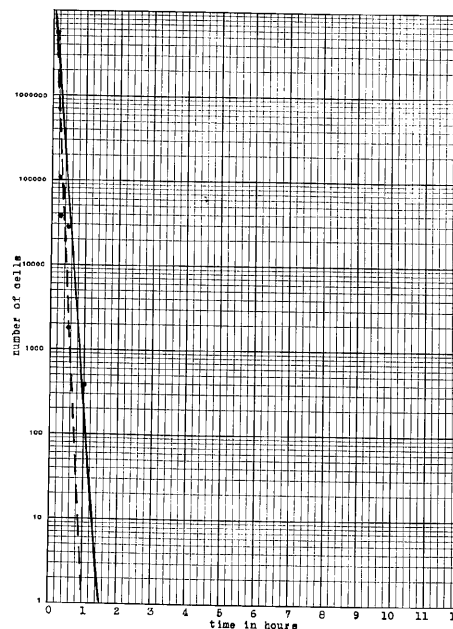


Figure 2.

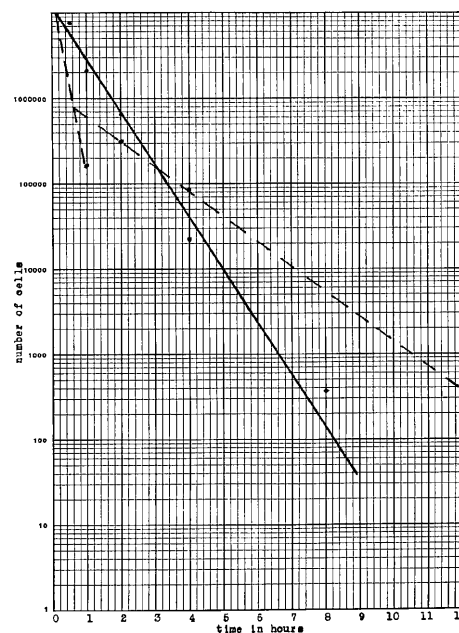


Table II.

Survival in canned lemon juice.
Vacuum 9 inches, pH 2.4, acidity
as citric acid 7.6.

Eberthella typhosa

	frozen	room temp.
Count per ml	3,700,000	3,700,000
Two minutes	147,000	1,300,000
Four minutes	120	74
Six minutes	10	0
Eight minutes	0	0
Ten minutes	0	0

Survival rate shown in figure 3.

Aerobacter aerogenes

	frozen	room temp.
Count per ml	18,000,000	18,000,000
Five minutes	17,000	1,600
Ten minutes	800	0
Fifteen minutes	0	0
Twenty minutes	0	0

Shigella paradysenteriae Sonne

	frozen	room temp.
Count per ml	16,400,000	16,400,000
Three minutes	54,200	274,000
Six minutes	8,500	42,000
Ten minutes	670	3,000
Fifteen minutes	40	230
Twenty minutes	0	0
Thirty minutes	0	0

Survival rate shown in figure 4.

Shigella dysenteriae

	frozen	room temp.
Count per ml	3,700,000	3,700,000
Three minutes	1,330,000	1,600,000
Six minutes	840,000	1,080,000
Ten minutes	72,000	440,000
Twenty minutes	1,400	11,000
Thirty minutes	0	310
Sixty minutes	0	0
Two hours	0	0

In a duplicate test, Shigella dysenteriae, with an initial count of 5,200,000, showed 290 cells viable for thirty minutes and 4 colonies survived sixty minutes of freezing. No bacteria were found after two hours at -10°C (14°F).

Figure 3.

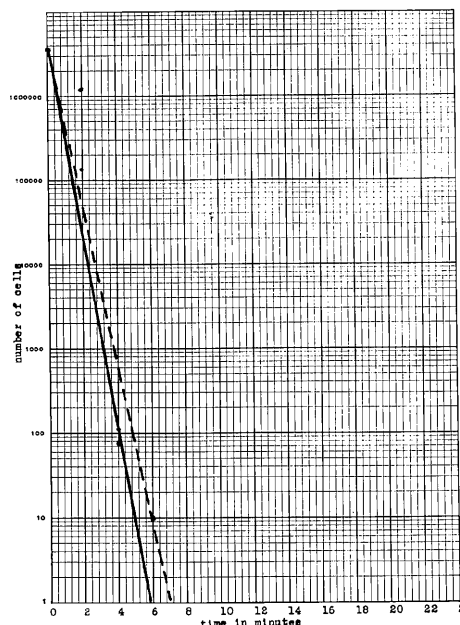


Figure 4.

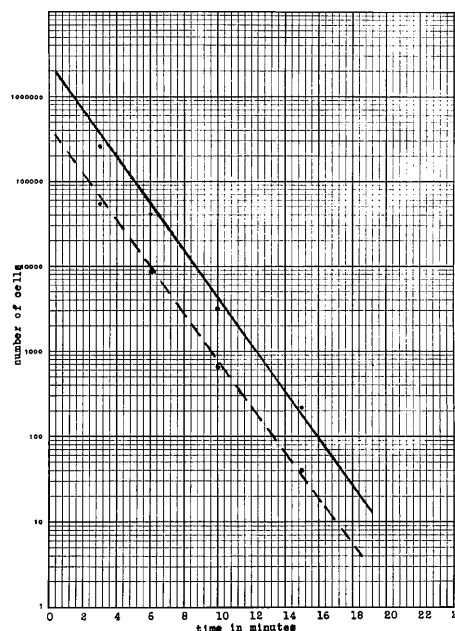


Table III.

Figure 5.

Survival in fresh lemon juice.
Acidity as citric acid 7.1, pH 3.3

Staphylococcus albus

	Frozen	Room temp.
Count per ml	4,300,000	4,300,000
Thirty minutes	74,000	611,000
Sixty minutes	220	40
Two hours	0	0
Four hours	0	0

Survival rate shown in figure 5.

Staphylococcus aureus

	Frozen	Room temp.
Count per ml	2,200,000	2,200,000
Thirty minute	1,350,000	-
Sixty minutes	910,000	-
Two hours	196,000	-
Four hours	27,300	6,000
Eight hours	0	22
One day	0	0
Two days	0	0

Escherichia coli

	Frozen	Room temp.
Count per ml	14,200,000	14,200,000
Ten minutes	5,300	3,800
Twenty minutes	0	30
Thirty minutes	0	0
Sixty minutes	-	0
Three hours	0	0

Sherrilla typhosa

	Frozen	Room temp.
Count per ml	2,800,000	2,800,000
Three minutes	56	7,000
Six minutes	0	100
Nine minutes	0	0
Twelve minutes	0	0

Win 1-10 dil'n.

Survival shown in figure 6.

Aerobacter aerogenes

	frozen	room temp.
Count per ml	11,800,000	11,800,000
Five minutes	480	310
Ten minutes	0	7
Fifteen minutes	0	0
Twenty minutes	0	0

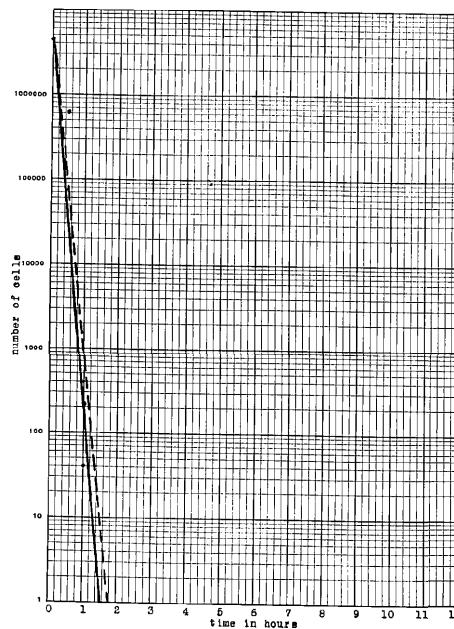


Figure 6.

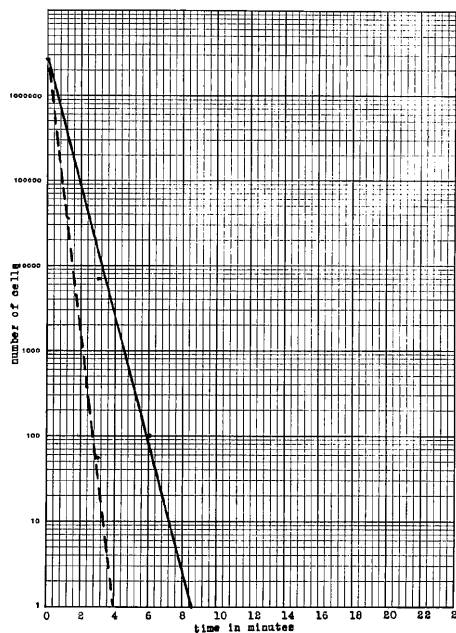


Table IV

Survival in fresh lemon juice.
Acidity as citric acid 7.1, pH 2.5

Salmonella paratyphi

	frozen	room temp.
Count per ml	2,600,000	2,600,000
Ten minutes	0	0
Twenty minutes	0	0
Thirty minutes	0	0

Salmonella schottmulleri

	frozen	room temp.
Count per ml	17,000,000	17,000,000
Ten minutes	0	*60
Twenty minutes	0	0
Thirty minutes	0	0

*in 1-10 dil'n.

Survival shown in figure 7.

Salmonella enteritidis

	frozen	room temp.
Count per ml	22,500,000	22,500,000
Ten minutes	71,000	40,000
Twenty minutes	67	225
Thirty minutes	0	20
Sixty minutes	0	0
Two hours	0	0

Shigella paradysenteriae Sonne

	frozen	room temp.
Count per ml	4,100,000	4,100,000
Ten minutes	33	3,300
Twenty minutes	0	0
Thirty minutes	0	0

Shigella dysenteriae

	frozen	room temp.
Count per ml	2,400,000	2,400,000
Three minutes	3,160	1,900
Six minutes	412	24
Ten minutes	110	3
Twenty minutes	*50	0
Thirty minutes	3	0
Sixty minutes	0	0
Two hours	0	0

*in 1-10 dil'n.

Survival shown in figure 8.

Figure 7.

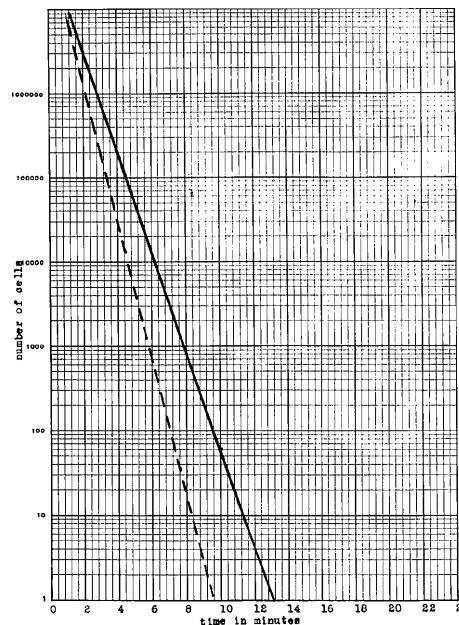


Figure 8.

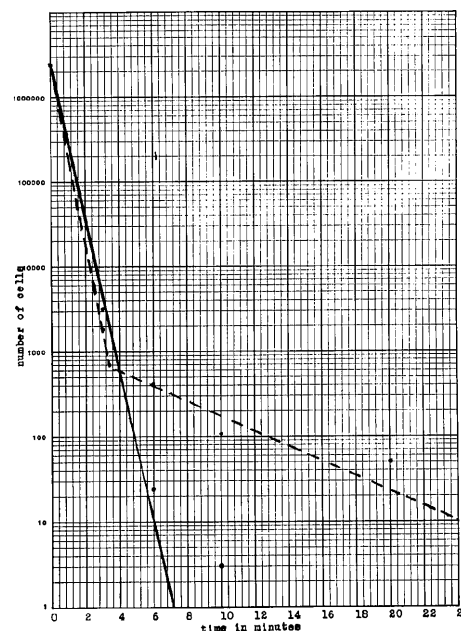


Table V.

Survival in fresh lime juice.
Acidity as citric acid 7.0, pH 2.5

Staphylococcus albus

	Frozen	room temp.
Count per ml	12,300,000	12,300,000
Thirty minutes	170,000	56,000
Sixty minutes	14	0
Ninety minutes	0	0
Two hours	0	0

Staphylococcus aureus

	Frozen	room temp.
Count per ml	135,000,000	135,000,000
Thirty minutes	98,000	*400,000
Sixty minutes	1,310	186
Ninety minutes	41	2
Two hours	0	0
Four hours	0	0

*approximately 4000 colonies in a
1-100 dilution.

Survival rate shown in figure 9.

Serratia marcescens

	Frozen	room temp.
Count per ml	15,800,000	15,800,000
Fifteen minutes	0	0
Thirty minutes	0	0
Sixty minutes	0	0

Escherichia coli

	Frozen	room temp.
Count per ml	5,700,000	5,700,000
Fifteen minutes	44	75
Thirty minutes	2	0
Sixty minutes	0	0
Two hours	0	0

Survival rate shown in figure 10.

Salmonella paratyphi

	Frozen	room temp.
Count per ml	2,030,000	2,030,000
Fifteen minutes	0	0
Thirty minutes	0	0
Sixty minutes	0	0

Salmonella schottmuelleri

	Frozen	room temp.
Count per ml	10,200,000	10,200,000
Fifteen minutes	1,402	380
Thirty minutes	0	0

Figure 9.

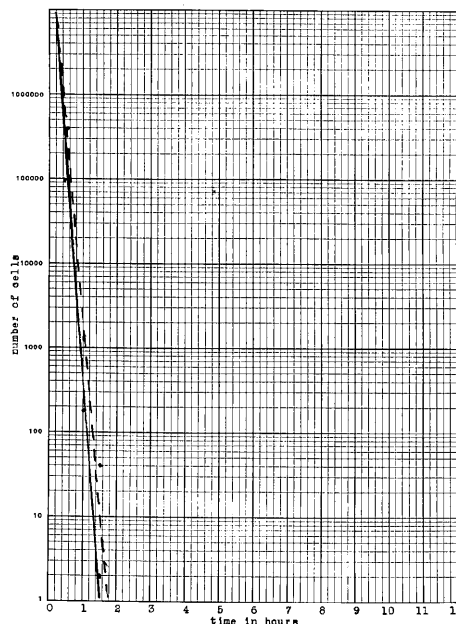


Figure 10.

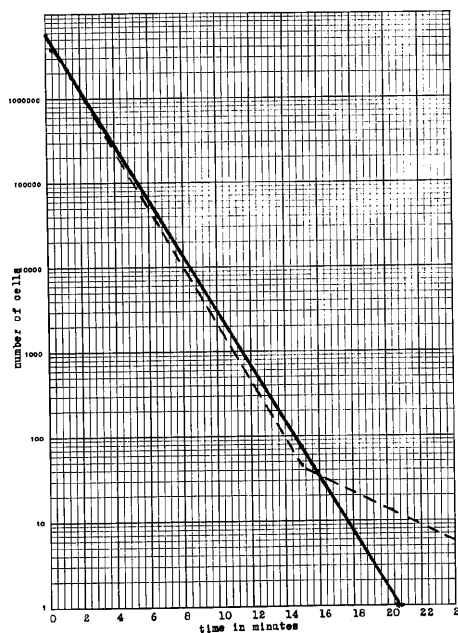


Table VI.

Survival in fresh lime juice.
Acidity as citric acid 7.0, pH 2.5

Salmonella enteritidis

	frozen	room temp.
Count per ml	23,100,000	23,100,000
Fifteen minutes	97,000	104,000
Thirty minutes	132	15
Sixty minutes	6	0
Two hours	0	0
Four hours	0	0

Eberthella typhosa

	frozen	room temp.
Count per ml	23,000,000	23,000,000
Fifteen minutes	830	238
Thirty minutes	170	0
Sixty minutes	0	0
Two hours	0	0

Survival rate shown in figure 11.

Aerobacter aerogenes

	frozen	room temp.
Count per ml	78,000,000	78,000,000
Fifteen minutes	4,600	2,400
Thirty minutes	30	0
Forty-five min.	1	0
Sixty minutes	0	0
Ninety minutes	0	0
Two hours	0	0

Survival rate shown in figure 12.

