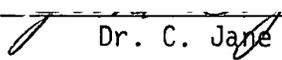


AN ABSTRACT OF THE THESIS OF

VICKI H. GUY for the degree of Master of Science  
in Food Science and Technology presented on \_\_\_\_\_

Title: THE GROWTH OF SALMONELLA TYPHIMURIUM, STAPHYLOCOCCUS AUREUS, AND  
BACILLUS CEREUS IN BAKERY PRODUCTS AS RELATED TO THE FOOD  
DISTRIBUTION SYSTEM.

Abstract APPROVED: \_\_\_\_\_

 Dr. C. Jane Wyatt

Current retail food distribution practices, microbial quality of bakery products, and the potential for growth of food borne pathogens (Staphylococcus aureus, Salmonella typhimurium, and Bacillus cereus) were examined using pumpkin pie as a model.

The pumpkin pies, as obtained from the retail outlets, showed contamination with food pathogens. The aerobic plate counts reached high numbers (up to  $10^9$ /g) when stored at 25<sup>0</sup>C for the specified shelf life as indicated by pull dates. S. aureus was isolated from one sample and B. cereus from two samples of pumpkin pie. Baking conditions were sufficient to destroy S. aureus and Salmonella typhimurium but not spores of B. cereus. The presence of S. aureus indicated post-processing contamination.

When inoculated with S. aureus, Salmonella typhimurium and B. cereus, pumpkin pie supported the growth of all these organisms when stored at 25<sup>0</sup>C. When stored at severe abuse conditions (35<sup>0</sup>C), pumpkin pie supported the growth of S. aureus and Salmonella typhimurium but not B. cereus. Refrigeration at 4<sup>0</sup>C controlled the growth of the pathogens studied. The addition of 0.25% potassium sorbate to the pie filling inhibited the growth of

Salmonella typhimurium and B. cereus but not S. aureus at 25<sup>0</sup>C.

The findings of this study indicated a lack of knowledge regarding safe food handling practices among bakers. Current distribution practices indicated that pumpkin pies were often displayed at room temperature from two to five days. If contaminated, the product could become a public health hazard.

The growth of Salmonella typhimurium, Staphylococcus aureus,  
and Bacillus cereus in bakery products as related  
to the food distribution system

by

Vicki H. Guy

A THESIS

submitted to

Oregon State University

in partial fulfillment of  
the requirements for the  
degree of

Master of Science

June 1981

APPROVED:

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in charge of major

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Date thesis is presented May 1, 1981

Typed by Jill Nowac for Vicki H. Guy

## ACKNOWLEDGEMENTS

I would like to thank the following people who helped in carrying out this study: Dr. W. Sandine, who provided advice and the Bacillus cereus culture used throughout the experiments; Dr. M. Woodburn, who provided the Staphylococcus aureus culture; the Oregon State Department of Agriculture, Laboratory Services, for the use of their water activity equipment and from which the Salmonella typhimurium culture was obtained; Dr. J. S. Lee for the use of equipment in addition to his advice; and R. W. Bennett for performing the Staphylococcal enterotoxin assays.

The people involved in the retail outlets were very helpful in providing samples and information on their food handling practices. A deep appreciation goes to those involved.

I also thank Dr. C. J. Wyatt for her invaluable assistance and advice from beginning to completion of this study.

Finally, I would like to thank my husband, Dan, for his support.

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THE GROWTH OF SALMONELLA TYPHIMURIUM, STAPHYLOCOCCUS AUREUS,  
AND BACILLUS CEREUS IN BAKERY PRODUCTS AS RELATED  
TO THE FOOD DISTRIBUTION SYSTEM

INTRODUCTION

It has been well documented that a major cause of foodborne illness is due to improper food handling in the home. In 1977, 25% of the foodborne illness outbreaks were caused by mishandling in the home (Center for Disease Control, 1979). Very little has been done, however, to determine if the responsibility lies with the homemaker or food retailer. If the consumer brings home contaminated food to start out with, along with the fact that he/she may abuse the food, public health problems of great significance may occur.

The Food and Drug Administration has published a model Retail Food Store Sanitation Ordinance (Federal Register, 1977) setting forth requirements for the retail food industry. The ordinance states that potentially hazardous foods are those with a pH above 4.6 or a water activity ( $a_w$ ) above 0.85. These foods need to be cooled and held at an internal temperature of 45<sup>0</sup>F or less, or held at 140<sup>0</sup>F or above. Currently, many products which would be considered potentially hazardous are not refrigerated in the retail store. This is especially true in the case of bakery items. The ordinance could force the retailer to make costly changes in the store by the addition of refrigeration units.

The purpose of this study was to examine the food handling and distribution practices of the food retail industry as it relates specifically to bakery products. The most common microorganisms of concern with bakery products have been Staphylococcus aureus and Salmonella.

Bacillus cereus is an organism that is only recently being looked at. In this study, all three organisms were inoculated into pumpkin pie which possessed pH, water activity, and ingredients conducive for the growth of the organisms and could present a potential health hazard. Possible solutions to eliminating the chances of the product becoming a health hazard are also included in the study.

## REVIEW OF THE LITERATURE

### Food Distribution

There are many different methods of distributing food in the United States to the consumer. Primarily, however, most consumers buy their food in the supermarket or eat out. How that food gets to the supermarket is of primary concern in this study, that is, the proper handling of food to minimize the potential for a public health hazard.

Although not much research has been conducted on the actual distribution of food, some studies have been made of in-plant sanitation in bakeries and knowledge assessments of both the consumer and the retail store managers regarding food handling practices. A study conducted by Abrahamson, et. al. (1952) in New York City involved 420 cream filled bakery products from 65 different wholesale bakeries. The authors concluded that sanitary conditions were satisfactory in only 18 of the bakeries examined. The microbial quality of the samples reflected the sanitary conditions of the bakery. Some of the problems encountered were: a) not cooking food long enough or at a high enough temperature, b) carelessness in cooling leading to recontamination, c) needless personal contact when transferring cooled custards to shells, d) no knowledge of sanitation, e) equipment difficult to clean, f) a 6-14 h or more time lapse from completion of the product to delivery, and g) no equipment for continuous refrigeration during transportation.

A problem in manufacturing and distributing bakery products is the lack of knowledge of sanitation. A survey of retail store managers conducted by Wyatt (1979) indicated a need for training in sanitation

and safe food handling practices exists. Although 72% of those surveyed thought their knowledge on sanitation was adequate, further questioning showed few understood basic principles of food safety and sanitation.

Another factor involved in food distribution concerns the consumer. What the consumer does with the food can also be a potential for abuse. A survey of households was conducted by the United States Department of Agriculture (Jones and Weimer, 1977) to determine homemaker's knowledge, attitudes, and practices regarding food safety. The findings showed that 63% of those surveyed could be categorized as "high risk" due to their food handling practices and/or knowledge.

## Organisms

### Growth of Organisms

Growth of bacteria depends on many factors -- temperature, pH, water activity ( $a_w$ ), and nutrients, to name a few. Bacteria have different requirements, depending on the type and species but all follow a characteristic growth curve (Figure 1). First, a lag phase (A) occurs which enables the organism to "adjust" to its new medium. Then the organism begins to multiply rapidly, or go through the exponential phase (B). After a while, growth levels off (C) and then death occurs (D) due to depletion of nutrients and/or toxin accumulation (Sokatch, 1969; Pelczar and Reid, 1972). The length of each phase varies depending on the environmental conditions of the medium.

In this study, the growth curves of Staphylococcus aureus, Salmonella typhimurium, and Bacillus cereus, in pumpkin pie are of concern.

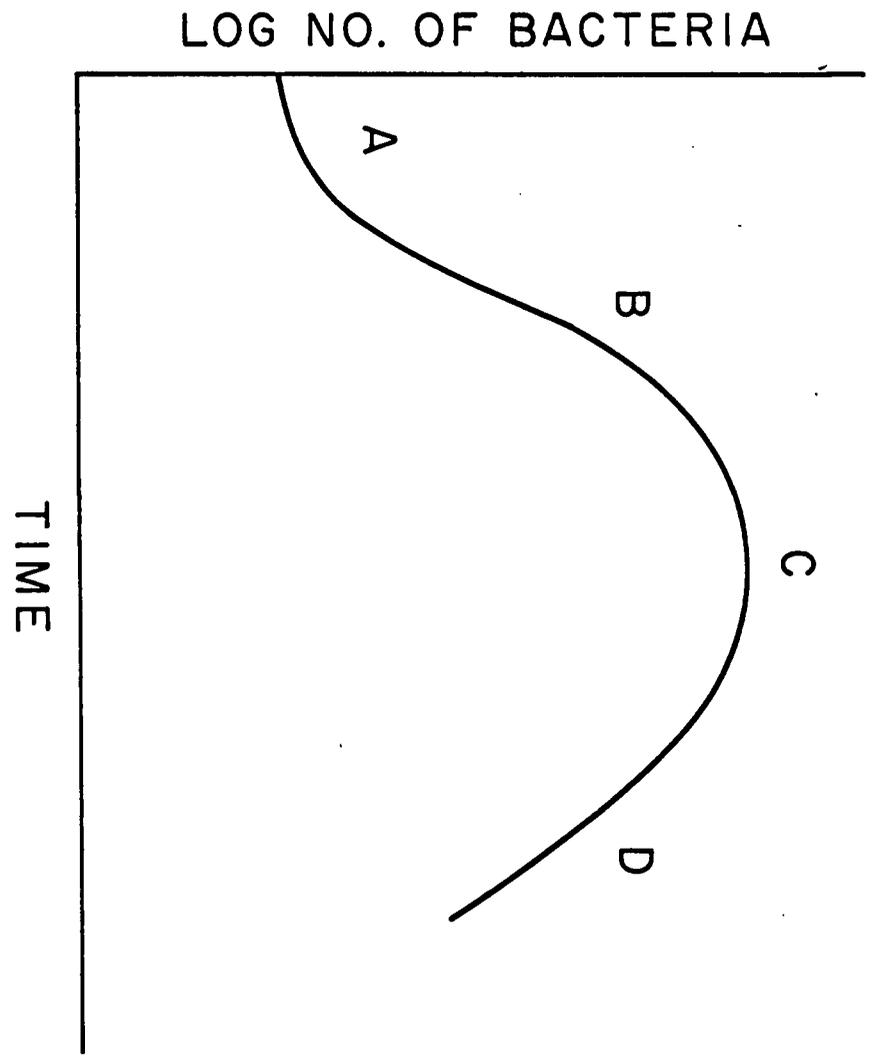


Figure 1. Growth curve of bacteria (Pelczar and Reid, 1972)

## Staphylococcus aureus

S. aureus is a gram positive cocci 0.8  $\mu\text{m}$  in diameter occurring singly, in pairs, or irregular clusters. It is a facultative anaerobe but grows best under aerobic conditions. Pigmentation of colonies is variable but most strains are orange (Buchanan and Gibbons, 1974).