Shigella paradysenteriae Sonne

	frozen	room temp.
Count per ml	750,000	750,000
Fifteen minutes	204	6,100
Thirty minutes	0	0
Forty-five minutes	0	0
Sixty minutes	0	0

Shigella dysenteriae

	frozen	room temp.
Count per ml	5,800,000	5,800,000
Fifteen minutes	0	0
Thirty minutes	0	0
Forty-five minutes	0	0
Sixty minutes	0	0

Figure 11.

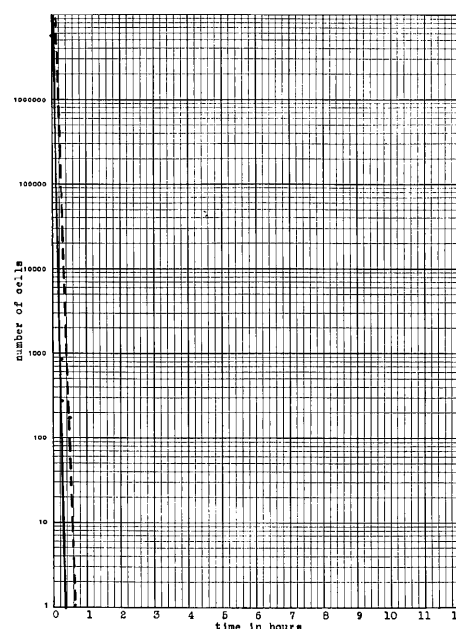


Figure 12.

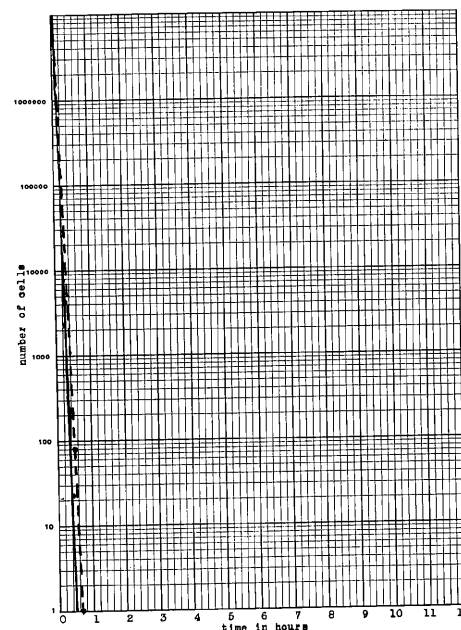


Table VII.

Survival in canned grapefruit juice. Vacuum 7.5", pH 5.3.

Serratia marcescens

	Frozen	room temp.
Count per ml	1,980,000	1,980,000
Thirty minutes	20,000	9,800
Sixty minutes	690	310
Two hours	8	0
Four hours	1	0
Eight hours	0	0
One day	0	0

Staphylococcus albus

	Frozen	room temp.
Count per ml	2,680,000	2,680,000
One hour	190,000	38,000
Two hours	21,000	1,400
Four hours	270	2
Eight hours	2	0
One day	0	0
Two days	0	0

Survival rate shown on figure 13.

Staphylococcus aureus

	Frozen	room temp.
Count per ml	950,000	950,000
Two hours	26,500	3,370
Four hours	18,000	560
Eight hours	8,600	30
One day	740	5
Two days	12	0
Three days	0	0
Four days	0	0

Survival rate shown on figure 14.

Escherichia coli

	Frozen	room temp.
Count per ml	610,000	610,000
Two hours	5,800	13,800
Four hours	960	-
Eight hours	250	-
One day	70	4
Two days	0	0
Three days	0	0
Four days	0	0

Figure 13.

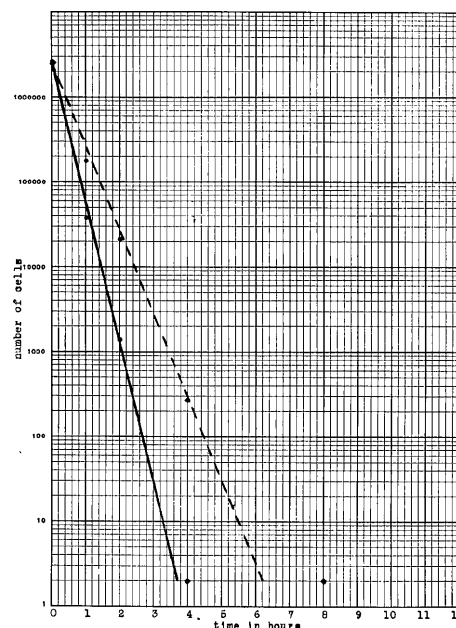


Figure 14.

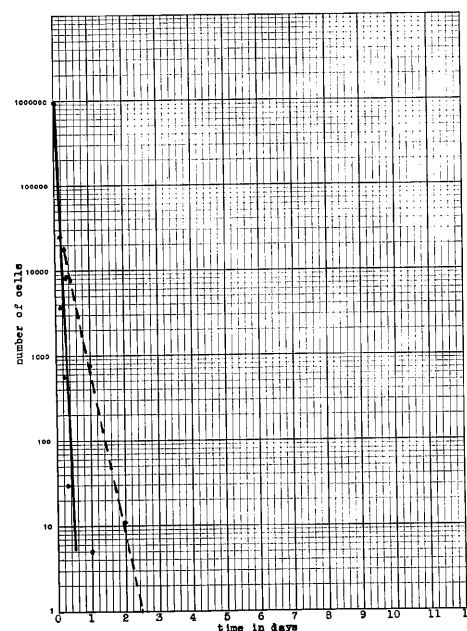


Table VIII.

Survival in canned grapefruit juice. Vacuum 7.5", pH 3.3.

Escherichia typhosa

	frozen	room temp.
Count per ml	830,000	830,000
One hour	80	5,800
Two hours	1,220	710
Four hours	52	9
Eight hours	4	0
One day	0	0
Two days	0	0

Salmonella paratyphi

	frozen	room temp.
Count per ml	1,820,000	1,820,000
One half hour	4,400	2,400
One hour	1,290	480
Two hours	82	*10
Four hours	0	0
Eight hours	0	0

*in 1-10 dil'n.

Survival rate shown on figure 15.

Aerobacter aerogenes

	frozen	room temp.
Count per ml	72,000,000	72,000,000
One-half hour	15,200,000	-
One hour	4,300,000	6,300,000
Two hours	260,000	108,000
Four hours	1,270	720
Six hours	20	77
One day	0	0
Two days	0	0

Survival shown on figure 16.

Shigella dysenteriae

	frozen	room temp.
Count per ml	560,000	560,000
One hour	20,000	13,400
Two hours	2,060	3,040
Four hours	0	123
One day	0	0
Two days	0	0

Figure 15.

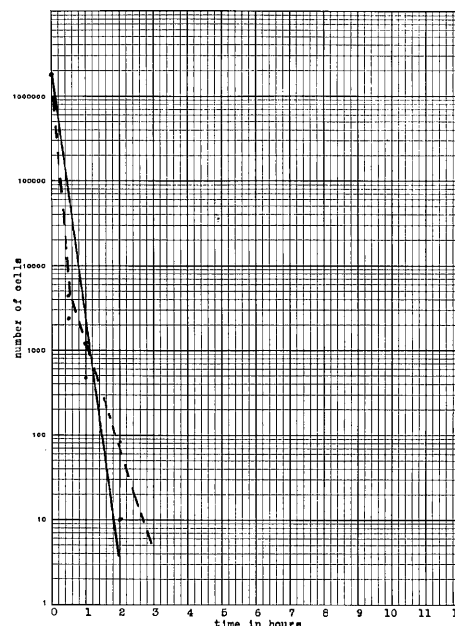


Figure 16.

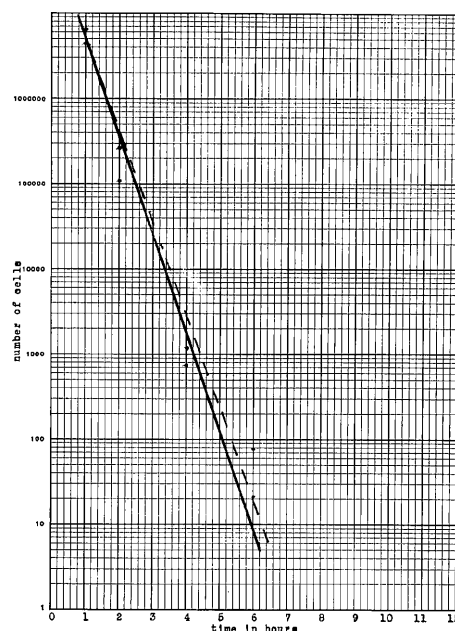


Table IX.

Survival in fresh grapefruit juice.
pH 3.2. Acidity as citric acid 1.7.

Serratia marcescens

	Frozen	Room temp.
Count per ml	810,000	810,000
Thirty minutes	300	3,000
Sixty minutes	0	25
Two hours	0	0
Four hours	0	0

Staphylococcus aureus

	Frozen	Room temp.
Count per ml	1,440,000	1,440,000
Two hours	11,300	51,000
Four hours	14,000	3,700
Eight hours	1,350	610
One day	40	0
Two days	0	0
Three days	0	0

Survival rate shown on figure 17.

Escherichia coli

	Frozen	Room temp.
Count per ml	524,000	524,000
Two hours	730	4,200
Four hours	0	95
Eight hours	0	0
One day	0	0

Sherrillia typhosa

	Frozen	Room temp.
Count per ml	1,970,000	1,970,000
One hour	1,610	860
Two hours	7	12
Four hours	0	0
Eight hours	0	0
One day	0	0

Salmonella paratyphi

	Frozen	Room temp.
Count per ml	3,780,000	3,780,000
One hour	29	147
Two hours	0	0
Four hours	0	0
Eight hours	0	0

Salmonella schottmuelleri

	frozen	Room temp.
Count per ml	402,000	402,000
One hour	8,700	12,300
Two hours	520	60
Four hours	15	0
Eight hours	0	0

Figure 17.

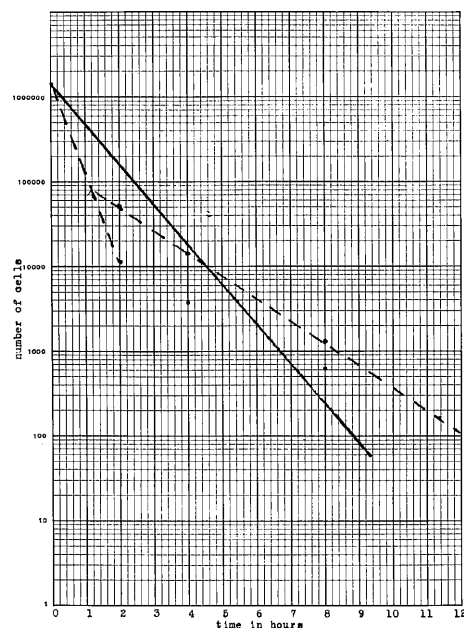


Figure 18.

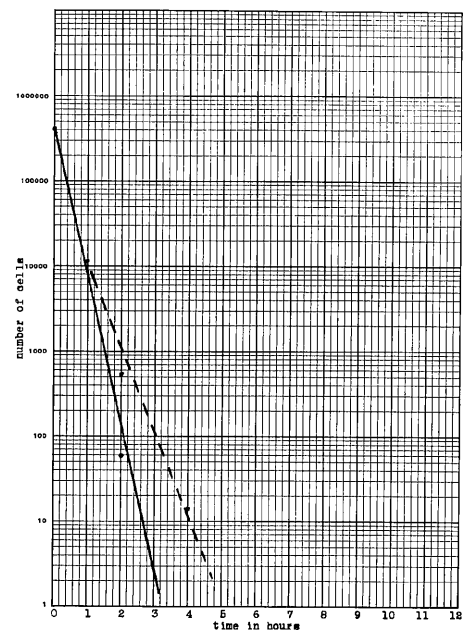


Table X.

Survival in fresh grapefruit juice.
pH 3.2. Acidity as citric acid 1.7.

Aerobacter aerogenes

	frozen	room temp.
Count per ml	3,400,000	3,400,000
One hour	750,000	332,000
Two hours	13,900	770
Four hours	22	0
Eight hours	0	0
One day	0	0

Survival rate shown in Figure 19.

Shigella paratyphosiae Sonne

	frozen	room temp.
Count per ml	332,000	332,000
One hour	257	1,090
Two hours	0	0
Four hours	0	0
Eight hours	0	0

Shigella dysenteriae

	frozen	room temp.
Count per ml	6,000,000	6,000,000
One hour	0	20
Two hours	0	0
Four hours	0	0

*in 1-10 dil'n.

Salmonella enteritidis

	frozen	room temp.
Count per ml	5,500,000	5,500,000
One hour	307,000	72,000
Two hours	42,000	500
Four hours	530,	0
Eight hours	1	0
One day	2	0
Two days	0	0

Survival rate shown on figure 20.

Figure 19.

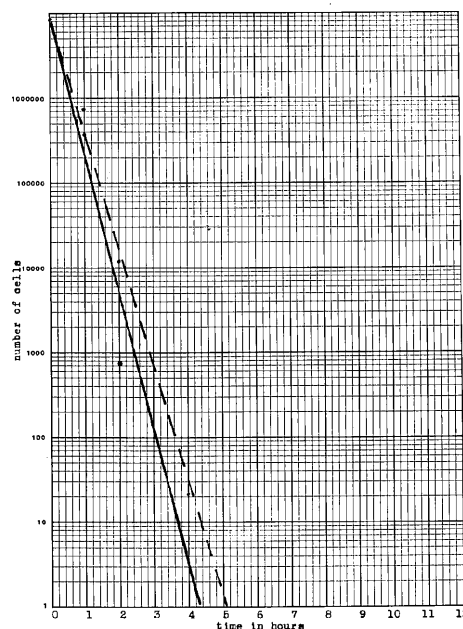


Figure 20.

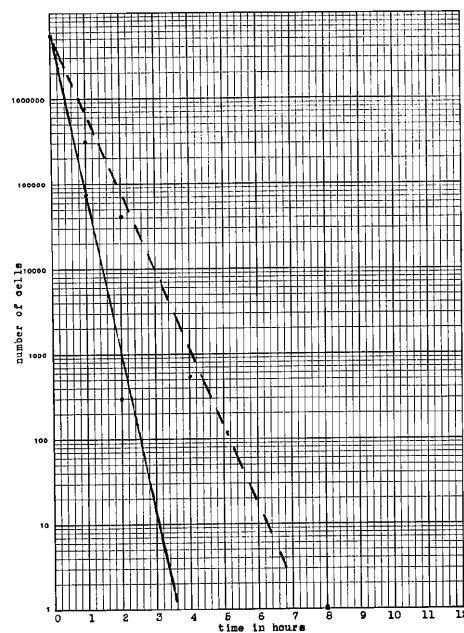


Table XI.

Survival in canned blended orange and grapefruit juice. Vacuum 7.4". pH 3.5.

Staphylococcus albus

	Frozen	room temp.
Count per ml	5,500,000	32,000,000
Ten minutes	3,230,000	2,660,000
Thirty minutes	520,000	-
Sixty minutes	106,000	160,000
Two hours	1,100	176,000
Three hours	47	93,000
Four hours	0	42,000
Eight hours	0	3,700
One day	0	5
Two days	0	0

Staphylococcus aureus

	Frozen	room temp.
Count per ml		9,100,000
Twenty minutes		3,400,000
One hour		780,000
Two hours		91,800
Four hours		19,200
Eight hours		720
One day		0
Two days		

Survival rate shown on figure 21.

Escherichia coli

	Frozen	room temp.
Count per ml		5,700,000
Fifteen minutes		3,400,000
Thirty minutes		512,000
Sixty minutes		190,000
Two hours		14,000
Four hours		110
Eight hours		0
One day		0

Shorthella typhosa

	Frozen	room temp.
Count per ml	6,700,000	2,600,000
Five minutes	290,000	-
Ten minutes	2,100	24,000
Twenty minutes	120	2,000
Thirty minutes	0	300
Sixty minutes	0	0
Two hours	0	0

Survival rate shown on figure 22.

Figure 21.