Over the years S. aureus has had many names including S. pyogenes aureus, S. pyogenes albus, S. albus, S. pyogenes citreus, Micrococcus aureus, M. pyogenes, M. pyogenes aureus, and M. pyogenes var. aureus. Although S. aureus is widely distributed in nature, man is a common source. The organism is commonly found on the skin, eyes, throat, intestinal tract, and mainly the nasal passages. Animals are also a common source. S. aureus can also be found on skin lesions, wounds, abrasions, and the like (Minor and Marth, 1976).

Any time a food is exposed to human handling there is a good possibility the food will become contaminated with staphylococci. Without proper handling, the organisms can multiply and produce enterotoxins. Heat treatments normally used in cooking or food processing are sufficient to destroy the organism but not the heat stable enterotoxins.

### Growth Requirements

Water Activity ( $a_w$ ). It is generally recognized that S. aureus is capable of growing at  $a_w$  levels down to 0.86 whereas the minimum requirement for most bacteria is 0.91. Related to its tolerance for such a low  $a_w$  is this organism is also very salt tolerant and, under certain

conditions, capable of surviving exposure up to 26% NaCl (Troller and Christian, 1978). Several studies have been reported on the salt tolerance of S. aureus. One study showed 15-20% NaCl was inhibitory at optimal growth temperatures but 20-25% NaCl was germicidal (Nunheimer and Fabian, 1940).

Enterotoxin production is also related to  $a_w$ . It has been found that decreasing the  $a_w$  in one medium from 0.99 to 0.98 and in another medium from 0.99 to 0.97 resulted in large numbers of S. aureus but reduced enterotoxin B production (Minor and Marth, 1972). In a similar study, enterotoxin A production was also decreased but to a lesser extent (Troller and Christian, 1978).

pH: According to Bergey's Manual for Determinative Bacteriology (Buchanan and Gibbons, 1974), the pH range for S. aureus is 4.2-9.3 with the optimum between 7.0-7.5. It has been reported, however, that a pH of 6.8 resulted in higher yields of enterotoxin B and C than at a pH of 6.0 or 5.3. At pH 5.3, enterotoxin A was not affected. The optimal pH for enterotoxin A production is reported to be 6.5-7.0 (Minor and Marth, 1972). S. aureus is apparently sensitive to acids. Nunheimer and Fabian (1940) reported that acetic, citric, lactic, tartaric, and hydrochloric acids, in order of decreasing effectiveness, were germicidal for S. aureus.

Temperature: Optimum temperature for growth of S. aureus is 35-40°C (Buchanan and Gibbons, 1974). Temperatures below 20°C or above 46°C seem to delay or inhibit enterotoxin production whereas temperatures of 40°C and 45°C seem to stimulate enterotoxin synthesis (Minor and Marth, 1972).

The D values for this organism vary with strains and conditions. The  $D_{60}$  value ranged from 0.5-2.5 min for S. aureus heated in buffer or

weak salt solutions at pH 6.5-7.0 (Cohen, 1972).

Nutrients. For aerobic growth, 12 amino acids along with adenine and thiamine are required. Uracil and a fermentable carbon source are also required for anaerobic growth (Buchanan and Gibbons, 1974).

### Pathogenicity

Illness due to S. aureus food poisoning is caused by ingesting a preformed enterotoxin. Six different enterotoxins have been isolated-- A, B, C<sub>1</sub>, C<sub>2</sub>, D, and E (Jay, 1978). A is the most common food poisoning type, causing 49% of the food poisoning outbreaks, and D is the next most common type (Minor and Marth, 1972). The enterotoxins are heat stable proteins. A considerably small amount of the toxin (< 1 µg) will cause illness although a large number of organisms is required to produce the minute quantity (Jay, 1978). The actual number of organisms necessary to produce enough toxin to cause illness is not known. Bryan (1978) reported as few as 500,000/g would cause illness. The mechanisms of toxicity are still largely unknown but the enterotoxins seem to destroy the cells which line the gastrointestinal tract. They also affect the circulatory system (Minor and Marth, 1976).

The typical symptoms of nausea, vomiting, severe abdominal cramps, diarrhea, headache, and sometimes a decrease in body temperature may occur 0.5 to more than 7 h after ingesting the food which contains the toxin (Jay, 1978). It has been found that the severity of the symptoms is dose dependent (Bergdoll, 1972).

### Salmonella

Salmonella are gram negative, facultative anaerobic rods and usually

motile (Buchanan and Gibbons, 1974). They normally do not ferment lactose. Of over 1700 serotypes, the five most commonly isolated in foods are S. infantis, S. oranienberg, S. typhimurium, S. montevideo, and S. heidelberg (Jay, 1978).

Salmonella is found in the intestinal tract of man and animals. They are excreted in the feces and can also be found in water. After contaminated food or water has been ingested, Salmonella is shed through the feces, completing the cycle. Foods most commonly contaminated with Salmonella include eggs, poultry, and meats (Jay, 1978).

### Growth Requirements

Water activity. For three Salmonella serotypes, the minimum  $a_w$  was found to be 0.941 and for one type, it was 0.945. In foods, however, the minimum  $a_w$  was found to be 0.93 (Troller and Christian, 1978). Inhibition of Salmonella occurs at  $a_w$  levels below 0.94 (Jay, 1978), however, Troller and Christian (1978) report a value of 0.95. Salmonella will not grow in a medium above 7-8% NaCl (Troller and Christian, 1978).

pH. The pH range for growth of Salmonella is 4.1-9.0 with the optimum close to 7.0 (Frazier and Westhoff, 1978).

Temperature. The temperature range depends on the medium and the strain. The minimum temperature for S. typhimurium has been reported to be 6.2°C by one study and 6.7°C in another. The upper limit is 45.6°C with optimum growth at 37°C (Frazier and Westhoff, 1978; Jay, 1978). In foods, the lower limit ranged from 6.7-7.8°C in chicken a la king to over 10°C in custard and ham salad (Angelotti, et. al., 1961). S. senftenberg is the most heat resistant of the Salmonella serotypes. It is destroyed at milk pasteurization temperatures (Jay, 1978).

The  $D_{60}$  values for Salmonella range from 0.06-11.3 min depending on the food and serotype (Frazier and Westhoff, 1978). Beloian and Schlosser (1963) considered baked goods to be free of Salmonella if the product reached an internal temperature of 160<sup>0</sup>F or higher.

Nutrients. Most strains will grow on defined media without special growth factors. Salmonella need a source of simple sugars, an ammonium compound, salts, and a few amino acids. Many strains, including S. typhimurium, do not require preformed vitamins. (Buchanan and Gibbons, 1974; Koser, 1968).

### Pathogenicity

Salmonellosis is caused by ingesting viable cells of Salmonella organisms. The actual number of cells necessary to cause illness ranges from several million to several billion depending on the strain. S. enteritidis and S. anatum are more infective than S. pullorum. The incubation time is 12-24 h and symptoms include nausea, vomiting, abdominal pain, headache, chills, fever, faintness, muscular weakness, restlessness, and drowsiness. Although the duration of the illness varies, symptoms usually persist for 2-3 days and about 5% of those infected can become carriers. Since the incubation period is rather short, the need for the organism to grow in the intestines has not been substantiated. The liberation of an endotoxin has been suggested as a cause of the illness (Jay, 1978).

### Bacillus cereus

B. cereus is a gram positive, sporeforming rod 1.0 $\mu$  to 1.2 $\mu$  by 3 $\mu$ -5 $\mu$  belonging to Group I of the Bacillus genus (Gordon, et. al., 1973).

It can be a strict aerobe or a facultative anerobe. Colony formation can vary. Sometimes outgrowths occur, irregularly tangled or curled predominantly in one direction. Othertimes no outgrowths occur (Buchanan and Gibbons, 1974).

B. cereus is ubiquitous and the incidence in various foods is being studied. Powers, et. al. (1976) found B. cereus in 53% of the spices they analyzed. The spices included bay leaves (7 out of 11 were positive) cayenne pepper (12 out of 18), chili powder (4 out of 16), cinnamon (15 out of 16), garlic powder (5 out of 17), mustard powder (1 out of 14), and oregano (14 out of 18). Although the levels found were relatively low (50-8500/g), the authors concluded that the potential for a public health problem exists if the food was mishandled. Eighty-nine percent of the isolates were capable of producing enterotoxin.