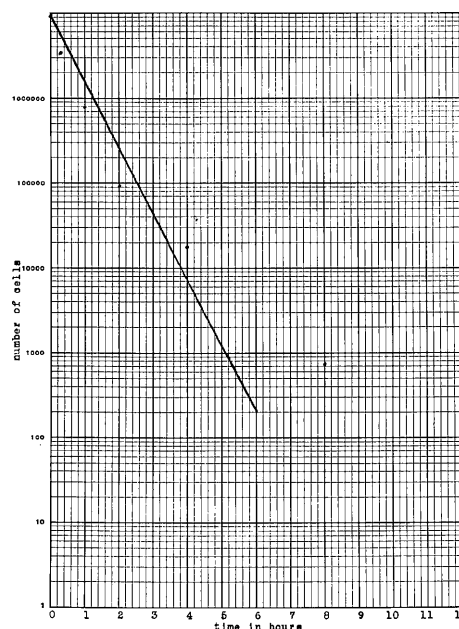


Figure 22.

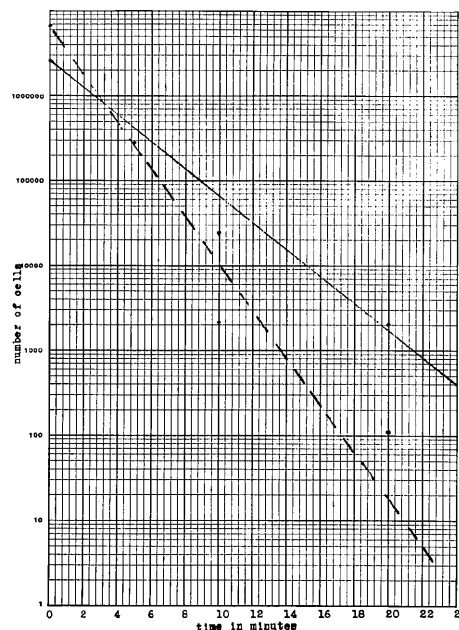


Table XII.

Survival in canned blended orange and grapefruit juice. Vacuum 7.4". pH 3.5.

Aerobacter aerogenes

	Frozen	room temp.
Count per ml	7,600,000	50,300,000
Ten minutes	1,500,000	1,310,000
Thirty minutes	81,000	3,500
Sixty minutes	16,000	200
Ninety minutes	4,300	0
Six hours	0	0
One day	0	0

Survival rate shown on figure 23.

Shigella paradysonteriae Sonne

	room temp.
Count per ml	5,200,000
One minute	4,400,000
Two minutes	530,000
Five minutes	90,000
Ten minutes	1,500
Twenty minutes	0
Thirty minutes	0
Sixty minutes	0

Survival rate shown on figure 24.

Shigella dysenteriae

	room temp.
Count per ml	603,000
One minute	4500,000
Two minutes	72,000
Five minutes	136
Ten minutes	0
Twenty minutes	0
Thirty minutes	0

Number given was approximated.

Figure 23.

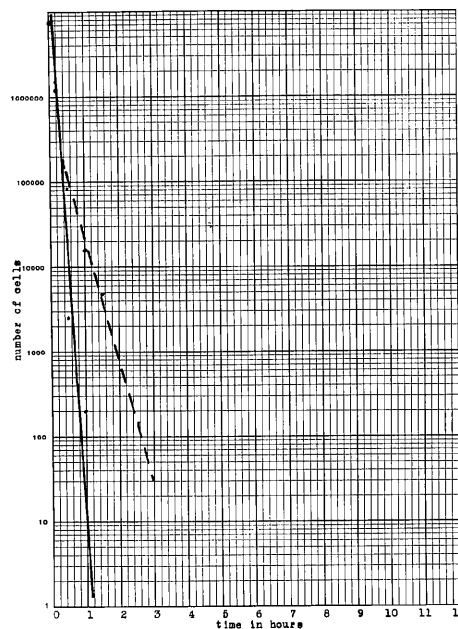


Figure 24.

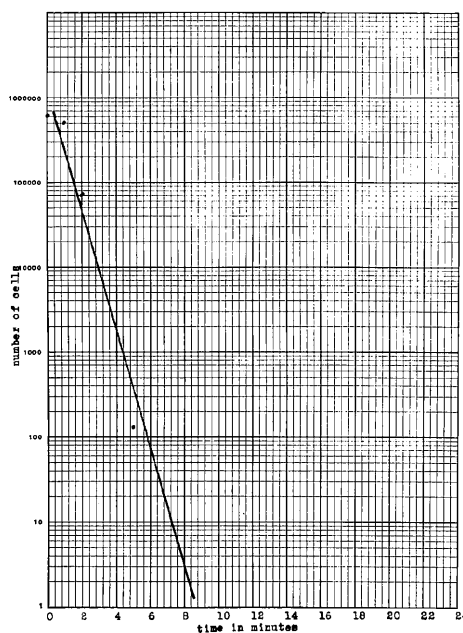


Table XIII.

Figure 25.

Survival in fresh orange juice.
Acidity as citric acid 1.7. pH 3.4.

Serratia marcescens

	frozen	room temp.
Count per ml	193,000	193,000
Thirty minutes	33,500	41,000
Twelve hours	1,200	530
One day	*10	0
Two days	0	0
Four days	0	0

*in 1-10 dil'n

Staphylococcus aureus

	frozen	room temp.
Count per ml	70,000,000	70,000,000
Twelve hours	660,000	*500,000
One day	190,000	30,000
Two days	20,000	1,000
Four days	370	12
Ten days	20	0
Fifteen days	0	0
Twenty days	0	0

*number given was approximated.

Survival rate shown in Figure 25.

Staphylococcus albus

	frozen	room temp.
Count per ml	19,400,000	19,400,000
One hour	3,450,000	5,100,000
Four hours	450,000	379,000
One day	1,360	105
Two days	610	0
Four days	59	0
Six days	2	0
Eight days	0	0
Ten days	0	0

Escherichia coli

	frozen	room temp.
Count per ml	9,120,000	9,120,000
Four hours	47,000	19,000
Twelve hours	104	12
One day	6	0
Two days	0	0
Four days	0	0

A control of pH 3.7, frozen in the same manner and containing 2,650,000 cells, showed five cells viable after five days, but none by ten days.

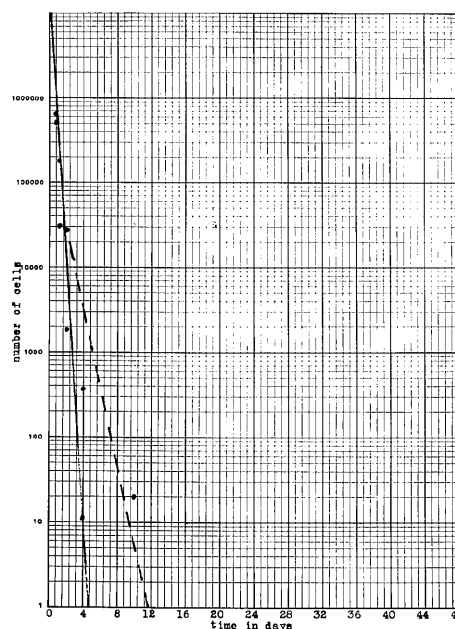


Figure 26.

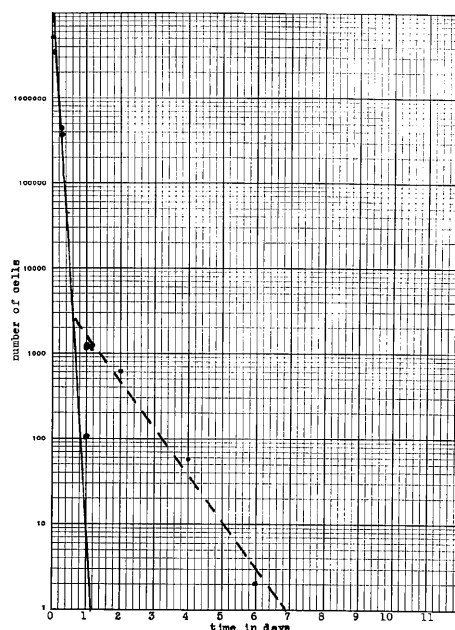


Table XIV.

Figure 27.

Survival in fresh orange juice.
Acidity as citric acid 1.4%. pH 3.4.

Escherichia typhosa

	Frozen	room temp.
Count per ml	66,800,000	66,800,000
Thirty minutes	2,460,000	-
Four hours	1,460	60,000
Twelve hours	490	60
One day	50	4
Two days	0	0
Three days	0	0
Four days	0	0

Survival rate shown in figure 27.

Salmonella paratyphi

	Frozen	room temp.
Count per ml	2,120,000	2,120,000
Thirty minutes	30,200	92,000
Four hours	86	2
Eight hours	3	0
Twelve hours	0	0
One day	0	0
Two days	0	0

Salmonella schottmuelleri

	Frozen	room temp.
Count per ml	87,000,000	87,000,000
One hour	2,570,000	-
Four hours	3,100,000	146,400
Twelve hours	2,000	48,000
One day	3	24
Two days	0	0
Four days	0	0

Aerobacter aerogenes

	Frozen	room temp.
Count per ml	26,000,000	26,000,000
Four hours	1,090,000	740,000
Twelve hours	33,000	9,700
One day	28	0
Two days	1	0
Four days	0	0
Six days	0	0

Survival rate shown in figure 28.

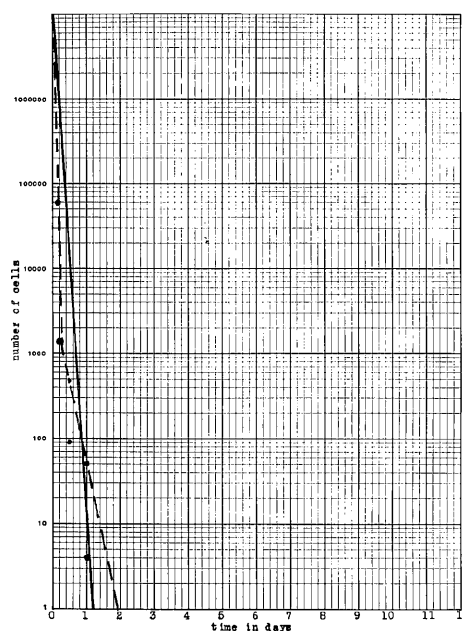


Figure 28.

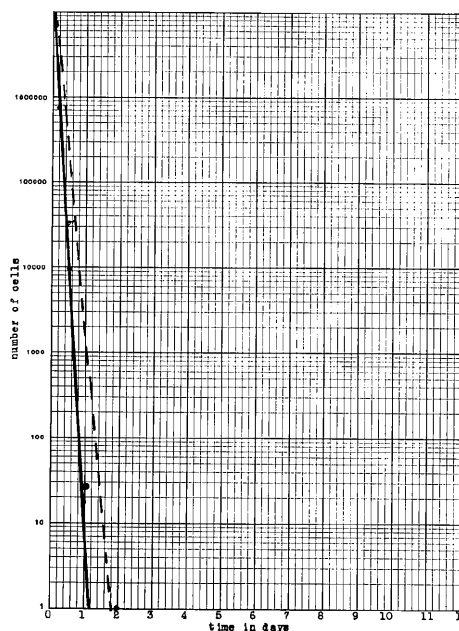


Table XV.

Figure 29.

Survival in fresh orange juice.
Acidity as citric acid 1.7, pH 3.4.

Shigella paratyphosiae Sonne

	frozen	room temp.
Count per ml	1,730,000	1,730,000
Four hours	1,580	~ 7,000
Twelve hours	140	81
One day	0	0
Two days	0	0
Four days	0	0

Shigella dysenteriae

	frozen	room temp.
Count per ml	6,800,000	6,800,000
Four hours	4,100	8,800
Twelve hours	80	0
One day	0	0
Two days	0	0

Survival rate shown in figure 29.

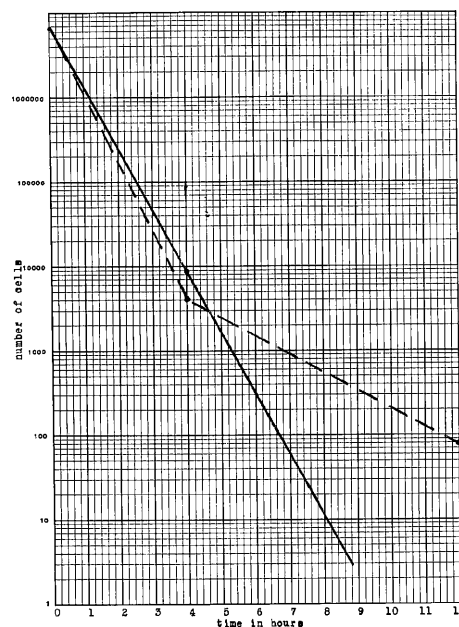


Table XVI.

Figure 30.

Survival in bottled grape juice.
 32.5 mls N/10 NaOH required to
 neutralize ten ml grape juice.
 Vacuum 11.4", pH 5.3, added sugar.

Serratia marcescens

	frozen	room temp.
Count per ml	6,500,000	6,300,000
Twenty minutes	12,640	19,000
Forty minutes	30	150
One hour	2	60
Two hours	0	0
Four hours	0	0

Staphylococcus aureus

	frozen	room temp.
Count per ml	23,000,000	23,000,000
Thirty minutes	2,760,000	5,200,000
One hour	1,750,000	2,520,000
Two hours	1,600,000	1,360,000
Four hours	640,000	9,400
Eight hours	88,000	340
One day	1,350	0
Two days	15	0
Four days	0	0

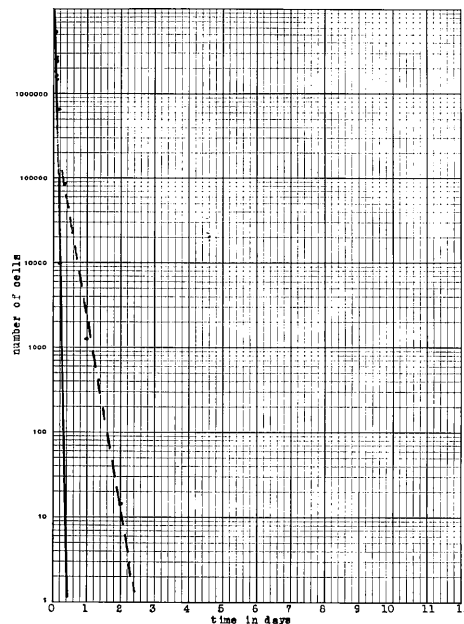


Figure 31.

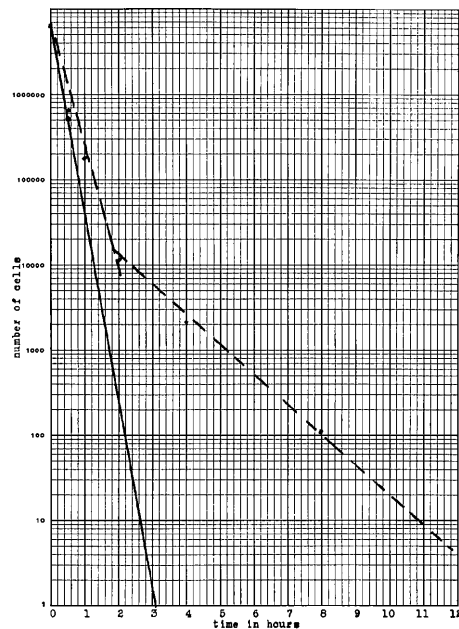
Survival rate shown in figure 30.

Escherichia coli

	frozen	room temp.
Count per ml	11,800,000	11,800,000
One hour	4,800,000	8,300,000
Two hours	1,300,000	300,000
Four hours	120,000	260,000
Eight hours	37,000	13,300
One day	2,720	20
Two days	0	0
Four days	0	0

Shorthella typhosa

	frozen	room temp.
Count per ml	6,200,000	6,200,000
Thirty minutes	510,000	670,000
One hour	125,000	46,500
Two hours	10,800	260
Four hours	2,200	0
Eight hours	112	0
One day	0	0
Two days	0	0



Survival rate shown in figure 31.

Table XVII.

Survival in bottled grape juice.
 88.6 ml 2/12 NaOH required to
 neutralize ten ml grape juice.
 Vacuum 11.4", pH 3.2, added sugar.

Salmonella paratyphi

	frozen	room temp.
Count per ml	15,100,000	15,100,000
Thirty minutes	890,000	241,000
One hour	652,000	1,300
Two hours	87,000	0
Four hours	5,500	0
Eight hours	7	0
One day	0	0
Two days	0	0

Survival rate shown in figure 32.

Salmonella schottmuelleri

	frozen	room temp.
Count per ml	8,500,000	8,500,000
Thirty minutes	870,000	955,000
Four hours	910	18,500
Eight hours	144	420
One day	0	0
Two days	0	0

Aerobacter aerogenes

	frozen	room temp.
Count per ml	39,600,000	39,600,000
Thirty minutes	5,200,000	4,500,000
One hour	1,300,000	980,000
Two hours	710,000	50,000
Four hours	65,000	140
Eight hours	420	0
One day	*20	0
Two days	0	0
Four days	0	0

*in 1-10 dil'n; none in 1 ml sample.

Survival rate shown in figure 33.

Shigella paradysenteriae Sonne

	frozen	room temp.
Count per ml	67,000,000	67,000,000
One day	5,400	15,700
Two days	0	0
Four days	0	0

Figure 32.

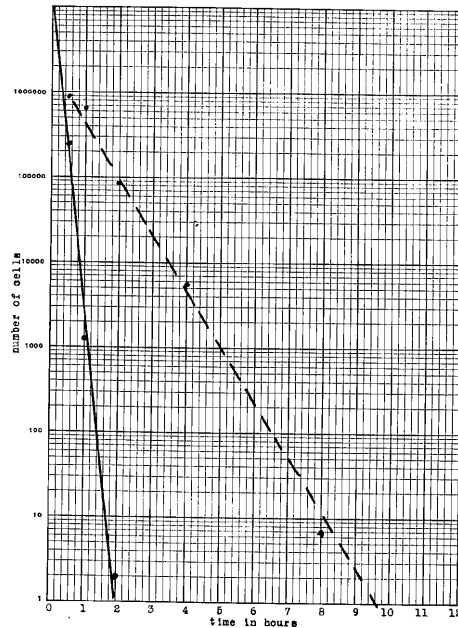


Figure 33.

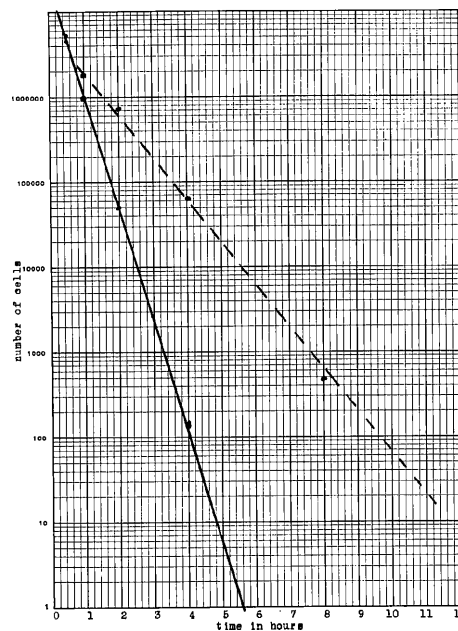


Table XVIII.

Survival in bottled grape juice.
38.5 mls N/10 NaOH required to
neutralize ten ml grape juice.
Vacuum 11.4", pH 3.2, added sugar.

Shigella dysenteriae

	frozen	room temp.
Count per ml	4,100,000	4,100,000
Thirty minutes	310,000	610,000
One hour	303,000	380,000
Two hours	3,620	248,000
Four hours	40	2,700
Eight hours	2	32
One day	0	0
Two days	0	0

slake neutralized.

Table XIX.

Survival in bottled grape juice. Un-
sweetened. 21.8 mls N/10 NaOH re-
quired to neutralize ten ml diluted
grape juice. Vacuum 17", pH 3.7.

Serratia marcescens

	frozen	room temp.
Count per ml	1,620,000	1,620,000
One hour	36	4
Two hours	5	0
Four hours	0	0

Staphylococcus aureus

	Frozen	room temp.
Count per ml	1,020,000	1,020,000
One hour	640,000	324,000
Two hours	430,000	205,000
Four hours	210,000	77,000
Eight hours	62,000	1,200
One day	120	4
Two days	0	0

Survival rate shown in figure 34.

Escherichia typhosa

	frozen	room temp.
Count per ml	9,200,000	9,200,000
One hour	410,000	302,000
Two hours	78,000	940
Eight hours	66	1
One day	2	0
Two days	0	0

Survival rate shown in figure 35.

Figure 34.

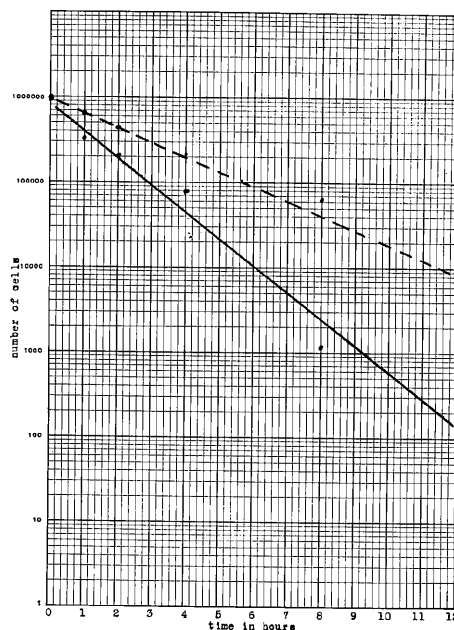


Figure 35.

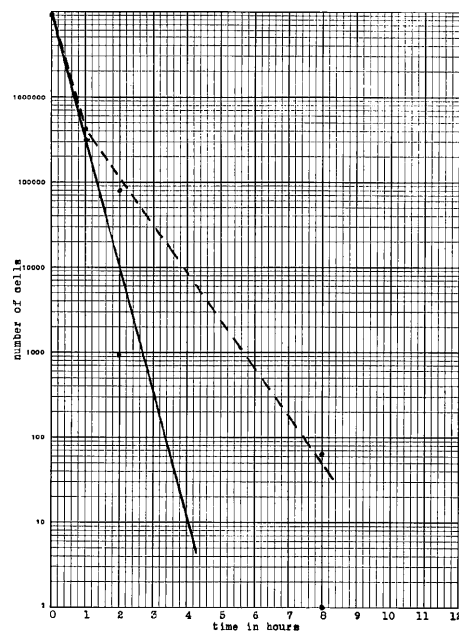


Table VI.

Survival in canned apple juice.
 0.03 mls N/10 NaOH required to
 neutralize ten ml apple juice.
 Vacuum 8", pH 3.45.

Staphylococcus aureus

	Frozen	room temp.
Count per ml	7,400,000	7,400,000
Eight hours	2,170,000	278,000
One day	630,000	16,900
Two days	600	10
Three days	47	1
Four days	1	0
Five days	0	0

Survival rate shown in Figure 36.

Escherichia coli

	Frozen	room temp.
Count per ml	18,100,000	18,100,000
Eight hours	2,000	-
One day	140	117,000
Two days	7	8,100
Three days	0	317
Four days	0	15
Five days	0	0

Salmonella paratyphi

	Frozen	room temp.
Count per ml	27,600,000	27,600,000
Eight hours	526,000	940,000
One day	0	1,200
Two days	0	0

Salmonella schottmulleri

	Frozen	room temp.
Count per ml	16,200,000	16,200,000
Eight hours	5	14,500
One day	0	3,100
Two days	0	170
Three days	0	0
Four days	-	0

Survival rate shown in figure 37.

Eberthella typhosa

	Frozen	room temp.
Count per ml	2,700,000	2,700,000
Eight hours	4	9
One day	0	0
Two days	0	0

Figure 36.

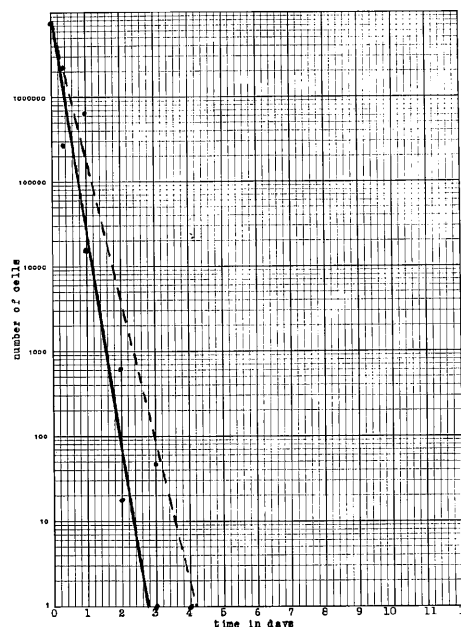


Figure 37.

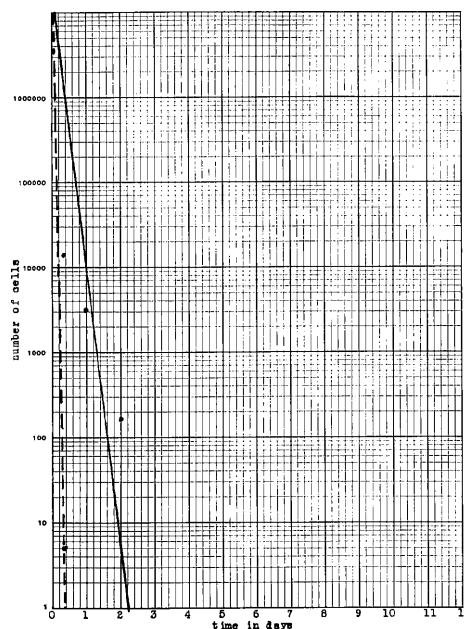


Table XXI.

Survival in canned apple juice.
 9.00 ml 5/10 NaOH required to
 neutralize ten ml apple juice.
 Vacuum 5", pH 3.45.

<u>Shigella paradysenteriae</u> Sonne		
	frozen	room temp.
Count per ml	750,000	750,000
Eight hours	123	409
One day	0	0
Two days	0	0
Three days	0	0

<u>Shigella dysenteriae</u>		
	frozen	room temp.
Count per ml	270,000	270,000
Eight hours	83	28,000
One day	0	280
Two days	0	0
Three days	0	0

approximate number.

Survival rate shown in figure 33.

Figure 33.

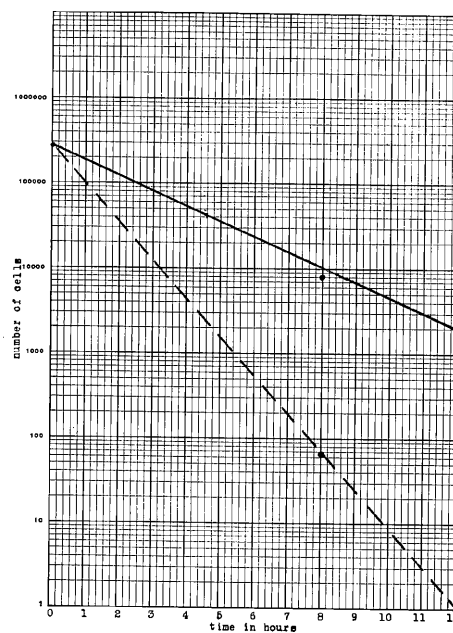


Table XXII.

Survival in canned cherry juice
with 2% sugar added. 12.8 mls
N/10 NaOH required to neutralize
ten ml cherry juice. Vacuum $7\frac{1}{2}$ ",
pH 3.3.

Serratia marcescens

	frozen	room temp.
Count per ml	10,200,000	10,200,000
One day	0	0
Two days	0	0

Staphylococcus albus

	frozen	room temp.
Count per ml	79,000,000	79,000,000
One day	8,600	510
Two days	0	0

Staphylococcus aureus

	frozen	room temp.
Count per ml	33,400,000	33,400,000
One day	590,000	112,000
Two days	4,100	2,700
Three days	510	173
Four days	50	3
Five days	6	0
Ten days	0	0

Survival rate shown in figure 39.

Escherichia coli

	frozen	room temp.
Count per ml	890,000	890,000
One day	1,500	2,200
Two days	10	0
Three days	0	0
Four days	0	0

Sherrthella typhosa

	frozen	room temp.
Count per ml	14,600,000	14,600,000
One day	232	1,180
Two days	12	0
Three days	0	0
Four days	0	0

Survival shown in figure 40.

Salmonella paratyphi

	frozen	room temp.
Count per ml	780,000	780,000
One day	0	0
Two days	0	0

Figure 39.

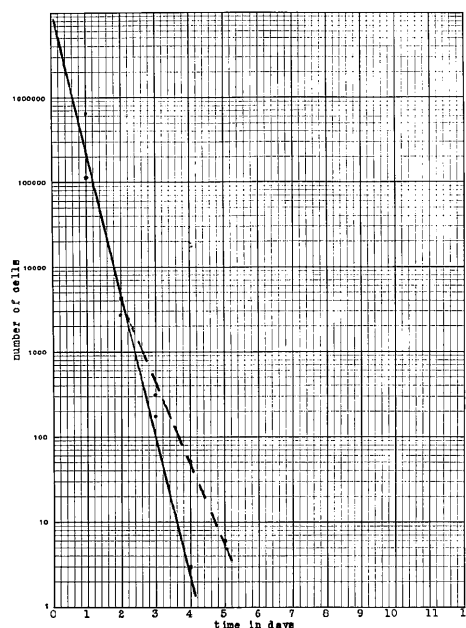


Figure 40.

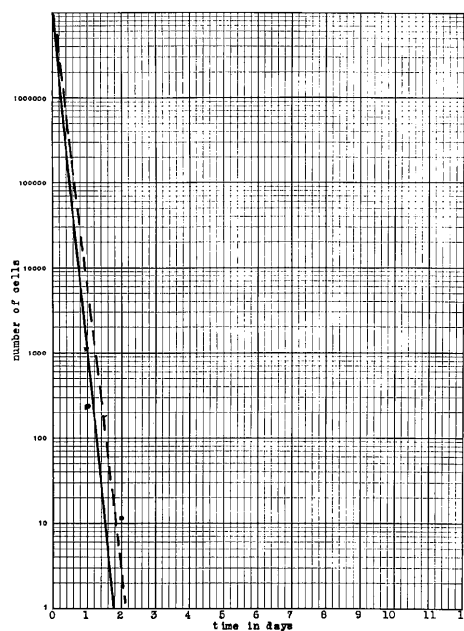


Table XIII.

Figure 41.

Survival in canned cherry juice
with 26 sugar added. 10.9 mlc
N/10 NaOH required to neutralize
ten ml cherry juice. Vacuum $7\frac{1}{2}$ "
pH 5.3.

Salmonella schottmuelleri

	frozen	room temp.
Count per ml	81,000,000	81,000,000
One day	16,000	20,000
Two days	20	80
Three days	3	3
Four days	0	0
Five days	0	0

Salmonella aertryke

	frozen	room temp.
Count per ml	85,000,000	85,000,000
One day	120,000	64,000
Two days	1,400	250
Three days	340	70
Four days	10	1
Five days	0	0
Ten days	0	0

Aerobacter aerogenes

	frozen	room temp.
Count per ml	47,400,000	47,400,000
One day	780	3,930
Two days	10	45
Three days	0	3
Four days	0	0
Five days	0	0

Survival rate shown in figure 41.

Shigella dysenteriae

	frozen	room temp.
Count per ml	8,200,000	8,200,000
One day	43	323
Two days	3	0
Three days	0	0
Four days	0	0

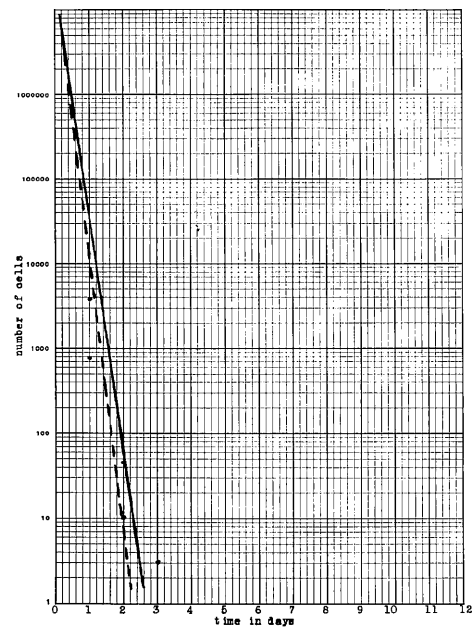


Figure 42.