In a similar study, Kim and Goepfert (1971) examined the incidence of B. cereus in 170 dried food samples. They found B. cereus in seasoning mixes (55%), spices (40%), dehydrated potatoes (40%), milk powder (37.5%), and spaghetti sauce mixes (37.5%). To a lesser degree, they also found B. cereus in soup mixes (1 out of 22), gravy mixes (2 out of 23), cream and BBQ sauces (1 out of 16), and flour and starch (1 out of 6). None was detected in 14 samples of puddings and custards.

There have been several reports that indicate the presence of B. cereus in milk and milk products (Davies and Wilkinson, 1973; Stone and Rowlands, 1952). It has also been shown that B. cereus in milk causes "broken" or "bitty" cream (Stone and Rowlands, 1952). Fried rice seems to be a common source of B. cereus as evidenced by several food poisoning outbreaks in Europe (Raevuori, et. al., 1976).

## Growth Requirements

Water activity. The minimum  $a_w$  for B. cereus growth in 8% NaCl containing medium was found to be 0.95. Spore germination occurred at 0.99-0.98 in the presence of NaCl and KCl. Total inhibition occurred at 0.95 (Troller and Christian, 1978).

pH. The pH range for growth of B. cereus is 4.9-9.3 (Jay, 1978; Frazier and Westhoff, 1978).

Temperature. Growth of B. cereus has been reported at 10°C to a high of 49°C, with 30°C as the optimum temperature. (Goepfert, et. al., 1972). Incubation at 37°C produced twice as much toxin as when incubated at 30°C (Bonventre and Johnson, 1970).

A  $D_{100}$  value of 2.7-3.1 min has been reported in skim milk but in low acid foods, the  $D_{100}$  value was 5 min. In soybean and olive oils, the  $D_{121}$  values were 30 and 17.5 min respectively (Goepfert, et. al., 1972). Vas and Proszt (1957) observed that at 90°C the heat destruction curve of B. cereus spores was not logarithmic. About one in  $10^7$  or  $10^8$  possessed extreme heat resistance. Bradshaw, et. al. (1975) found that from six B. cereus strains isolated from abnormal commercially canned foods, one strain was more heat resistant than the others.

Nutrients. Strains differ as to their nutrient requirements. The organism has an absolute requirement for one to several amino acids. Vitamins, however, are not required (Buchanan and Gibbons, 1974). Toxin production seems to depend on nutrients present in fresh beef infusion broth, although other media (trypticase soy broth, nutrient broth, beef infusion broth, and bacto peptone) support the growth of B. cereus (Bonventre and Johnson, 1970).

## Pathogenicity

B. cereus elicits two different types of illness. One is similar to C. perfringens food poisoning and the other, more severe type is similar to S. aureus. The incubation period for the mild type syndrome is 8-16 h and causes nausea but not vomiting, abdominal pain, watery stools, and no fever. Symptoms usually last 6-12 h (Jay, 1978). The other type is evident 1-5 h after eating contaminated food with symptoms of nausea, abdominal pain, and vomiting but rarely diarrhea. This type is associated with fried and boiled rice (Raevuori, et. al., 1976).

The mode of infection is largely unknown, however, it is known that B. cereus produces extracellular products that include hemolysin, a soluble toxin, proteolytic enzymes, and phospholipase C. The relationship between these substances and illness is unknown (Jay, 1978; Goepfert, et. al., 1972). Goepfert, et. al. (1972) believe that due to the large number of cells (more than  $10^6$ /g) needed to cause illness, the evidence points to the enterotoxin being released upon lysis of the cell.

A summary of the growth characteristics of the organisms discussed is shown in Table I.

## Bakery Products

### Epidemiology

Cream filled bakery products are notorious for their history involving foodborne illness. In the United States, from 1938 to 1972, S. aureus was responsible for 65.3% of the outbreaks of foodborne disease caused by cream filled pastries (Bryan, 1976). In the 1930's and 40's, a large portion of foodborne disease was attributed to cream filled pastries. For instance,

Table 1. Growth Characteristics of S. aureus, Salmonella, and B. cereus.

Organism	Temperature °C		pH		a <sub>w</sub>
	Range	Optimum	Range	Optimum	
<u>S. aureus</u>	6.5-46.0 <sup>1</sup>	35-40 <sup>1</sup>	4.0 <sup>2</sup> -9.3 <sup>1</sup>	7.0-7.5 <sup>1</sup>	0.86 <sup>2</sup>
<u>Salmonella</u>	6.2-45.6 <sup>3,4</sup>	37 <sup>3,4</sup>	4.1-9.0 <sup>3</sup>	7.0 <sup>3</sup>	0.94-0.95 <sup>2</sup>
<u>B. cereus</u>	10.0-49.0 <sup>5</sup>	30 <sup>5</sup>	4.9-9.3 <sup>3,4</sup>		0.95 <sup>2</sup>

<sup>1</sup>Buchanan and Gibbons, 1974

<sup>2</sup>Troller and Christian, 1978

<sup>3</sup>Frazier and Westhoff, 1978

<sup>4</sup>Jay, 1978

<sup>5</sup>Goepfert, et. al., 1972

in New York, S. aureus was believed to be the cause of 17 outbreaks involving 1246 cases of gastroenteritis from 1935-1939. Chocolate eclairs and cream puffs were the most common vehicles involved (Coughlin and Johnson, 1941).

Recently, the number of foodborne disease outbreaks due to bakery products has significantly decreased since the earlier outbreaks. For instance, in 1958, custard filled desserts were implicated in only one outbreak (23 cases) of salmonellosis and 6 outbreaks (40 cases) due to S. aureus (Dauer and Davids, 1958). Bryan (1975) states that the primary reason for the decline in these outbreaks is the rapid cooling of the fillings, and the refrigeration of pastries in the bakeries, retail stores, and the home. Other factors contributing to the decline is the use of pasteurized egg products as opposed to raw eggs, and the use of formulas with a significant amount of sugar to prevent the growth of S. aureus.

Salmonella has also been responsible for a significant number of outbreaks. A salmonella Surveillance Program initiated in 1963 has improved the reporting of Salmonella outbreaks (Cohen and Blake, 1977). Prior to 1964, egg products were the second most frequent food implicated in salmonellosis outbreaks--and these were mostly in cakes or custards (Sickenga, 1964). From 1973-75 however, only one outbreak was attributed to eggs (Cohen and Blake, 1977).

B. cereus has only recently been recognized as a food poisoning agent in the United States (since 1968). In Europe, however, it has been recognized since 1950. Eighteen outbreaks of B. cereus have occurred in England since 1971 implicating boiled and fried rice. In the United States, seven outbreaks of B. cereus food poisoning have been reported from 1968-1973 (Schmitt, et. al., 1976; MMWR, 1973). Because food samples and specimens

have not routinely been examined for B. cereus, it is possible that the organism could have been responsible for unidentified outbreaks. How many, and if any bakery products would have been implicated is unknown.

Food handling practices have changed over the years. Currently, fewer food processing establishments are guilty of causing foodborne illness. From 1969-1973 food processors were responsible for only 6% of the outbreaks. Homes were implicated in 16.5% of the outbreaks whereas food service establishments were responsible for 35.2% of the outbreaks. The unknown category accounted for 42.1% of the outbreaks (Bryan, 1975). Perhaps retail markets are responsible for some of these outbreaks. Inadequate cooking and/or refrigeration are the factors contributing to foodborne disease outbreaks from 1961-72. Food contamination by infected workers is also a major factor (Bryan, 1975).

### Microbial Quality

The microbial quality of bakery products can vary widely, depending on the conditions of manufacturing. In one study, Abrahamson et. al. (1952), found S. aureus in 30% of the eclairs, 12% of the napoleons, 3% of the coconut pies, and 30% of nesselrode pies tested. The authors recommended standards of not more than 100,000/g total count and 10/g coliform for bakery products.

In another study, Surkiewicz (1966), examined frozen imitation pies made with dried milk but no cream or eggs. E. coli was isolated from 4.9%, and S. aureus was isolated from 3.5% of the pies sampled. Pies produced in plants under sanitary conditions had an aerobic plate count of < 25,000/g, absence of S. aureus, and average MPN of < 50/g coliform, and E. coli of < 3/g.

Studies concerning frozen cream desserts indicate these products

contain S. aureus in varying degrees. One study showed 42% of the cream and custard pies contained S. aureus in the range of 5-80,000/g (Levine, 1973). Another study (Leininger, et. al., 1971) on frozen cream type pies showed that 84% of the samples had an APC of < 10,000/g and 77% had a coliform count of < 10/g.

As a result of several outbreaks of cream filled pastries in the 30's, the California State Board of Health recommended certain practices to prevent food poisoning. Those recommendations included: preparing fillings under clean conditions, using pasteurized milk and fresh cream, time and temperature requirements, cooling fillings rapidly, keeping fillings cooled, proper methods of cleaning the equipment, and prohibiting persons with skin infections from handling the food (Anon., 1937).

#### Growth of Pathogens in Bakery Products

The advent of using synthetic ingredients seemed to bring about a decrease in the number of foodborne disease outbreaks concerning bakery products. However, one S. aureus outbreak dealing with cream filled donuts in an unrefrigerated vending machine prompted a study by McKinley and Clarke (1964). The filling contained no milk or eggs and the manufacturer's directions stated the product could be used in all seasons without refrigeration. The researchers inoculated three strains of S. aureus into the filling, incubated the product at 25 and 32<sup>0</sup>C for 20 and 40 h. Findings showed that the filling supported growth in all cases.

A similar study by Crisley, et. al. (1964) used seven commercial synthetic cream mixes containing no milk or eggs. Mixes were reconstituted as directed by the manufacturer. One was rehydrated with milk, the others with water. Findings indicated that the fillings were capable of supporting

bacterial growth at room temperatures. When inoculated with S. aureus, five fillings did not support growth of the organism and two did. With the addition of dry whole milk, all were capable of supporting growth. The authors concluded "claims that synthetic cream fillings do not support growth at room-holding temperatures cannot be considered valid in connection with completed pies."