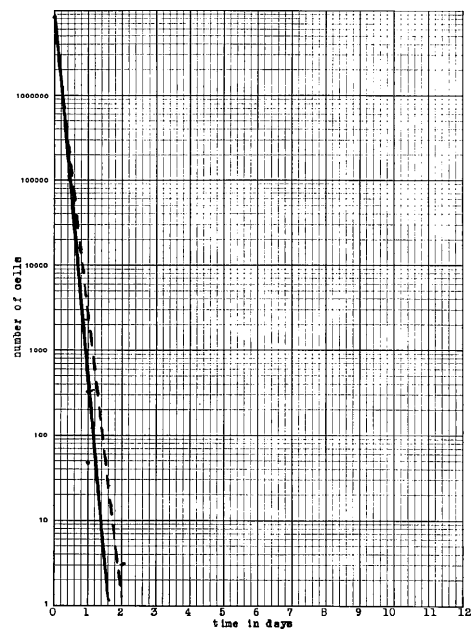


Table XXIV.

Figure 43.

Survival in canned cherry juice
with 2% sugar added. 12.3 mls
N/10 NaOH required to neutralize
ten ml cherry juice. Vacuum 7½",
pH 3.3; samples frozen at -16°C
(or 3.2°F)

Escherichia coli

	frozen	room temp.
Count per ml	6,900,000	6,900,000
One day	3,900	3,900
Two days	16	11
Three days	0	0
Four days	0	0

Staphylococcus aureus

	frozen	room temp.
Count per ml	63,000,000	63,000,000
One day	44,800	19,800
Two days	12,700	498
Three days	1,040	25
Four days	510	2
Five days	192	0
Ten days	9	0
Fifteen days	0	0

Survival rate shown in figure 43.

Escherichia typhosa

	frozen	room temp.
Count per ml	41,000,000	41,000,000
One day	62,000	38,000
Two days	2,100	440
Three days	17	2
Four days	1	0
Five days	0	0

Survival rate shown in figure 44.

Salmonella paratyphi

	frozen	room temp.
Count per ml	45,000,000	45,000,000
One day	36	0
Two days	0	0

Aerobacter aerogenes

	frozen	room temp.
Count per ml	3,900,000	3,900,000
One day	22,000	53,000
Two days	101	9
Three days	6	0
Four days	0	0
Five days	0	0

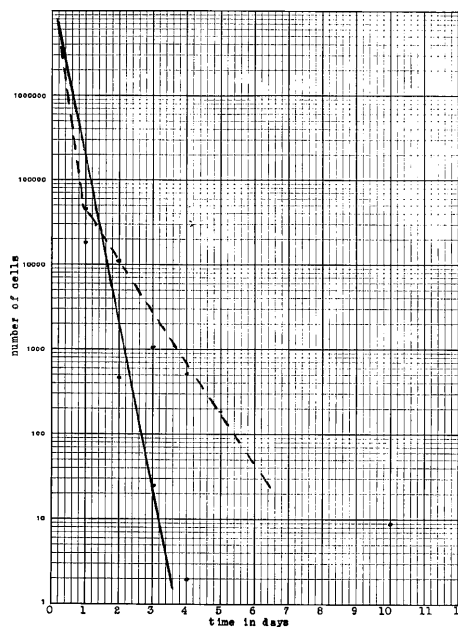


Figure 44.

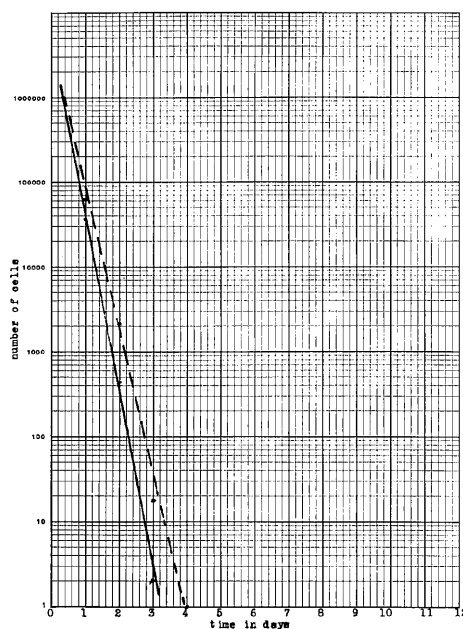


Table XIV.

Survived in canned cherry juice
with 20 sugar added. 12.5 mls
N/10 NaOH required to neutralize
ten ml cherry juice. Vacuum 7 $\frac{1}{2}$ "
pH 5.5; samples frozen at -80°C
(or 17.5°F)

Escherichia coli

	Frozen	room temp.
Count per ml	6,000,000	6,900,000
One day	700	1,790
Two days	0	0
Three days	0	0

Staphylococcus aureus

	Frozen	room temp.
Count per ml	63,000,000	63,000,000
One day	135,500	217,000
Two days	2,100	340
Three days	159	20
Four days	0	0
Five days	0	0

Survival rate shown in figure 45.

Shorthella typhosa

	Frozen	room temp.
Count per ml	41,000,000	41,000,000
One day	32	30,300
Two days	4	305
Three days	0	0
Four days	0	0

Survival rate shown in figure 46.

Salmonella paratyphi

	Frozen	room temp.
Count per ml	45,000,000	45,000,000
One day	0	0
Two days	0	0

Salmonella schottmulleri

	Frozen	room temp.
Count per ml	5,100,000	5,100,000
One day	2,600	1,900
Two days	32	14
Three days	9	0
Four days	0	0

Aerobacter aerogenes

	frozen	room temp.
Count per ml	3,900,000	3,900,000
One day	5,700	36,000
Two days	5	10
Three days	0	0

Figure 45.

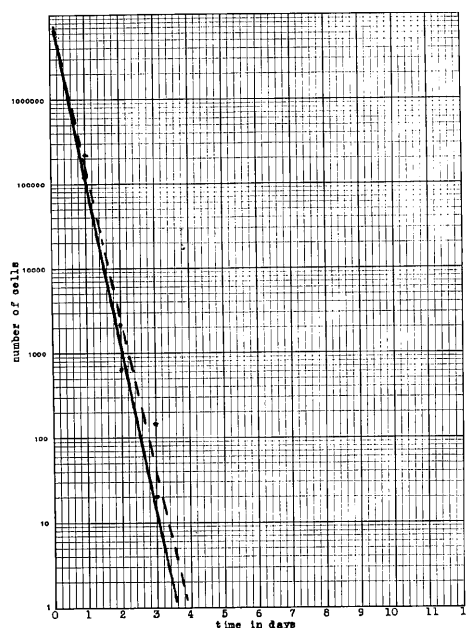


Figure 46.

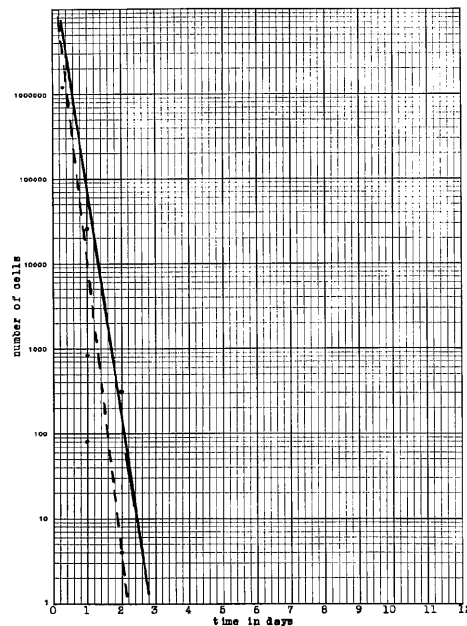


Table XXVI.

Survival in canned apricot nectar.
7.5 mls 3/10 NaOH required to
neutralize ten mls apricot nectar.
Vacuum 5.8", pH 3.7.

Staphylococcus aureus

	frozen	room temp.
Count per ml	26,200,000	26,200,000
Eight hours	9,800,000	14,100,000
One day	4,430,000	2,600,000
Two days	(?)7,200,000	40,000
Four days	96,000	18
Six days	20,800	0
Eight days	1,200	0
Ten days	630	0
Fifteen days	0	
Twenty days	0	

Survival rate shown in figure 47.

Escherichia coli

	frozen	room temp.
Count per ml	19,800,000	19,800,000
Eight hours	282,000	-
One day	137,000	1,420,000
Two days	132,000	710,000
Four days	3,300	4,700
Six days	490	212
Eight days	53	0
Ten days	0	0

Survival rate shown in figure 48.

Shorthella typhosa

	frozen	room temp.
Count per ml	20,500,000	20,500,000
Eight hours	44,200	3,000,000
One day	3	442,000
Two days	* 0	23,000
Four days	0	2
Six days	0	0

*in a duplicate run, S. typhosa were
viable in the frozen ampoules at the
end of two days.

Salmonella paratyphi

	frozen	room temp.
Count per ml	78,000,000	78,000,000
Eight hours	1,340,000	1,340,000
One day	14,800	234,000
Two days	76	0
Four days	0	0
Six days	0	0

Figure 47.

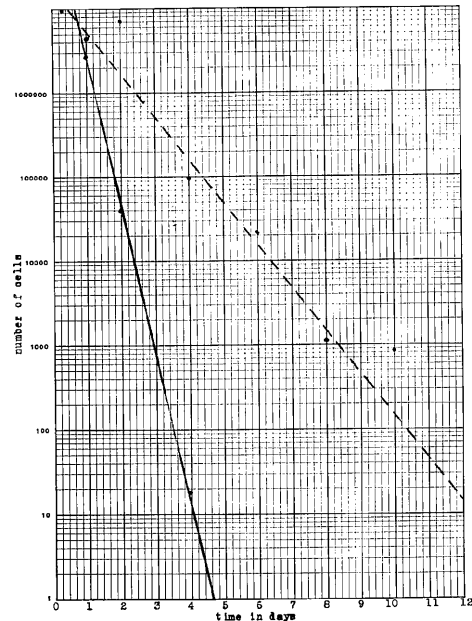


Figure 48.

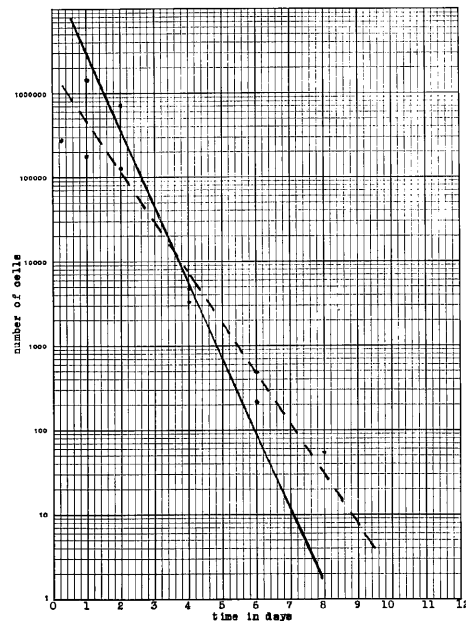


Table XXVII.

Survival in canned apricot nectar.
7.3 mlis N/10 NaOH required to
neutralize ten ml apricot nectar.
Vacuum 5.3", pH 3.7.

Salmonella schottmulleri

	frozen	room temp.
Count per ml	57,400,000	57,400,000
Eight hours	1,690,000	8,900,000
One day	220,000	5,700,000
Two days	17,400	250,000
Four days	820	*10,000
Six days	* 10	129
Eight days	0	* 20
Ten days	0	0
Fifteen days	0	0

sin 1-10 dil'n.

Survival rate shown in figure 49.

Shigella paradysenteriae Sonne

	frozen	room temp.
Count per ml	350,000	350,000
Eight hours	1,410	4,050
One day	11	52
Two days	1	0
Four days	0	0
Six days	0	0

Shigella dysenteriae

	frozen	room temp.
Count per ml	1,300,000	1,300,000
Eight hours	6,900	40,300
One day	102	6,900
Two days	43	71
Four days	0	0
Six days	0	0

Survival rate shown in figure 50.

Figure 49.

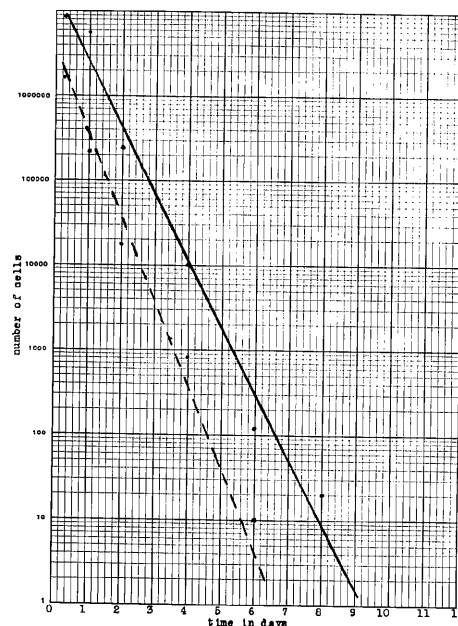


Figure 50.

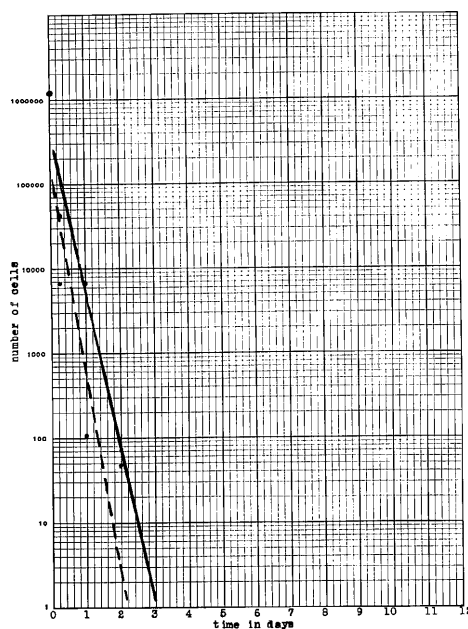


Table XVIII.

Survival in canned pineapple juice.
3.6 mls 1/10 NaOH required to neu-
tralize ten ml pineapple juice. Vac-
uum 8.6", pH 3.5.

Serratia marcescens

	<u>Frozen</u>	<u>room temp.</u>
Count per ml	2,300,000	2,500,000
One day	570,000	455,000
Two days	132,000	270,000
Three days	155,000	115,000
Five days	107,000	48,000
Ten days	36,200	0
Thirty days	24	0
Sixty days	3	
Ninety days	0	
120 days	0	

Staphylococcus albus

	<u>Frozen</u>	<u>room temp.</u>
Count per ml	24,000,000	24,000,000
One day	42,000	280,000
Two days	8,000	1,300
Three days	100	37
Five days	10	0
Ten days	0	0

Staphylococcus aureus

	<u>Frozen</u>	<u>room temp.</u>
Count per ml	6,100,000	6,100,000
One day	73,000	360,000
Two days	60,000	332,000
Three days	15,900	64,000
Five days	34,000	9,800
Ten days	1,900	202
Thirty days	85	0
Sixty days	7	0
Ninety days	0	
One-hundred twenty days	0	

Survival rate shown in figure 51.

Escherichia coli

	<u>Frozen</u>	<u>room temp.</u>
Count per ml	31,000,000	31,000,000
One day	161	9,300,000
Two days	5	8,900,000
Three days	2	6,100,000
Five days	1	7,700,000
Ten days	0	1,800,000
Thirty days		24,600,000
Sixty days		15,000,000
Ninety days		590,000
One-hundred twenty days		30,000

Figure 51.

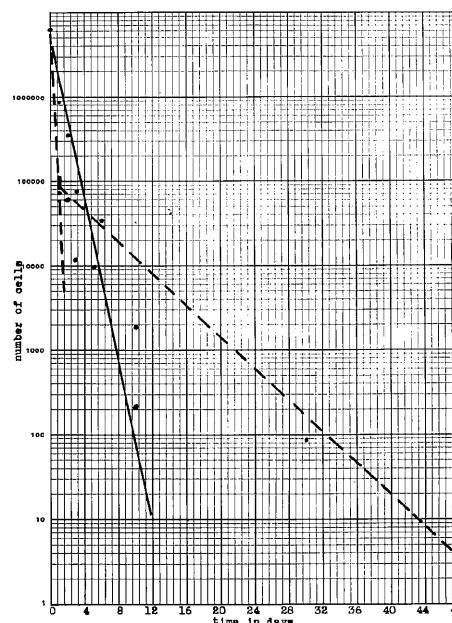
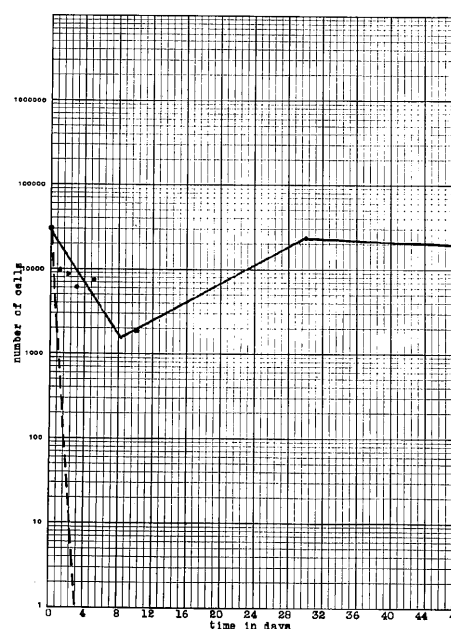


Figure 52.



Survival rate of E. coli.

Table XXII.

Survival in canned pineapple juice.
5.8 mls N/10 NaOH required to neu-
tralize ten ml pineapple juice. Vac-
uum 3.6", pH 3.5.

Salmonella paratyphi

	frozen	room temp.
Count per ml	9,800,000	9,800,000
Four hours	82,000	750,000
One day	16	380,000
Two days	0	43
Three days	0	0
Five days	0	0

Salmonella schottmuelleri

	frozen	room temp.
Count per ml	40,200,000	40,200,000
Four hours	1,110,000	9,500,000
One day	29,000	177,000
Two days	320	58
Three days	4	9
Five days	1	0
Ten days	0	0
Thirty days	0	0

Survival rate shown in figure 53.

Aerobacter aerogenes

	frozen	room temp.
Count per ml	21,900,000	21,900,000
One day	52,000	25,300,000
Two days	88	20,800,000
Three days	12	15,900,000
Five days	5	9,400,000
Ten days	0	11,600,000
Thirty days	0	45,600,000
Sixty days		7,800,000
Ninety days		640,000
One-hundred twenty days		290,000

Survival rate shown in figure 54.

Shigella dysenteriae

	frozen	room temp.
Count per ml	6,800,000	6,800,000
One day	910	227,000
Two days	40	133,000
Three days	5	75,000
Five days	0	700
Ten days	0	0
Thirty days	0	0

Figure 53.

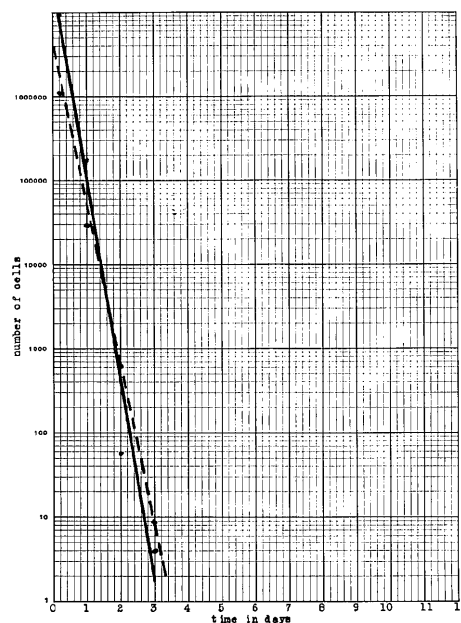


Figure 54.

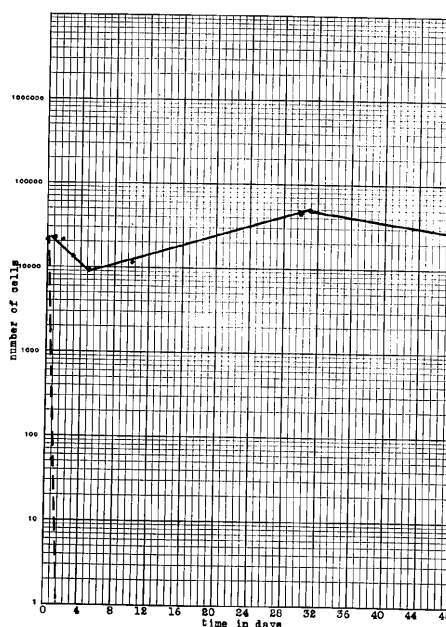


Table XXX.

Survival in canned pineapple juice.
5.8 mlc N/10 NaOH required to neutralize ten ml pineapple juice. Vacuum 2.6", pH 3.5.

Salmonella enteritidis

	frozen	room temp.
Count per ml	7,600,000	7,600,000
One day	41,000	530,000
Two days	1,100	1,970
Three days	128	266
Five days	6	0
Ten days	0	0
Thirty days	0	0

Survival rate shown in figure 55.

Shigella paradysenteriae Sonne

	frozen	room temp.
Count per ml	820,000	820,000
One day	112	4,300
Two days	20	65
Three days	0	0
Five days	0	0
Ten days	0	0

Survival rate shown in figure 56.

Eberthella typhosa

	frozen	room temp.
Count per ml	4,000,000	4,000,000
Four hours	243,000	1,960,000
Twelve hours	13,100	*170,600
One day	550	56,800
Two days	11	66
Three days	(?) 1	2
Five days	0	0
Ten days	0	0

*980 colonies in 1-200 dil'n

Figure 55.

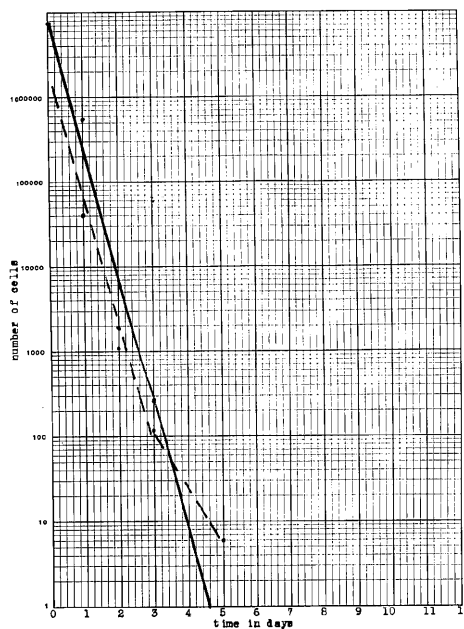


Figure 56.

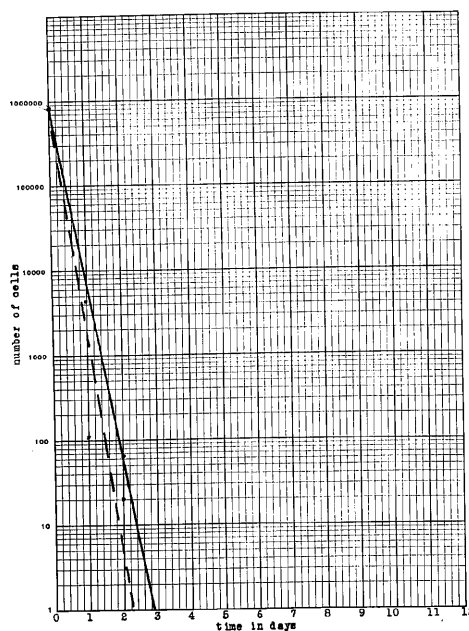


Table VIII.

Figure 57.

Survival in canned tomato juice.
 9.7 mlis N/10 NaOH required to
 neutralize ten ml tomato juice.
 Vacuum 6", pH 4.5.

Serratia marcescens

	Frozen	room temp.
Count per ml	1,560,000	1,560,000
One day	2,980	3,000,000
Two days	210	1,340,000
Three days	98	4,500,000
Four days	12	920,000
Five days	2	540,000
Ten days	0	3,700
Twenty days	0	42
Thirty days	0	0

Survival rate shown in figure 57.

Staphylococcus albus

	Frozen	room temp.
Count per ml	1,760,000	1,760,000
One day	680,000	1,500,000
Two days	290,000	970,000
Three days	96,000	540,000
Four days	138,000	102,000
Five days	85,000	160,000
Ten days	19	2,000
Twenty days	0	4
Thirty days	0	0

Staphylococcus aureus

	Frozen	room temp.
Count per ml	2,200,000	2,200,000
One day	1,070,000	870,000
Two days	310,000	250,000
Four days	38,000	71,000
Five days	1,390	5,300
Ten days	0	33
Twenty days	0	0
Thirty days	0	0

Survival rate shown in figure 58.

Salmonella paratyphi

	Frozen	room temp.
Count per ml	22,000,000	22,000,000
One day	38,000	1,400,000
Two days	0	433,000
Three days	0	95,000
Four days	0	6,300
Five days	0	5,200
Ten days		37
Twenty days		0

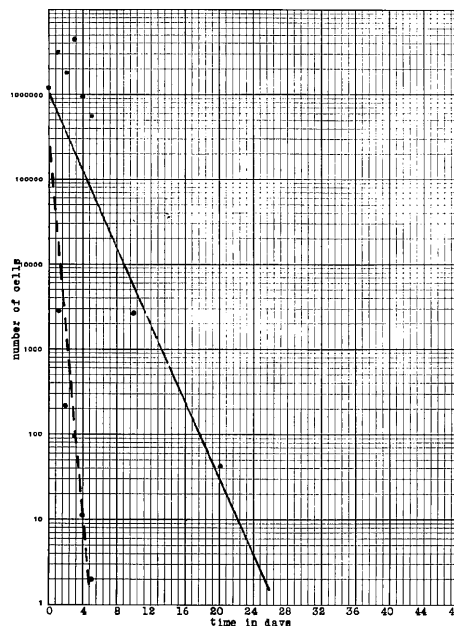


Figure 58.

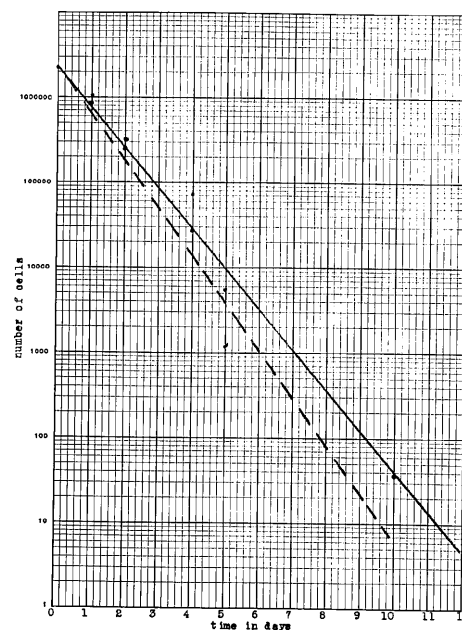


Table XXVII.

Survival in canned tomato juice.
9.7 mls N/10 NaOH required to
neutralize ten ml tomato juice.
Vacuum 6", pH 4.5.

Escherichia coli

	Frozen	room temp.
Count per ml	10,100,000	10,100,000
One day	47,000	140,000,000
Two days	36,000	208,000,000
Three days	-	230,000,000
Four days	1,400	164,000,000
Five days	900	306,000,000
Ten days	4	94,000,000
Twenty days	0	8,000,000
Thirty days	0	1,400,000
Sixty days		80,000
Ninety days		55,000

Survival rate shown in figure 59.

Aerobacter aerogenes

	Frozen	room temp.
Count per ml	51,000,000	51,000,000
One day	5,800,000*	200,000,000
Two days	365,000	472,000,000
Five days	900	600,000,000
Ten days	10	920,000,000
Twenty days	0	290,000,000
Thirty days	0	460,000,000
Sixty days		214,000,000
Ninety days		75,000,000
One hundred twenty days		34,000,000

*Estimate from a 1-100,000 plate.

Survival rate shown in figure 60.

Salmonella suispestifer

	Frozen	room temp.
Count per ml	4,700,000	4,700,000
One day	1,030,000	8,150,000
Five days	240,000	1,670,000
Ten days	52,700	360,000
Twenty days	7,000	65,000
Thirty days	830	1,620
Sixty days	0	15
Ninety days	0	0
One hundred twenty days		0

Figure 59.

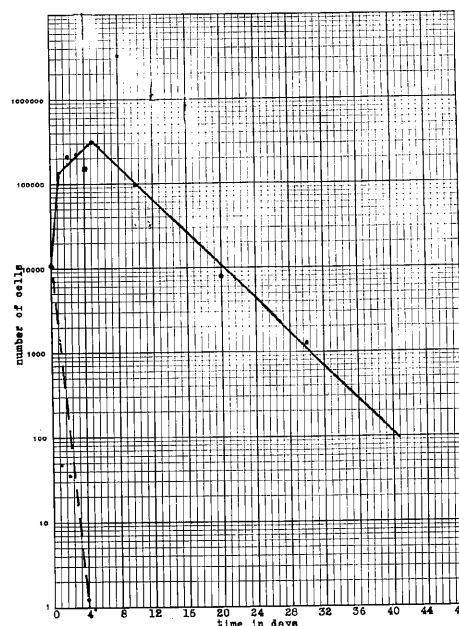


Figure 60.

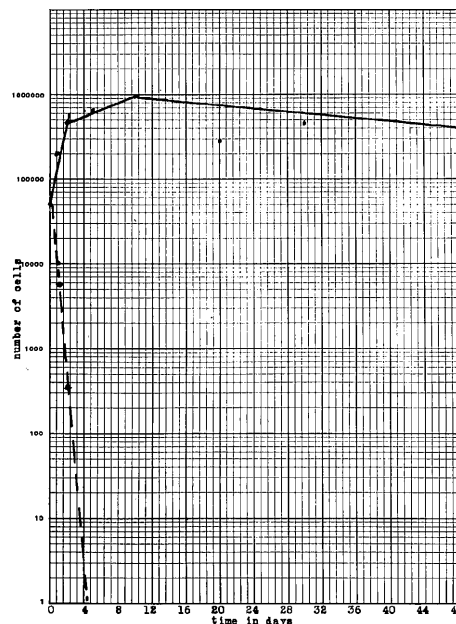


Table XXXIII.

Survival in canned tomato juice.
0.7 ml. N/10 NaOH required to
neutralize ten ml tomato juice.
Vacuum 6", pH 4.8.

Sherrthella typhosa

	Frozen	room temp.
Count per ml	7,800,000	7,800,000
One day	4,000	3,250,000
Two days	600	5,000,000
Three days	320	3,500,000
Four days	-	1,320,000
Five days	14	930,000
Ten days	1	80,000
Twenty days	0	1,400
Thirty days	0	0
Sixty days	0	0

Survival rate shown in figure 61.

Shigella dysenteriae

	Frozen	room temp.
Count per ml	10,600,000	10,000,000
One day	44,000	1,340,000
Two days	6,100	2,100,000
Three days	590	1,200,000
Five days	10	77,000
Ten days	0	63
Twenty days	0	0
Thirty days	0	0

Survival rate shown in figure 62.

Shigella paradysenteriae Sonne

	frozen	room temp.
Count per ml	4,100,000	4,100,000
One day	29,000	560,000
Two days	7,300	110,000
Three days	1,950	44,000
Five days	100	800
Ten days	0	26
Twenty days	0	1
Thirty days	0	0

Figure 61.

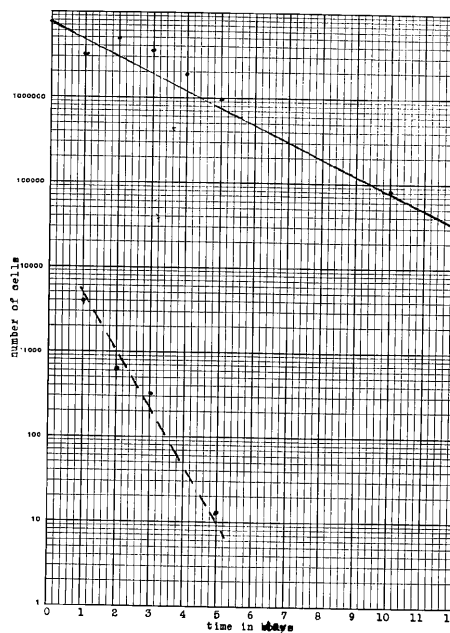


Figure 62.