A study by Scheusner and Harmon (1973) showed that enterotoxin A from S. aureus was evident in canned vanilla pudding at 24-48 h after incubating at 26°C. The level of S. aureus present at the time of toxin detection was  $4.8 \times 10^7$ /g. Keoseyan and Bennett (1972) found that enterotoxin was evident in reconstituted non-egg custard after 24 h at 25°C, and in 9 h at 35°C. Counts were  $6.9 \times 10^6$ /g and  $4.9 \times 10^6$ /g respectively.

Silliker (1969) states the presence of pathogenic microorganisms in bakery products is due to the use of raw materials which are contaminated, processing failure, or post-processing contamination. The author attributes the stability of bakery products to many factors. One is that the baking temperatures are high enough to destroy non-sporeforming bacteria in addition to the vegetative cells of sporeformers. Also, the cooking of fillings at 170-180°F is likely to destroy non-sporeforming bacteria.

### Inhibition of Pathogens in Bakery Products

The foodborne disease outbreaks of the 1930's and 40's implicating bakery products led to numerous studies on the prevention of growth of pathogens in these products. Most of these experiments dealt with inhibiting S. aureus, a few involved Salmonella, and none dealt with B. cereus since it was not considered a pathogen at the time. One of the earliest studies was conducted by Dack, et. al. (1931). As a result of a

staphylococcal food poisoning outbreak of a bakery cake, this experiment was performed to determine if the organism survived the baking process. It was found that the organism was killed during baking. However, the authors concluded that the filling was capable of supporting growth if inoculated after cooking.

Stritar, et. al. (1936) proved if custard filled puffs and eclairs were baked after filling, S. aureus would be killed and no apparent change resulted in the product. The authors found that S. aureus was killed in 25 min at 375 and 400<sup>0</sup>F, and 20 min at 425<sup>0</sup>F. Data indicated the same pattern for Salmonella. Gilcreas and Coleman (1941) confirmed this work. Along the same line, Cathcart, et. al. (1942a) found that bringing custard to a second boil was sufficient to kill S. aureus and Salmonella enteridis. Pies baked at 475<sup>0</sup>F for 25-35 min also killed both organisms.

The use of ultraviolet (UV) rays and ozone was examined by Cathcart, et. al. (1942b). Although ozone was ineffective against S. aureus and Salmonella enteridis, UV rays decreased the counts on smooth surfaces. UV rays were ineffective below the surface and imparted an off aroma.

Studies conducted by Ryberg and Cathcart (1942) showed Salmonella enteridis and S. aureus were inhibited in fruit pies (lemon, orange, pineapple, apricot, and strawberry). Lemon rind added to a standard custard had no effect on S. aureus until the concentrations were high enough to impart an off flavor. Cathcart and Merz (1942) found that S. aureus was inhibited by fillings made with natural chocolate and cocoa. The authors thought this was due to the pH and inhibitory substances in the nonfat portion of the chocolate or cocoa. Salmonella was not included in the experiment.

Cathcart, et. al. (1947) found that commercial dry mix puddings supported growth of S. aureus when made with milk. If water was substituted, no growth occurred but the flavor was less desirable. Vanilla, pumpkin, squash, sweet potato pie fillings, cheese cake fillings, and whipping cream mixes all supported growth of S. aureus. When milk was eliminated, S. aureus still grew as the products also contained eggs. The authors also found if citric acid was added to vanilla filling to decrease the pH to 3.43-3.65, no growth occurred but a sour taste resulted. The use of lactic acid yielded a better tasting product and retarded growth at a pH of 4.42-4.67. Peach and raspberry fillings were inhibitory by themselves.

Several amino acids and related compounds were tested by Castellani (1953) to determine if any exhibited an inhibitory effect on S. aureus in cream pastries. He found thioglycollic, DL-serine, and L cysteine HCl were effective, however, cysteine and thioglycollic acid imparted an undesirable flavor, and serine did not inhibit enterococci or Salmonella.

The use of antibiotics as a means of control was investigated by Godkin and Cathcart (1952). They found 100 ppm of 70% potency subtilin retarded growth of S. aureus and heat resistant flora for 48-72 h. A greater effect was observed when 1 ppm terramycin was combined with the subtilin. The authors indicated the use of antibiotics as a food preservative was questionable.

The use of chemical preservatives has been looked into as a possible means of control. Schmidt, et. al. (1969) found if the cream filling was acidified to a pH of 4.5-5.0, both potassium sorbate and sodium benzoate effectively inhibited the growth of S. aureus. The filling contained no

egg or milk products. Potassium sorbate was more effective than sodium benzoate if incubated at 22<sup>0</sup>C but sodium benzoate was more effective at 37<sup>0</sup>C.

Preonas, et. al. (1969), studied the effects of  $a_w$  and preservatives on the growth of S. aureus in southern custard pies. They showed that by using dextrose instead of sucrose to decrease the  $a_w$  to the point of inhibiting S. aureus, the product was organoleptically unacceptable. They also found sorbic and propionic acids did not inhibit S. aureus alone but were effective in combination. In addition, they found the lag time for the top surface was greater than that for the cut surface. This was due to a skin layer formed during baking which established a moisture gradient.

#### Potassium Sorbate

Sorbate has been used as a preservative in foods since 1930. It is most effective at a low pH and its upper range limit is pH 6.5. It is primarily effective against yeasts and molds. It has been reported that spores resist concentrations of sorbic acid which are inhibitory to other bacteria (Furia, 1968).

A study conducted by Pierson, et. al. (1979) showed potassium sorbate was effective against S. aureus in bacon when stored at 27<sup>0</sup>C for 14 days. Concentrations of potassium sorbate were 0.13 and 0.26% with no nitrite. At 13<sup>0</sup>C, potassium sorbate and nitrite were more effective than potassium sorbate alone.

Davidson et. al. (1979) found that potassium sorbate was effective in inhibiting the growth of both S. aureus and Salmonella typhimurium in trypticase soy broth at a pH of 6.0 only when used in combination with

butylated hydroxyanisole (BHA) and tertiary butylhydroquinone (TBHQ).

A similar study by Robach and Stateler (1980) showed 0.2% sorbate in trypticase soy broth (pH 6.0) slowed the growth and extended the lag phase of the two strains of S. aureus tested. However, inhibition resulted for one strain when sorbate was combined with NaCl (7%). Inhibition of both strains was evident when sorbate was combined with 100 ppm BHA.

## MATERIALS AND METHODS

### Food Distribution Systems and Sample Procurement

Five retail outlets in Corvallis, Oregon were selected for sampling of bakery products to represent a cross section of food distribution practices. The retail outlets selected included: two major supermarket chains with in-store bakeries, an independently owned supermarket with an in-store bakery, a major supermarket that distributes products from a central location, and a retail bakery. Procedures for preparation and distribution of product was obtained from each outlet.

The products used in the study were purchased directly from the outlet. Upon receipt in the laboratory, samples were stored at  $-23^{\circ}\text{C}$ . Prior to analysis, samples were thawed overnight at  $4^{\circ}\text{C}$ .

### Water Activity and pH

Water activity ( $a_w$ ) was measured using a Beckman Model HMP-1 Laboratory Moisture Measuring System located at the Oregon State Department of Agriculture in Salem, Oregon. Samples were purchased the day of testing and transported from Corvallis to Salem, Oregon without refrigeration. Standards provided with the instrument were run at the time of analysis.

A Perkin-Elmer Metrion IV pH Meter was used to determine pH. Prior to analysis, the instrument was calibrated using a pH 7.0 buffer solution.

### Microbial Quality

#### General

The microbial quality of the product was determined by analyzing for

aerobic plate count (APC), coliform, Staphylococcus aureus, Salmonella, and Bacillus cereus. The products were stored at 25°C simulating conditions of the food distribution system. Aliquots were taken at 24 h intervals for 72 h.

Samples of 50 g were weighed and blended in a Waring Blendor for 1 min with 450 ml 0.1% sterile peptone<sup>1</sup> water. A duplicate 50 g sample was blended with 450 ml lactose broth. Subsequent tenfold dilutions were made as necessary in 90 ml sterile dilution blanks containing 0.1% peptone. Duplicates were run on all tests with the exception of Salmonella.

### Aerobic Plate Count

One milliliter of each dilution was placed into each of two separate petri dishes. Plates were poured with tempered plate count agar (PCA) within 20 min. After cooling and solidifying, plates were incubated at 35°C for 48 h. A Quebec Colony Counter was used to count colonies and final calculations were made in accordance with the rules outlined in Standard Methods for the Examination of Dairy Products (Hausler, 1972).

### Coliform

Inocula of 1 ml from the first three dilutions (1:10, 1:100, 1:1000) were placed into each of three replicate tubes containing lauryl tryptose broth (LST) with invert tubes. Tubes were incubated at 35°C for 24-48 h and examined for gas production. A loopful from each gasing tube was transferred to brilliant green bile broth (BGB) with invert tubes. After

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<sup>1</sup>Unless otherwise noted, all media was prepared using Difco products (Difco Laboratories, Detroit, Michigan).

incubating at 35<sup>0</sup>C for 24-48 h, tubes were examined for gas production. Final counts were based on the number of positive BGB tubes and the Most Probable Number (MPN) tables in Compendium of Methods for the Microbiological Examination of Foods (Mayou, 1976).

### Staphylococcus aureus

Three tubes of BBL trypticase soy broth (TSB) with 10% NaCl were inoculated with 1 ml from each of the first three dilutions (1:10, 1:100, 1:1000) and incubated 48 h at 35<sup>0</sup>C, ~~for 48 h.~~ A loopful from each tube was streaked onto pre-poured Staphylococcus medium 110 (staph 110). Plates were incubated at 35<sup>0</sup>C for 48 h. (Baer, et. al., 1976).

The coagulase test was performed by transferring two colonies from each plate with growth to brain heart infusion broth (BHI) and incubating overnight at 35<sup>0</sup>C. To a small test tube, 0.5 ml coagulase plasma EDTA and two drops of the broth culture were added. The tubes were incubated at 35<sup>0</sup>C and examined periodically for 24 h for clot formation (Difco, 1978). Only tubes with a firm clot were considered positive (Sperber and Tatini, 1975). MPN's were calculated based on the number of coagulase positive tubes, and using the tables as previously described (Mayou, 1976).

### Salmonella

The lactose broth samples were incubated at 35<sup>0</sup>C for 24 h. One milliliter each was transferred to tubes of selenite cystine broth (SC) and tetrathionate broth (TT) to which a 1% brilliant green solution and a potassium iodide solution had been added. These were incubated for 24 h at 35<sup>0</sup>C after which a loopful from each tube was streaked onto pre-poured plates of brilliant green agar (BG) and xylose-lysine-desoxycholate

agar (XLD). Plates were incubated at 35<sup>0</sup>C for 24 h and examined for typical colonies. On BG, typical colonies appear fusia, translucent to opaque, with the surrounding medium pink to red (Poelma and Silliker, 1976; FDA, 1978). Typical Salmonella colonies on XLD after 24 h appear yellow with a black center and the surrounding medium is pink (Williams, 1978). Since no typical colonies appeared, further testing was discontinued.

### Bacillus cereus

From the 1:10 dilution, 0.1 ml was spread with a glass bent rod onto pre-poured plates of KG agar (Kim and Goepfert, 1971a). Plates were incubated at 30<sup>0</sup>C for 24 h. Following incubation, plates were examined for typical colonies which are surrounded by a zone of turbidity. Presumptive B. cereus colonies were chosen for confirmation tests.

Colonies were purified by inoculating slants of BBL nutrient agar and incubating at 30<sup>0</sup>C for 24 h. Slants were used to inoculate biochemical tests and to perform a gram and/or spore stain.

Carbohydrate utilization. The ability of the organism to utilize certain carbohydrates was tested for by inoculating tubes of phenol red carbohydrate broth containing 0.5-1.0% glucose, sucrose, glycerol, or salicin. After 24-48 h at 30<sup>0</sup>C, tubes were examined for acid (yellow) and gas production.

Nitrate reduction. The reduction of nitrate to nitrite was examined for by inoculating tubes of nitrate broth and incubating 24 h at 30<sup>0</sup>C. One-half milliliter of sulfanilic acid solution and 0.5 ml of alpha-naphthol solution were added to each tube. If the organism reduces nitrate, a red color is evident.

Acetylmethylcarbinol production. The production of acetylmethylcarbinol was tested for by using VP broth-modified for B. cereus (Leininger, 1976). After inoculating and incubating 48 h at 30°C, 5 ml of a 40% potassium hydroxide (KOH) solution was added to 5 ml of broth. Creatine (0.5-1 mg) was also added. The development of a red color within 60 min is a positive test.

Gelatin liquefaction. The ability of the organism to liquefy gelatin was tested by stabbing tubes of nutrient gelatin and incubating at 30°C for 1-7 days. Before reading results, tubes were held at 4°C for at least 1 h. (Goepfert, 1976; FDA, 1978).

### Pathogenicity

A mouse lethality test was performed to determine the pathogenicity of the B. cereus isolates. Intraperitoneal injections of 0.75 ml of either a broth or filtrate preparation were used. Four white mice (18-22 g) were utilized for each treatment. Mice were observed for 32 h to determine if death occurred.

The broth and filtrate were prepared by first inoculating tubes of BHI broth from nutrient agar slants and incubating overnight at 30°C. One-half milliliter of the BHI broth was added to 50 ml fresh beef infusion broth (Lennette, et. al., 1974) in 250 ml erlenmeyer flasks. Flasks were placed on a G24 Environmental Shaker (New Brunswick Scientific Co.) at 37°C, 90 rpm for 12 h. The broth was then centrifuged at 2300 rpm for 10 min using an International Centrifuge Model HN and supernatant was filtered through a fine fritted-glass filter. Positive and negative controls were prepared in the same manner. (Bonventre and Eckert, 1963). The number of

organisms in the broth was determined at the end of the 12 h period by plating 0.1 ml of the appropriate dilutions onto prepared BHI agar plates and spreading with a glass bent rod. After incubating 24 h at 30<sup>0</sup>C, plates were counted.

### Growth of Pathogens

#### Culture Preparation

Pure cultures of Staphylococcus aureus 265-1 A strain, Salmonella typhimurium, and Bacillus cereus were stored on nutrient agar or plate count agar slants at 4<sup>0</sup>C and transferred regularly. An inoculum of the culture to be used was prepared by transferring to BHI broth and incubating overnight at 35<sup>0</sup>C for S. aureus and Salmonella typhimurium, and 30<sup>0</sup>C for B. cereus. The broth was then centrifuged 10 min at 2300 rpm. The supernatant was discarded and cells resuspended in sterile phosphate buffer. After mixing thoroughly, tubes were centrifuged repeatedly to be certain any adhering medium was removed. The resulting suspension was adjusted to the appropriate optical density using a Bausch and Lomb Spectronic 20 spectrophotometer set at 500nm. Standardization of the optical density of each organism had been previously carried out using the same procedure. Appropriate dilutions were made to achieve the proper inoculum. Counts were determined from the suspension on BHI agar and incubated at 35<sup>0</sup>C for 24 h to determine more precisely the number of organisms inoculated (Lee, 1978).

#### Inoculations

Growth studies were carried out by inoculating samples with the culture preparation described above. Products were inoculated with a low level of

organisms (< 250/g). Samples inoculated with S. aureus, Salmonella typhimurium, or B. cereus were stored at 4, 25, or 35°C. Growth curves were determined by taking aliquots at certain intervals in a 0-10 day period and enumerating for the organism according to procedures described below.

### Staphylococcus aureus

Duplicate 25 g aliquots of product inoculated with S. aureus were taken at regular intervals for enumeration. The sample was blended with 225 ml 0.1% sterile peptone water for 1 min. Tenfold dilutions were made in 90 ml sterile 0.1% peptone dilution blanks. For each dilution, 1 ml was equally distributed over three pre-poured Staph 110 plates and spread using sterile glass bent rods. Plates were incubated 48 h at 35°C and counted (Baer, et. al., 1976).

The coagulase test, as described previously, was used for confirmation. Final counts were based on the number of coagulase positive colonies.

### Salmonella

Salmonella was enumerated in the inoculated product using a three tube MPN method. Duplicate 25 g portions, taken at regular intervals, were blended 1 min in 225 ml sterile 0.1% peptone water and subsequent tenfold dilutions were made. For each dilution, 1 ml was transferred to each of three tubes containing 10 ml selenite cystine broth. Tubes were incubated 24 h at 35°C. A loopful from each tube was streaked onto XLD agar and incubated 24 h at 35°C. Typical colonies were noted and examined

further (Poelma and Silliker, 1976; Sharf, 1966).

Since organisms other than Salmonella can grow on XLD agar (Citrobacter, Proteus, Arizona) the following confirmation tests were performed.

Differential media. Typical colonies on XLD plates were inoculated onto slants of triple sugar iron agar (TSI) and lysine iron agar (LIA) by stabbing the butt and streaking the slant. Tubes were incubated 24 h at 35<sup>0</sup>C and examined for typical reactions.

Biochemical tests. Malonate broth tubes were inoculated and incubated 24-48 h at 35<sup>0</sup>C. A change in color from green to blue indicates a positive reaction. Phenol red carbohydrate broth with 0.5-1% dulcitol tubes were inoculated and incubated 24-48 h at 35<sup>0</sup>C. Acid (yellow) and gas production were noted. Calculations were based on the MPN table and the number of positive Salmonella tubes. (Poelma and Silliker, 1976; Williams, 1978).

### Bacillus cereus

B. cereus was enumerated in the inoculated product by taking duplicate 25 g aliquots at regular intervals. The sample was blended with 225 ml sterile 0.1% peptone water for 1 min. Subsequent tenfold dilutions were made and 0.1 ml of each dilution was spread over prepared KG agar plates with sterile glass bent rods. Plates were incubated at 30<sup>0</sup>C for 24 h and typical colonies were counted.

Final counts were determined based upon the number of confirmed colonies as described previously (Goepfert, 1976; FDA, 1978).

### Staphylococcal Enterotoxin

Samples to be tested for the presence of staphylococcal enterotoxin A were inoculated with  $1.0 \times 10^4$ /g S. aureus and held at 25°C for 24, 48, and 72 h. One sample was held at 35°C for 48 h. At the end of the incubation period, the samples were placed in sterile Whirl Pak bags and frozen. Samples were then packed in dry ice and shipped to the testing laboratory via air freight. Extraction of the enterotoxin was carried out using the Casman-Bennett method (Bergdoll and Bennett, 1976). Sample elutes were tested serologically by the procedure outlined by Bennett and McClure (1976).

### Baking

Retail baking conditions were simulated in the laboratory to determine internal temperatures and ability of organisms to survive the cooking process. Uncooked filling and pie shells were purchased at a local retail outlet involved in the study, and stored at 4°C until used. Magic Chef Series VI ovens were preheated to 450 and 375°F. Thermocouples attached to a Honeywell Brown Electronic temperature recorder were placed in the center of the pie. The product was baked until done as indicated by a knife coming out clean after inserting in the center. Cooking and cooling temperatures and times were recorded.