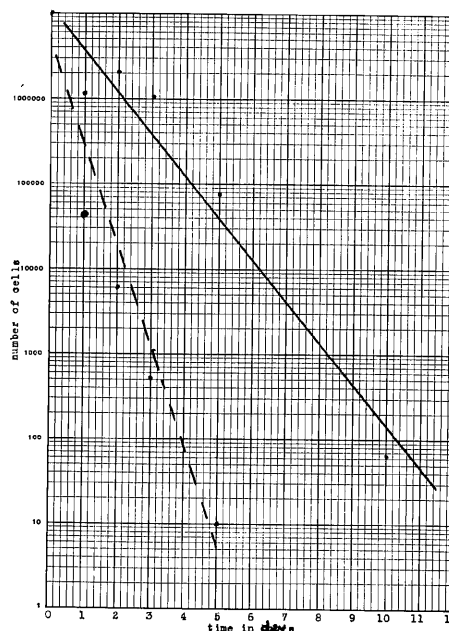


Table XXXIV.

Survival in fresh tomato juice.
6.95 mls N/10 NaOH required to
neutralize ten ml tomato juice.
pH 4.35.

Corratia marcescens

	frozen	room temp.
Count per ml	1,180,000	1,180,000
One day	32,800	710,000
Two days	4,900	600,000
Three days	1,200	340,000
Four days	90	27,400
Five days	135	10,900
Ten days	300	300
Thirty days	14	4
Sixty days	1	0
Ninety days	0	0
One hundred twenty days	0	0

Survival rate shown in figure 63.

Staphylococcus albus

	frozen	room temp.
Count per ml	31,800,000	31,800,000
One day	2,800,000	17,500,000
Two days	1,020,000	1,580,000
Three days	-	142,000
Four days	223,000	108,000
Five days	170,000	-
Ten days	20,400	50,000
Thirty days	7	35
Sixty days	0	0
Ninety days	0	0

Staphylococcus aureus

	frozen	room temp.
Count per ml	19,100,000	19,100,000
One day	1,970,000	10,600,000
Two days	1,153,000	3,300,000
Three days	755,000	1,200,000
Four days	230,000	840,000
Five days	-	210,000
Ten days	29,600	55,000
Thirty days	10,200	13
Sixty days	760	0
Ninety days	8	0
One hundred twenty days	0	0

Survival rate shown in figure 64.

Figure 63.

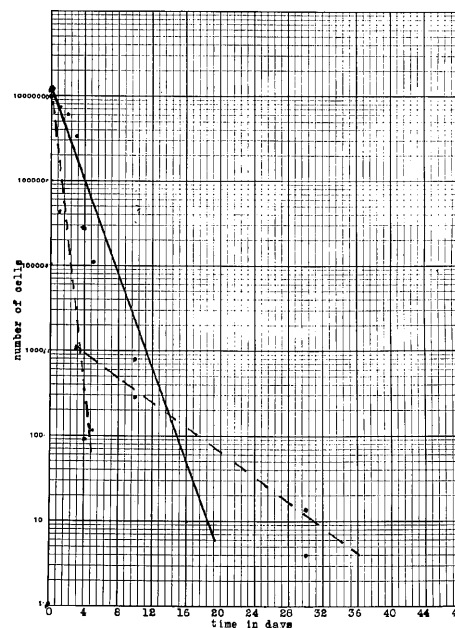


Figure 64.

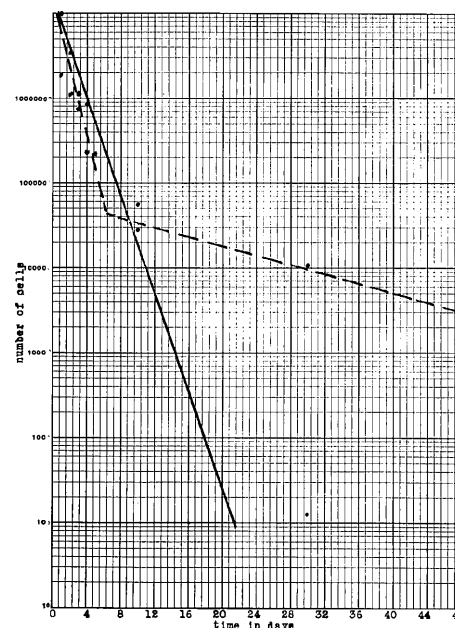


Table XXIV.

Survival in fresh tomato juice.
0.35 lbs 7/10 NaOH required to
neutralise ten ml tomato juice.
pH 4.55.

Escherichia coli

	Frozen	Room temp.
Count per ml	24,300,000	24,300,000
One day	455,000	34,400,000
Two days	640,000	28,400,000
Four days	250,000	14,300,000
Five days	310,000	24,500,000
Ten days	110,000	9,900,000
Twenty days	1,830	690,000
Thirty days	242	33,000
Sixty days	310	3170
Ninety days	0	111

sin 1-10 dil'n.

Survival rate shown in figure 65.

Shorthella typhosa

	Frozen	room temp.
Count per ml	6,400,000	6,400,000
One day	90,000	16,400,000
Two days	67,000	4,600,000
Three days	-	690,000
Four days	72,000	117,000
Five days	42,000	45,500
Ten days	14,200	16
Thirty days	6	0
Sixty days	0	0
Ninety days	0	0

Survival rate shown in figure 66.

Salmonella paratyphi

	frozen	room temp.
Count per ml	1,720,000	1,720,000
One day	58,000	540,000
Two days	24,100	350,000
Three days	13,200	-
Four days	9,400	37,000
Ten days	360	91
Twenty days	7	0
Thirty days	0	0
Sixty days	0	0

Figure 65.

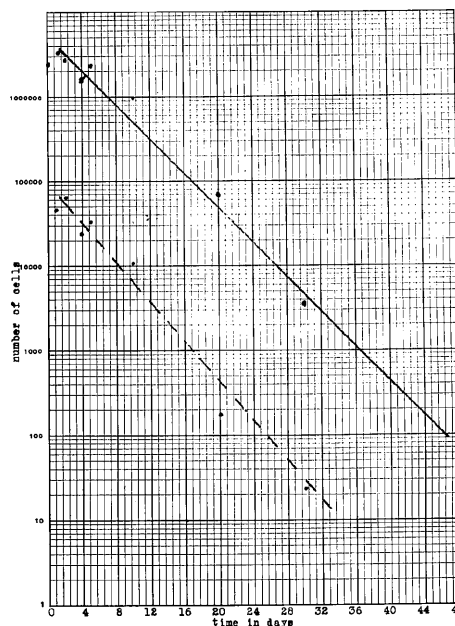


Figure 66.

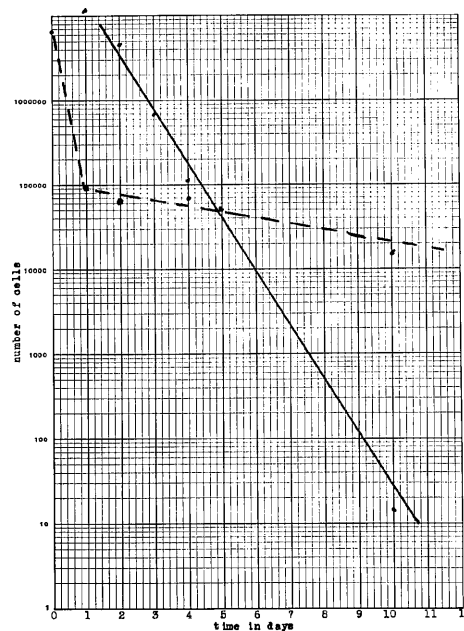


Table XVI.

Figure 67.

Survival in fresh tomato juice.
 8.85 mlis 2/10 N/10 required to
 neutralize ten ml tomato juice.
 pH 4.55.

Salmonella schottmuelleri

	Frozen	room temp.
Count per ml	35,000,000	35,000,000
One day	104,000	19,400,000
Two days	85,000	30,600,000
Three days	-	21,000,000
Four days	57,700	-
Ten days	33,000	91,000
Thirty days	171	32
Sixty days	0	0
Ninety days	0	0

Aerobacter aerogenes

	Frozen	room temp.
Count per ml	134,000,000	134,000,000
One day	6,800,000	562,000,000
Two days	4,800,000	1860,000,000
Three days	700,000	1600,000,000
Four days	422,000	1300,000,000
Ten days	5,300	3800,000,000
Twenty days	1,700	3350,000,000
Thirty days	60	802,000,000
Sixty days	1	140,000,000
One hundred twenty days	0	910,000

Survival rate shown in figure 67.

Shigella dysenteriae

	Frozen	room temp.
Count per ml	11,450,000	11,450,000
One day	6,100	5,500,000
Two days	2,600	4,000,000
Three days	760	950,000
Ten days	50	370
Thirty days	0	0
Sixty days	0	0

Survival rate shown in figure 68.

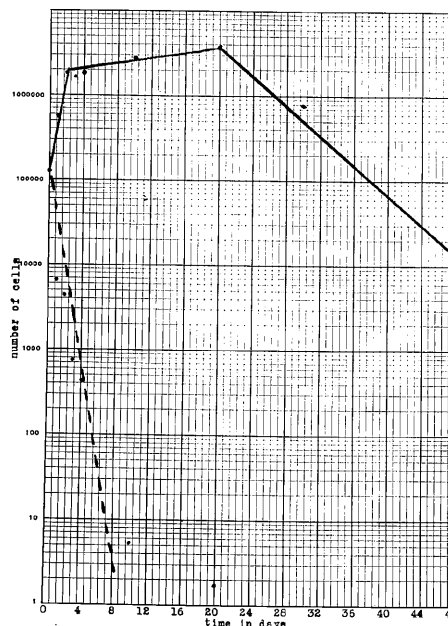


Figure 68.

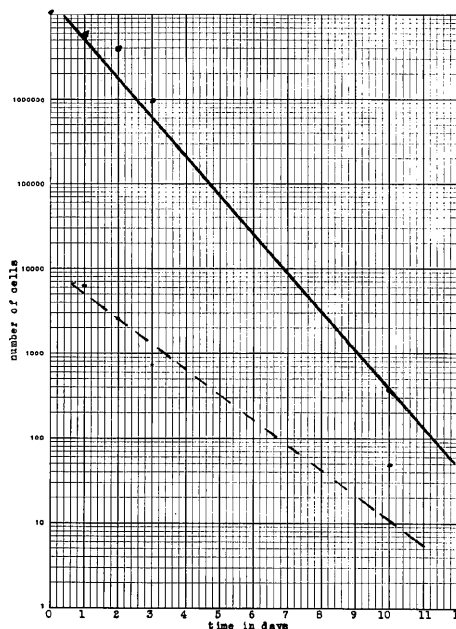


Table XXXVII.

Survival in canned unsweetened pineapple juice. Vacuum 2", pH 5.4. Comparison of water dilution blanks with water blanks to which 10% sucrose solution has been added.

	frozen		room temp.	
	water	10% sucrose	water	10% sucrose
<u><i>Escherichia coli</i></u>				
Count per ml	12,100,000	12,100,000	12,100,000	12,100,000
One day	250	110	3,080,000	6,000,000
Two days	0	10	2,880,000	550,000
Three days	0	0	900,000	1,400,000
Four days	0	0	710,000	670,000
Ten days			1,030,000	849,000
Thirty days			390	350
<u><i>Salmonella paratyphi</i></u>				
Count per ml	7,400,000	7,400,000	7,400,000	7,400,000
One day	0	0	480,000	1,640,000
Two days	0	0	830	990
Three days			0	0
<u><i>Salmonella schottmuelleri</i></u>				
Count per ml	44,000,000	44,000,000	44,000,000	44,000,000
One day	2,600	16,500	3,000,000	3,220,000
Two days	50	71	52,000	46,000
Three days	2	1	141	160
Four days	0	0	0	0
<u><i>Eberthella typhosa</i></u>				
Count per ml	4,100,000	4,100,000	4,100,000	4,100,000
One day	600,	360	150,000	710,000
Two days	59	65	20	5
Three days	7	0	0	0
Four days	0	0	0	0
<u><i>Aerobacter aerogenes</i></u>				
Count per ml	8,900,000	8,900,000	8,900,000	8,900,000
One day	25,300	47,800	1,386,000	1,560,000
Two days	80	44	1,100,000	2,320,000
Three days	12	10	3,700,000	2,100,000
Four days	0	1	3,550,000	3,130,000
Ten days	0	0	7,600,000	2,400,000
Thirty days	0	0	14,000,000	11,700,000
<u><i>Shigella dysenteriae</i></u>				
Count per ml	980,000	980,000	980,000	980,000
One day	420	1,320	57,000	22,000
Two days	9	15	208	250
Three days	0	0	0	0
Four days	0	0	0	0

Table XXXVIII.

Survival in canned grapefruit juice. Vacuum 7.5", pH 3.3. Comparison of water dilution blanks with water blanks to which 10% sucrose has been added.

	frozen		room temp.	
	water	10% sucrose	water	10% sucrose
<u>Serratia marcescens</u>				
Count per ml	1,960,000	1,960,000	1,960,000	1,960,000
Thirty minutes	20,000	18,000	9,800	8,900
Sixty minutes	690	540	310	89
Two hours	8	1	0	0
Four hours	0	0	0	0
<u>Staphylococcus albus</u>				
Count per ml	2,680,000	2,680,000	2,680,000	2,680,000
One hour	190,000	132,000	38,000	50,000
Two hours	21,000	16,300	1,400	1,900
Four hours	270	330	2	0
Eight hours	2	0	0	1
One day	0	0	0	0
<u>Staphylococcus aureus</u>				
Count per ml	950,000	950,000	950,000	950,000
Two hours	26,500	25,100	3,870	4,800
Four hours	17,000	13,400	560	190
Eight hours	8,600	5,020	30	8
One day	740	600	5	0
Two days	12	18	0	0
Three days	0	0	0	0
<u>Escherichia coli</u>				
Count per ml	610,000	610,000	610,000	610,000
Two hours	5,800	3,100	13,800	12,000
Four hours	950	240	-	-
Eight hours	250	210	-	-
One day	70	67	4	0
Two days	0	0	0	0
<u>Sherrillia typhosa</u>				
Count per ml	830,000	830,000	830,000	830,000
One hour	*0	*0	5,800	7,200
Two hours	1,280	1,550	710	635
Four hours	52	37	9	3
Eight hours	4	1	0	0
One day	0	0	0	0
Two days	0	0	0	0
*Error in sampling				
<u>Salmonella paratyphi</u>				
Count per ml	1,820,000	1,820,000	1,820,000	1,820,000
Thirty minutes	4,400	3,900	2,400	3,000
One hour	1,290	7,000	480	660
Four hours	0	0	0	0

Table XXXIX.

Survival in canned grapefruit juice. Vacuum 7.5", pH 3.3. Comparison of water dilution blanks with water blanks to which 10% sucrose has been added.