### Potassium Sorbate Treatment

S. aureus, Salmonella typhimurium, and B. cereus were inoculated into product containing 0.25% potassium sorbate and stored at 25°C. Organisms

were enumerated according to procedures described above to determine if growth occurred. Aliquots were taken at regular intervals in a 0-3 day period.

#### D values of Bacillus cereus

D values of the B. cereus isolates were determined by the Thermal Death Time (TDT) tube method described below. Inocula of the organisms were prepared and enumerated by the optical density method previously outlined. Triplicate samples (5 ml) of inoculated filling in screwcapped test tubes were placed in a mineral oil bath and subjected to various times and temperatures. Tubes were heated at 100°C for 5, 10, 30, and 60 min; 108°C for 1, 5, 15, and 25 min; and 124°C for 1, 5, 10, and 15 min. After heat treatment, tubes were immediately placed in an ice bath.

After cooling, enumeration was carried out with duplicate tubes by making the appropriate dilutions (1:2, 1:10, 1:100) and plating on KG agar. Plates were incubated 24-48 h at 30°C. Biochemical confirmation was performed in addition to spore stains. The actual D value was calculated from a survivor versus time graph (Stumbo, 1965). One tube from each treatment was stored at 30°C to determine if thermal injury and recovery would take place. After incubating for 48 h, enumeration procedures were performed.

## RESULTS AND DISCUSSION

### Water Activity and pH

Water activity ( $a_w$ ) and pH of several bakery products were analyzed to determine which products would be conducive to further study. Actual  $a_w$  and pH values of the products examined are in Table II. All of the products had an  $a_w$  of 0.85 or above and/or a pH of 4.5 or above. These are the limits set forth in the proposed FDA model retail food store sanitation ordinance (Federal Register, 1977) for "potentially hazardous foods" to be refrigerated. Pumpkin pie was chosen as a model for bakery products for further study.

### Baking

Pumpkin pies were baked at two different temperatures simulating the range of baking condition used in the bakery. Time and temperature of the baking and cooling period were recorded. Table III shows pies reached an internal temperature of 222-223<sup>0</sup>F (105.56-106.11<sup>0</sup>C) in 39 and 49 min and took 2.1 and 1.9 h to cool to room temperature when baked at 450 and 375<sup>0</sup>F (232.22 and 190.56<sup>0</sup>C) respectively. It should be noted that the room temperature was rather warm (84<sup>0</sup>F) -- a condition not uncommon in bakeries.

### Food Distribution

#### Practices

In the retail markets studied, pumpkin pies were distributed by two different systems. In one, pies were baked at the retail market whereas

Table II. Water Activity ( $a_w$ ) and pH of Bakery Products.

Product	$a_w$	pH
Custard Filled Donut		
Filling	0.98	6.4
Crust	0.95	-
Pumpkin Pie		
Filling	0.97	5.6-6.6
Lemon Meringue Pie		
Filling	0.99	-
Meringue	0.85	-

Table III. Baking Conditions for Pumpkin Pie.

Factor	Oven Temperature	
	450°F (232.22°C)	375°F (190.56°C)
Time to doneness	40 min	60 min
Maximum internal temperature	223°F (106.11°C)	222°F (105.56°C)
Time to reach maximum temperature	39 min	49 min
Time held at maximum temperature	1 min	1 min
Time to reach room temperature (84°F) (28°C)	2.1 h	1.9 h

in the other, pies were baked at a central location and transported to the retail market by truck. Figures 2-6 illustrate the distribution practices of each store included in the study. Stores A and B represent supermarket chains, store C is an independently owned supermarket, store D is a supermarket that distributes products from a central location, and store E is a retail bakery. The figures show that there are different methods of preparation and baking. Most of the bakers preferred to store the filling overnight in the refrigerator (stores A, C, and E), but others bake it immediately (store B).

The time and temperatures of storage of any perishable food are very important. Display and holding conditions of pumpkin pie after processing varied in this study. Cooling times in the retail bakeries were said to range from 0.5-1.5 h, however, under controlled laboratory conditions, it was found to take 2.1 h when baked at 450<sup>0</sup>F and 1.9 h when baked at 375<sup>0</sup>F. Only one store (store A) consistently displayed pies in the refrigerated cases. The other stores kept the products at room temperature. One baker indicated pies would be placed in a refrigerated case if space allowed. Store E's volume of pumpkin pies was very low. The baker indicated that the pies were displayed under refrigeration, however the author did not verify this statement. Pumpkin pies for store D were made at a central bakery and transported to the store. Although the truck was refrigerated, the pies were displayed at room temperature. Procedures in handling old product also varied from store to store. Pull dates ranged from two to five days.

Table IV shows the ingredients used in the pies. All used canned pumpkin, a source of milk and eggs, sugar, and spices. Three of the

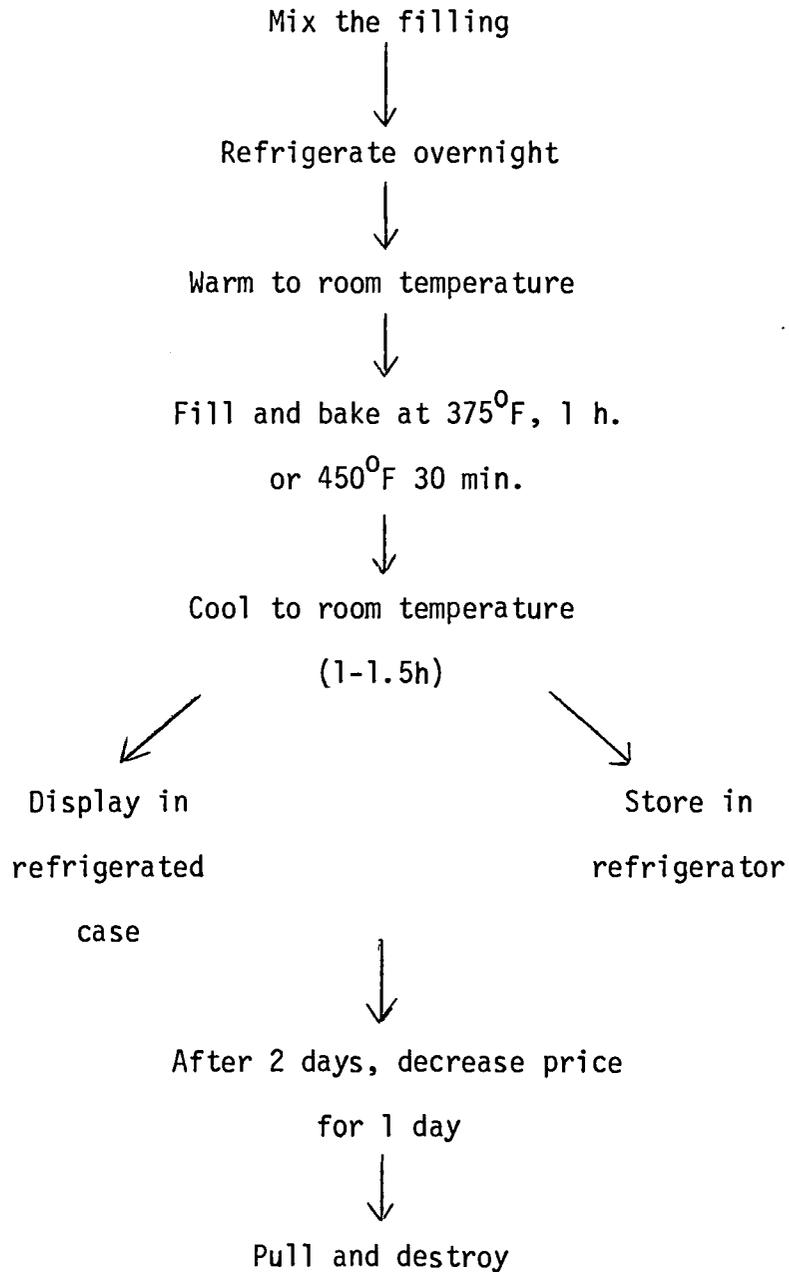


Figure 2. Store A distribution pattern for pumpkin pie.

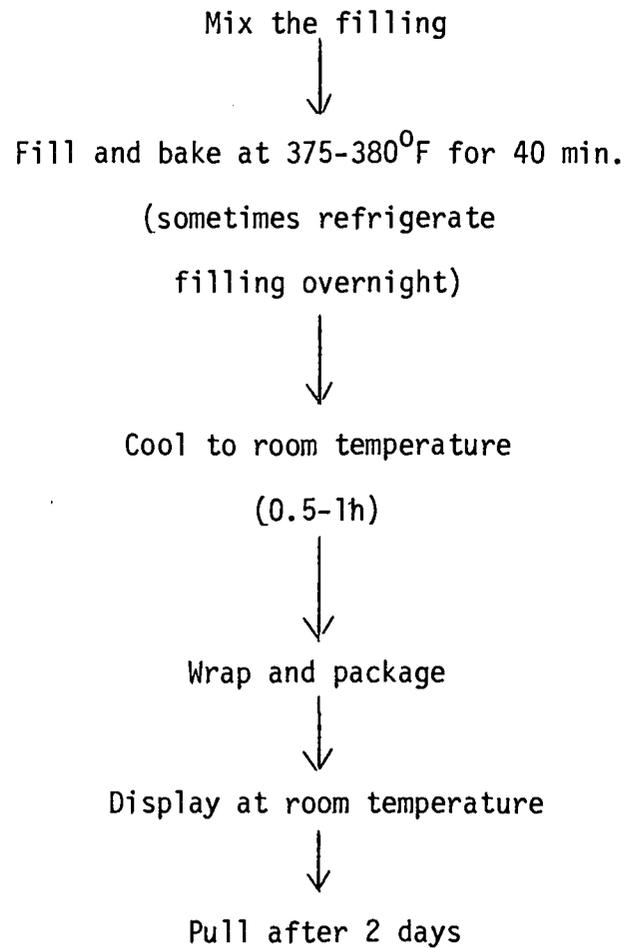


Figure 3. Store B distribution pattern for pumpkin pie.

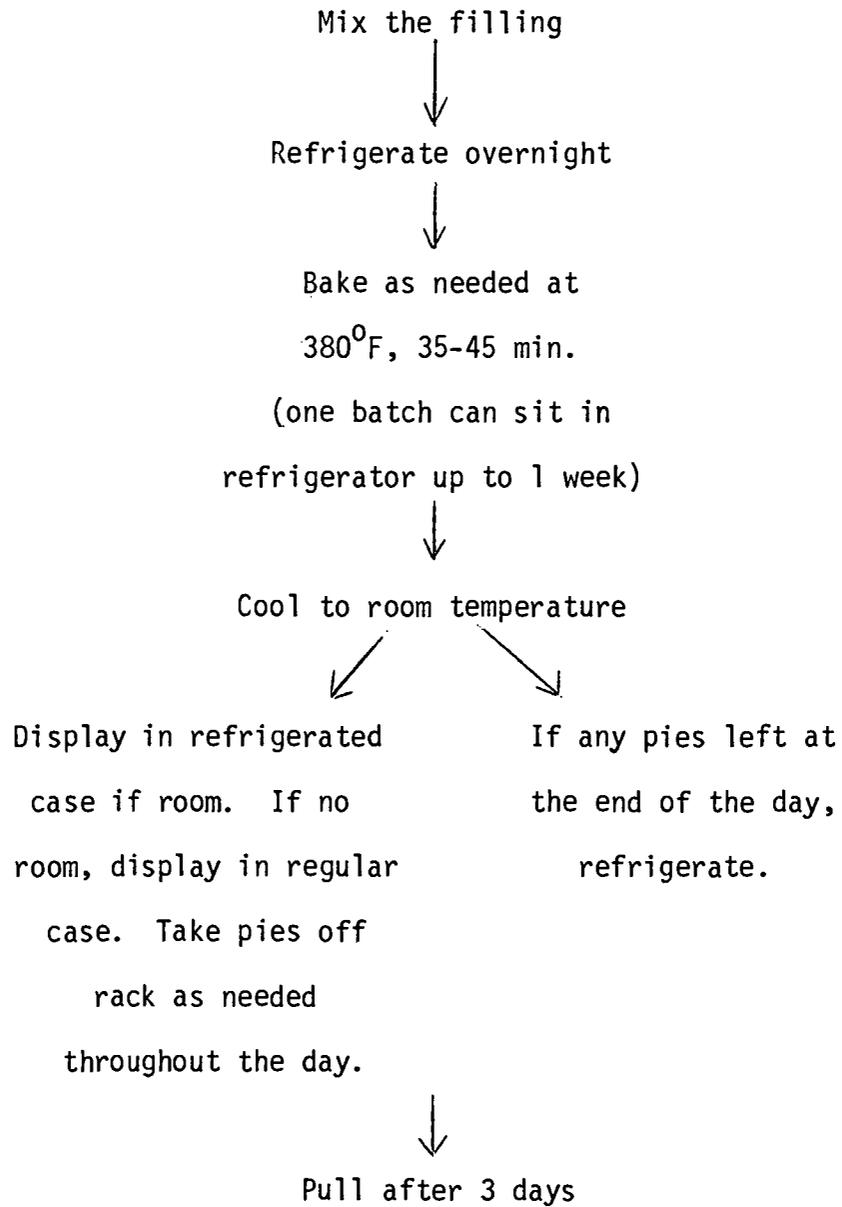


Figure 4. Store C distribution pattern for pumpkin pie.

Baked and packaged at central bakery (Portland area)

(8:00 pm-4:00 am)



Placed in refrigerated truck and

transported



Delivered to Corvallis

7-7:30 am



Displayed at room

temperature



Pull after 5 days

Figure 5. Store D distribution pattern for pumpkin pie.

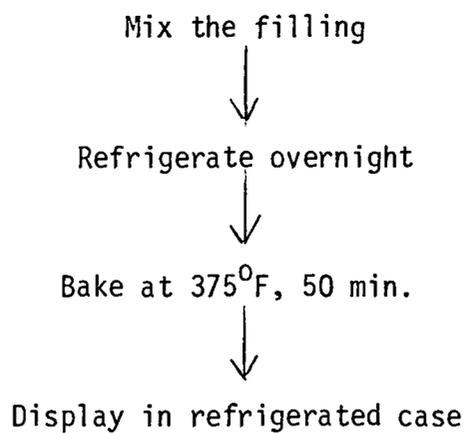


Figure 6. Store E distribution pattern for pumpkin pie.

Table IV. Pumpkin Pie Ingredients.

Ingredient	Store				
	A	B	C	D	E
Pumpkin-canned	X	X	X	X	X
Whole milk	X		X		X
Milk powder		X			X
Condensed milk				X	
Whole eggs-frozen	X	X	X		X
Egg-o-mix				X	
Granulated sugar	X	X	X	X	X
Brown sugar	X			X	
Honey	X				
Butter-melted		X			
Oil			X		
Flour		X			
Bread flour			X		
Vanilla			X	X	
Salt	X	X	X	X	X
Pumpkin pie spice	X	X	X		
Nutmeg				X	
Cinnamon				X	X
Allspice				X	
Cloves					X
Water		X			

bakers used whole milk, one used milk powder, and one used whole frozen eggs whereas the other baker used egg-o-mix.

### Summary

There seem to be two critical factors involved in the safe handling of bakery products. One is the knowledge of the baker and employees regarding food handling practices. For instance, the baker for store C did not seem concerned about leaving products with egg and milk at room temperature for an extended period of time. On the other hand, the baker for store A seemed fairly knowledgeable. It was observed that there was a considerable lack of knowledge among bakers in regard to safe food handling practices.

The second factor involved the physical design of the bakery. Lack of refrigeration space in both the preparation area as well as display cases was noted in every instance except store E which was a retail bakery.

### Microbial Quality

The microbial quality of pumpkin pie from stores A, B, C, and D was determined. Store E product was eliminated due to its unavailability. Pies were stored at 25<sup>0</sup>C for 72 h to simulate the distribution practices most commonly found. Table V summarizes the results.

### Aerobic Plate Count

All the pies tested showed an increase in aerobic plate count. In addition, after 72 h at 25<sup>0</sup>C, a significant change in odor and occasionally some mold growth was evident. Figure 7 illustrates the increase in growth.

Table V. Microbial Quality and pH of Pumpkin Pies Stored at 25°C.

Store	Days	APC/gm	Coliform MPN/g	<u>S. aureus</u> MPN/g	<u>Salmonella</u>	<u>B. cereus</u>	pH
A	0	25 <sup>7</sup>	<3	<3	ND <sup>2</sup>	ND	6.2
	1	9.0 x 10 <sup>9</sup>	<3	<3	ND	Positive	-
	2	3.0 x 10 <sup>9</sup>	<3	<3	ND	ND	-
	3	4.2 x 10 <sup>9</sup>	<3	<3	ND	ND	4.2
B	0	75	<3	<3	ND	ND	5.6
	1	1.3 x 10 <sup>5</sup>	<3	<3	ND	ND	5.6
	2	9.9 x 10 <sup>6</sup>	<3	<3	ND	ND	5.5
	3	7.0 x 10 <sup>7</sup>	<3	<3	ND	ND	4.3
C	0	75	<3	<3	ND	ND	6.1
	1	3.4 x 10 <sup>5</sup>	6.7	460	ND	ND	6.1
	2	6.2 x 10 <sup>7</sup>	>2400	1100	ND	ND	6.0
	3	1.9 x 10 <sup>8</sup>	>2400	>2400	ND	ND	4.8
D	0	< 10	<3	<3	ND	ND	6.1
	1	< 10	<3	<3	ND	ND	6.0
	2	6.6 x 10 <sup>3</sup>	<3	<3	ND	ND	5.9
	3	4.5 x 10 <sup>5</sup>	<3	<3	ND	Positive	5.9