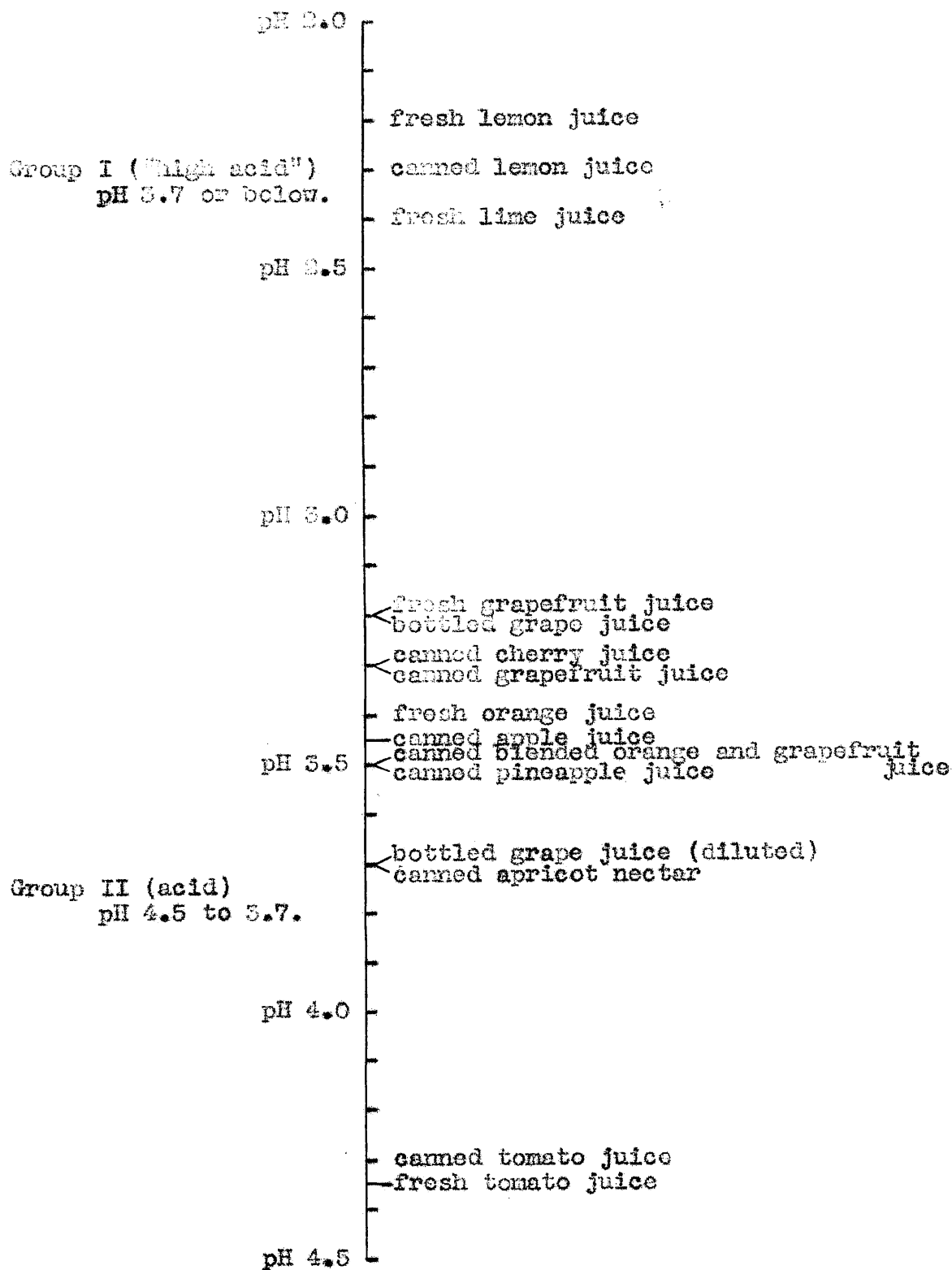
	frozen		room temp.	
	water	10% sucrose	water	10% sucrose
<i>Aerobacter aerogenes</i>				
Count per ml	72,000,000	72,000,000	72,000,000	72,000,000
One-half hour	15,200,000	14,200,000	-	-
One hour	4,300,000	3,300,000	6,300,000	6,300,000
Two hours	200,000	119,000	103,000	171,000
Four hours	1,270	1,100	720	930
Six hours	20	15	77	104
One day	0	0	0	0
Two days	0	0	0	0
<i>Shigella dysenteriae</i>				
Count per ml	560,000	560,000	560,000	560,000
One hour	20,000	16,000	13,400	36,000
Two hours	2,060	1,300	3,040	2,410
Four hours	0	22	123	111
One day	0	0	0	0
Two days	0	0	0	0

Table XL.

Survival in canned pineapple juice. Vacuum 5.4", pH 5.4. Temperatures are approximate (accurate within a plus or minus two degrees Centigrade).

	-8°C.	20°C.	37°C.	55°C.
<u>Serratia marcescens</u>				
Count per ml	6,300,000	6,300,000	6,300,000	6,300,000
One day	340,000	505,000	116,000	25
Two days	122,000	300,000	45,000	0
Three days	102,000	93,000	400	0
Four days	48,000	7,100	0	0
Five days	9,000	20	0	
<u>Staphylococcus albus</u>				
Count per ml	12,000,000	12,000,000	12,000,000	12,000,000
One day	44,000	179,000	54,000	10,800
Two days	6,800	910	3	0
Three days	530	16	0	0
Four days	2	0	0	0
Five days	0	0	0	
<u>Staphylococcus aureus</u>				
Count per ml	2,800,000	2,800,000	2,800,000	2,800,000
One day	33,000	200,000	115,000	2,200
Two days	35,000	84,000	3,070	7
Three days	11,400	10,000	55	0
Four days	2,300	780	0	0
Five days	80	0	0	0
Ten days	0	0		
<u>Eberthella typhosa</u>				
Count per ml	750,000	730,000	730,000	750,000
One day	460	8,100	50	0
Two days	5	32	0	0
Three days	0	0	0	0
Four days	0	0		
<u>Salmonella paratyphi</u>				
Count per ml	2,570,000	2,570,000	2,570,000	2,570,000
One day	(?)1	31	0	0
Two days	4	0	0	0
Three days	0	0		
Four days	0	0		
<u>Salmonella schottmuelleri</u>				
Count per ml	16,300,000	16,300,000	16,300,000	16,300,000
One day	720,000	1,030,000	570,000	0
Two days	35,000	58,000	6,200	0
Three days	770	65	0	0
Four days	3	0	0	
Five days	1	0		

Figure 69. Hydrogen Ion Concentrations of Various Juices.



IV. DISCUSSION

Marked differences among various fruit juices have been clearly demonstrated. In accordance with the view of Cameron and Eddy (1940), the acid group of foods may be roughly divided into two groups--the acid and the high acid foods. This scheme was observed in figure 69 on page 94 which ranks the fruit juices in order of hydrogen ion concentration. All of the juices and nectars studied were "high acid" with the exception of tomato juice.

Distinction on the basis of hydrogen ion concentration alone, however, does not encompass the marked bactericidal variance of fruits of different viscosities, titratable acidities, and proportions of protective substances in the product. Longevity of bacteria depends as much upon any or all of these factors as upon the pH of the substrate.

Fresh and canned lemon juice and fresh lime juice were extremely acid, having approximately the same pH and titratable acidities. The juices were rapidly bactericidal--fresh juice of lemon completely destroying pathogenic loads, excepting staphylococci, in less than one hour. This observation agrees with results of Alliot (1919) and Douglas and Edin (1930). The former investigator found that it took at least fifteen minutes for complete destruction of five cc of a twenty-four hour culture of Eberthella typhosa and Salmonella paratyphi in a liter of lemon juice. This survival rate was slightly higher than that observed by the author, but most probably was due to a larger inoculum or to introduction of a small amount of protective substrate along with the cells. Douglas and Edin noted that two billion cells of Eberthella typhosa in two

teaspoonfuls of lemon juice were killed within five to nine and one-half minutes, while Salmonella paratyphi and Salmonella schottmuelleri could not be found three hours after mixing with lemon juice. Numbers of Escherichia coli were quite small at three hours and none were present when tested six hours after inoculation.

The period of viability in canned lemon juice was slightly longer than in the fresh fluid. Such slight variations in cell counts are to be expected and may be attributed to errors in sampling, differences in size of inoculum, age and viability, strain of cells, effect of heat or cooking on the composition of the food, available hydrogen ion, total acidity, viscosity, or solids in the substrate.

Action of fresh lime juice also was quite severe on all bacteria. Even the two Staphylococci were destroyed in less than two hours. In some of the samples where cells survived until solidly frozen, the period of survival was of slightly longer duration. In the main, freezing contributed very little positive or negative bactericidal effect on lemon juice or lime juice since most of the samples were sterile before the samples had become frozen solidly. Staphylococcus aureus was an exception.

Grapefruit juice, while not as rapidly bactericidal as the fluid expressed from lemons or limes, was sterile by forty-eight hours in all cases at room temperature and by seventy-two hours when frozen at -8° to -10°C (17.6° to 14°F). None of the pathogenic gram-negative, non sporing rods lasted four hours in fresh grapefruit juice held at room temperature. Salmonella enteritidis and Salmonella schottmuelleri were viable at the end of one day and four hours respectively when stored at -8° to -10°C .

Bottled grape juice of the same hydrogen ion concentration as grapefruit juice was not as effective as a killing agent at either room temperature or freezing temperature. Nevertheless, the lower temperature was a more favorable medium insofar as the bacteria were concerned. Shigella spp. and Salmonella schottmuelleri did not conform to this generalization--living as long at room temperature as in the frozen medium. Tests performed by Rochaix and Jaqueson (1932) showed the same bactericidal effect of grape juice upon Eberthella typhosa, Salmonella paratyphi, Salmonella schottmuelleri, Shigella dysenteriae, and Escherichia coli at room temperature as has been reported in this study. From results appearing in Table XVII comparison of survival time in frozen samples with fluid stored at twenty degrees Centigrade (68°F) again demonstrated that the higher temperature produced complete sterilization more quickly.

Orange juice allowed a few cells of Eberthella typhosa, Escherichia coli, Shigella paradysenteriae Sonne, and Salmonella paratyphi to remain viable longer than one day in frozen or unfrozen orange juice but not two days. Staphylococcus aureus lived at least ten days and Aerobacter aerogenes at least two days in frozen orange juice. Only twelve of an initial seventy million cells of the former were not killed by two days when stored at twenty degrees while all Aerobacter aerogenes individuals were destroyed before one day at the same temperature. Douglas and Edin (1930), using two billion bacteria in two teaspoonfuls of orange juice and incubating at room temperature, gave survival periods of nine to twenty-two hours for Eberthella typhosa, nine to thirty hours for Salmonella paratyphi, and two to five days for Salmonella schottmuelleri. These results closely conform with those revealed in

Tables VIII, XIV, and XV. Beard and Cleary (1933) were able to isolate Eberthella typhosa and Shigella dysenteriae for a period of seven days while Salmonella paratyphi lasted three days and Salmonella schottmuelleri four days in orange juice held at -4°C (24.8°). Staphylococci lived fifty hours. They considered the hydrogen ion concentration of orange juice rapidly bactericidal at ordinary temperatures but published no reports of such determinations.

Results with canned apple juice showed the material to be quite germicidal. Eberthella typhosa could not be isolated one day after incubation, while Salmonella paratyphi could for one day but not for two. Salmonella schottmuelleri lived at least two days in canned apple juice. Bodin (1928) noted that all of one and one quarter million cells of Eberthella typhosa were destroyed by sixty hours in cider. Paquet (1925) found that both this organism and Salmonella paratyphi were killed within a week.

Frozen cherry juice harbored Eberthella typhosa at least two days while Salmonella schottmuelleri and Salmonella aertrycke survived three and four days respectively in the fluid. None of the organisms tested were found after five days. Survivals were somewhat longer in frozen samples than in those maintained at room temperature though the difference was not marked. These results contrast with those of Wallace and Park (1935) who worked with heavy inoculums of Escherichia coli, Salmonella aertrycke, Salmonella schottmuelleri, and Eberthella typhosa, and found that E. coli lasted at least three weeks while the two Salmonella species were viable when tested after two weeks storage at -17.3°C (0°F). Eberthella typhosa was not isolated after one week.

In order to determine whether this disparity was due to the difference of storage temperatures, another experiment was conducted at -16°C (3.2°F). In every case (see table) the organisms were viable over a longer period. Hence, it may be stated that temperatures nearer to the freezing point are more destructive to intestinal pathogens than lower temperatures. This is in accordance with the observation of Tanner and Wallace, Berry, Prescott, Bates and Highlands, and others.

Canned pineapple juice and canned apricot nectar were not as bactericidal as any of the previously named juices. The acidity was sufficiently high to inhibit growth of most of the organisms, but was not high enough to be bactericidal except for the least resistant. For this reason the added effect of concentrating the acidity and destruction during freezing were more detrimental than the effect of acidity at a higher temperature. Aerobacter aerogenes was able to reproduce in canned pineapple juice. Although Escherichia coli is said to be unable to carry on metabolism below a pH 4.2, evidence in pineapple juice at pH 3.5 seems to the contrary.

Tomato juice was least germicidal of any of the juices and for that reason destruction was more rapid in frozen suspension than in unfrozen samples. Both Aerobacter aerogenes and Escherichia coli were able to reproduce. The former found tomato juice quite a favorable medium and growth was marked. It is possible that, under certain conditions, some of the pathogens are able to reproduce. Salmonella schottmuelleri and Serratia marcescens showed small increases in one sample tested. This may have been due to error in sampling or to growth.

Those organisms able to withstand the critical temperature or time period just before freezing and during crystal formation found low temperatures more favorable than temperatures above freezing in the highly acid juices.

A number of factors must be considered before any definite conclusion can be drawn. If a logarithmic rate of death is assumed for bacterial cells in fruit juices held at room temperature, experimental evidence, as here presented, indicates a shift or even a break in the curve when organisms are inoculated and stored in juices held at subfreezing temperatures. In some cases the initial curves for samples undergoing freezing may be expected to drop more rapidly due to the hyper-acid condition caused by concentration of cells and acids. McFarlane (1940), in "Malt quarts" of frozen cider, found that frozen microbial forms, soluble solids, and total acids were concentrated in central cone-shaped areas and in horizontal surface areas while in other foods there was no such marked freezing redistribution. This fact may be used to explain the failure of pathogenic forms to survive in high acid juices such as lemon, lime and grapefruit for as long a time as at room temperature where no such concentration has been effected.

Even though cells are able to withstand mounting acidity, many are ruptured by large crystal formations. This phenomenon is more prevalent in pure liquids than in juices containing a higher proportion of solid particles.

Keith (1913) discussed the probability that water-bearing food materials freeze in such a manner that most of the bacteria and other non-watery matters are extruded from the water crystals and "lie in or among these matters without being crushed or injured"

while in pure water suspensions the whole mass becomes solidly crystalline and the particles and bacteria are caught and are destroyed between the growing crystals.

Of the experimental juices studied, apple juice was the most watery while a sweet nectar and tomato juice were quite viscous, and although there was not much difference in the acidity of the first two juices, survival rates varied markedly. Much of this difference may be assumed to be due to difference in kind and amount of acid, but a portion must be attributed to protective action of solids present in the medium. On the other hand, once the cells have entered the frozen state, metabolism proceeds more slowly. They no longer absorb acids at the rapid rate possible at room temperature since metabolism proceeds at a much slower rate and ions and gases do not diffuse across the cell membranes as quickly as before. In fact, some workers believe that the cells go into a state of suspended animation below the freezing point.

In view of the fact that bacteria die gradually, it is probable that there is a slow exchange of gases and that the cell dies from starvation. It is also possible that acid ions enter the cell and gradually denature the protein, either by coagulating a large protein fraction or by altering the molecule.

V. CONCLUSIONS.

The assumption that bactericidal action of fruits renders them innocuous after short storage periods, either at room temperature or in the frozen state, is dependent upon a number of factors including the contaminative load, the species and strain of organism entering the fluid, and the degree of temperature at which the juice is preserved.

Some juices are more germicidal than others. Lemon and lime juice were highly bactericidal and were able to destroy most bacteria within a very few minutes after inoculation. Apricot nectar and pineapple and tomato juice were the least severe in their action of pathogenic organisms. Aerobacter aerogenes and Escherichia coli found the latter two juices favorable for preservation and even reproduction.

In some of the fruit juices, the added effect of freezing was harder on bacterial cells than was the action of fruit acid at room temperature.

Effects of freezing were variable. In lemon, lime, and grapefruit juice, freezing did not increase longevity since the fluid was so highly toxic that few cells were viable by the time that crystallization had become noticeable. In grape juice, apple juice, and cherry juice survival rates in frozen juices as compared to fluids stored at room temperature varied with the organism. In many cases longevity was more prolonged at the lower temperature.

Organisms stored in cherry juice and held at -16°C (3.2°F) and -8°C (17.6°F) remained viable for a longer period at the lower degree.

Ten per cent sucrose diluting fluid was not shown to be a more favorable diluting medium than distilled water. Calcium carbonate added to water used in diluting high acid samples allowed more abundant growth upon subculture.

Staphylococcus aureus was usually more resistant to fruit juices than any of the other pathogenic forms. Salmonella paratyphi did not persist as long as other members of the genus. Eberthella typhosa remained viable for a longer period than Salmonella paratyphi but not as long as Salmonella schottmuelleri.

While storage of lemon and lime juices at either room temperature or in the frozen state is comparatively safe, apricot and pineapple juice may harbor dangerous organisms for weeks. Tomato juice subjected to contamination by intestinal pathogens, should not be used without employing some means of sterilization.

If juice is frozen, it is advisable to store the material at a point immediately below the limit of crystallization rather than at a lower temperature.

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