<sup>1</sup>Counts based on the average of duplicate samples

<sup>2</sup>ND = none detected

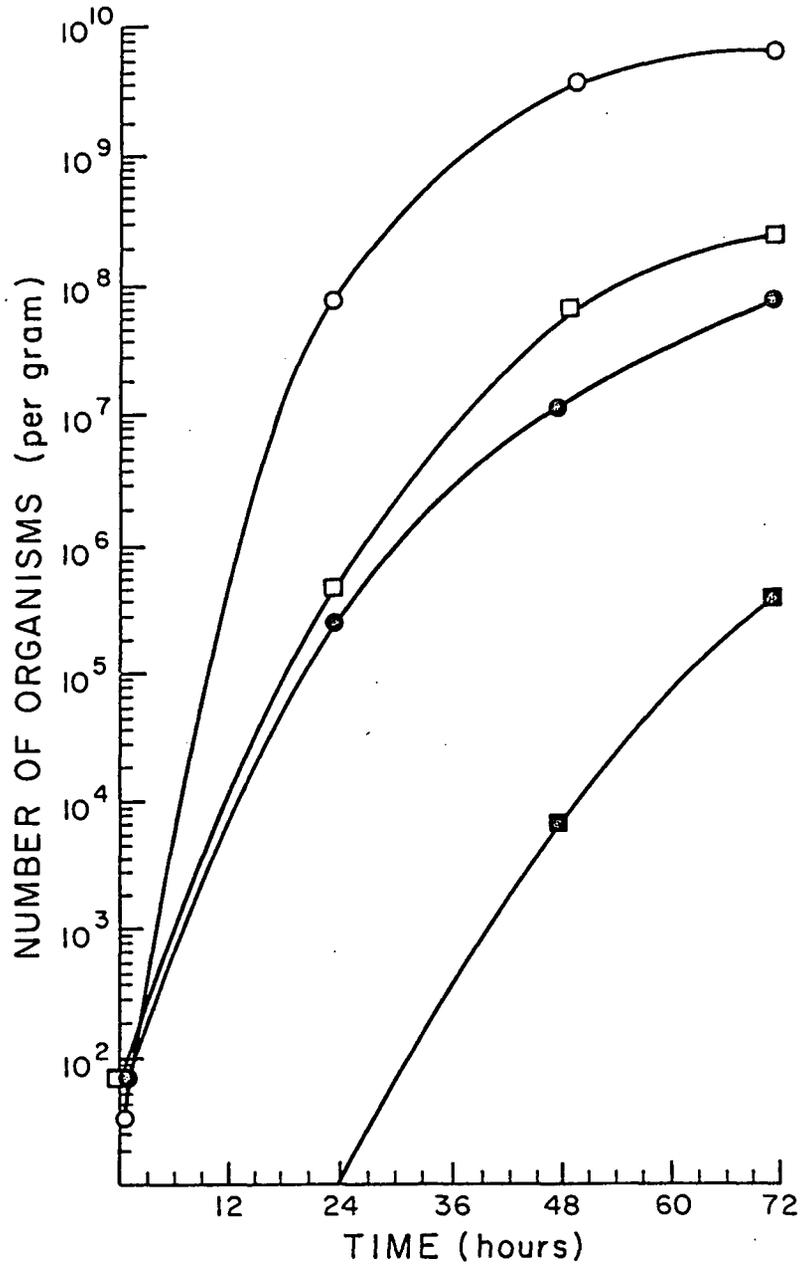


Figure 7. APC of pumpkin pie stored at 25°C for 72 h.  
 ○ - Store A, ● - Store B, □ - Store C,  
 ■ - Store D.

Although all followed the same growth pattern, the count from store D pie started off at a lower count and did not reach the high levels that the other pies did. This could be due to the use of more processed ingredients in store D product. For instance, the use of condensed milk versus whole milk and egg-o-mix versus whole frozen eggs. One would normally expect processed foods to have lower bacterial counts than their fresh counterparts.

Related to the increase in aerobic plate count is the significant decrease in pH after 72 h in product from stores A, B, and C (Table V). The milk and eggs in the pies could act as buffers to delay the pH from decreasing due to acids produced by bacteria. However, the pH would eventually decrease--by 72 h in this case. This theory is substantiated by the fact that pie from store D did not show a significant decrease in pH because the APL count was two to four log cycles lower than the others. The bacterial load was low enough for the buffers to keep the pH at the same level.

### Summary

Quality of perishable bakery products is dependent on many factors, only one being microbial. This factor strongly influences acceptability of the product. Shelf life of most perishable food is determined by microbial quality. There is lack of agreement on what the limits should be for different foods. For some foods, attempts have been made to more clearly define these limits than others. For fluid dairy products, off-flavors are detected at  $10^4$ - $10^7$  organisms/ml, fresh meat should not exceed  $10^6$  organisms/g and poultry is considered spoiled at  $10^7$ - $10^8$  organisms/cm<sup>2</sup>

(OTA, 1979).

Based on APC levels alone, one could question some of the pull date practices of the stores that are not refrigerating these products. Store A normally refrigerates its product and pulls after three days. This is a good practice as the product reached an APC of  $9.0 \times 10^7$  organisms/g after one day at  $25^{\circ}\text{C}$ . Store B does not refrigerate the product and pulls after two days of display. After two days at  $25^{\circ}\text{C}$ , the APC level was  $9.9 \times 10^6$  organisms/g. One might question the display practice on this product, especially if the consumer might not consume the product right away and could realistically add another day or two storage at room temperature. Store C pulls the product after three days of display and may use some refrigerated storage. An APC of  $1.9 \times 10^8$  organisms/g was obtained on this product after incubation at  $25^{\circ}\text{C}$  for three days. In addition, high levels of coliform organisms were detected ( $> 2400$  MPN/g) after three days. This suggests post-processing contamination. Jay (1978) indicates that coliforms do not survive temperatures greater than  $50^{\circ}\text{C}$ . No coliforms were detected in any of the other samples studied. Store D displays the product for five days at room temperature. The product is baked at a central bakery and delivered to the store in a refrigerated truck. Initial bacterial load on this product was lower than values obtained for all other products. Incubation did not extend beyond three days but after that time the product had an APC level of  $4.5 \times 10^5$  organisms/g. Again one could question the five day pull date practice.

#### Presence of Pathogens

Staphylococcus aureus. All samples were screened by the MPN method

for the presence of S. aureus. Only one sample (product from store C) contained detectable levels of S. aureus (Table V). After incubating at 25<sup>0</sup>C for 1 day, a S. aureus count of 460 MPN/g was obtained. S. aureus is destroyed at temperatures normally used in food processing and should have been inactivated by the baking conditions. It is probable that the presence of S. aureus was due to post-processing contamination. Humans are the main reservoir and source of contamination of S. aureus in processed foods.

Salmonella. Salmonella was not detected in any of the samples tested. Although Salmonella is a fecal organism and could potentially be found in some of the ingredients used, it is unlikely that this organism would survive the processing temperature.

Bacillus cereus. Bacillus cereus was isolated from product from two of the four stores sampled, stores A and D (Table V). It was previously mentioned that this organism grows at 10-49<sup>0</sup>C with an optimum of 30<sup>0</sup>C (Goepfert, et. al., 1972)

D values of the B. cereus isolate from product from store D were determined to see if the organism would survive the cooking process. Figures 8-10 show the actual D values obtained for 100, 108, and 124<sup>0</sup>C respectively. The temperature of 108<sup>0</sup>C approximates the internal temperature reached during a normal baking process. It was determined that the D<sub>108</sub> value was 10.5 min. Therefore, B. cereus could very well survive the heat process of baking pumpkin pies to an internal temperature of 106<sup>0</sup>C for 1 min. An aliquot was incubated for 48 h after the heat treatment. Growth occurred in all the samples suggesting that the

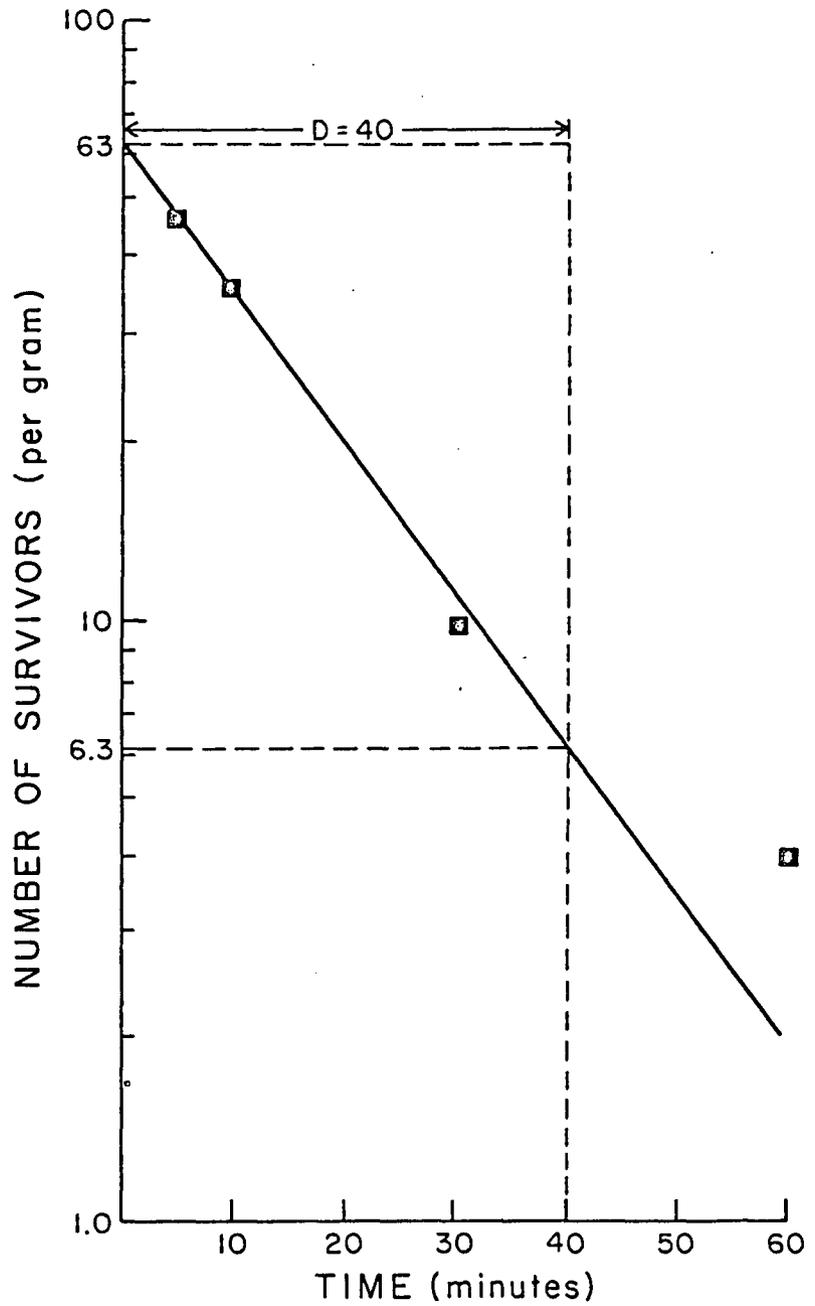


Figure 8. Death rate curve of *B. cereus* isolate in pumpkin pie at 100°C.

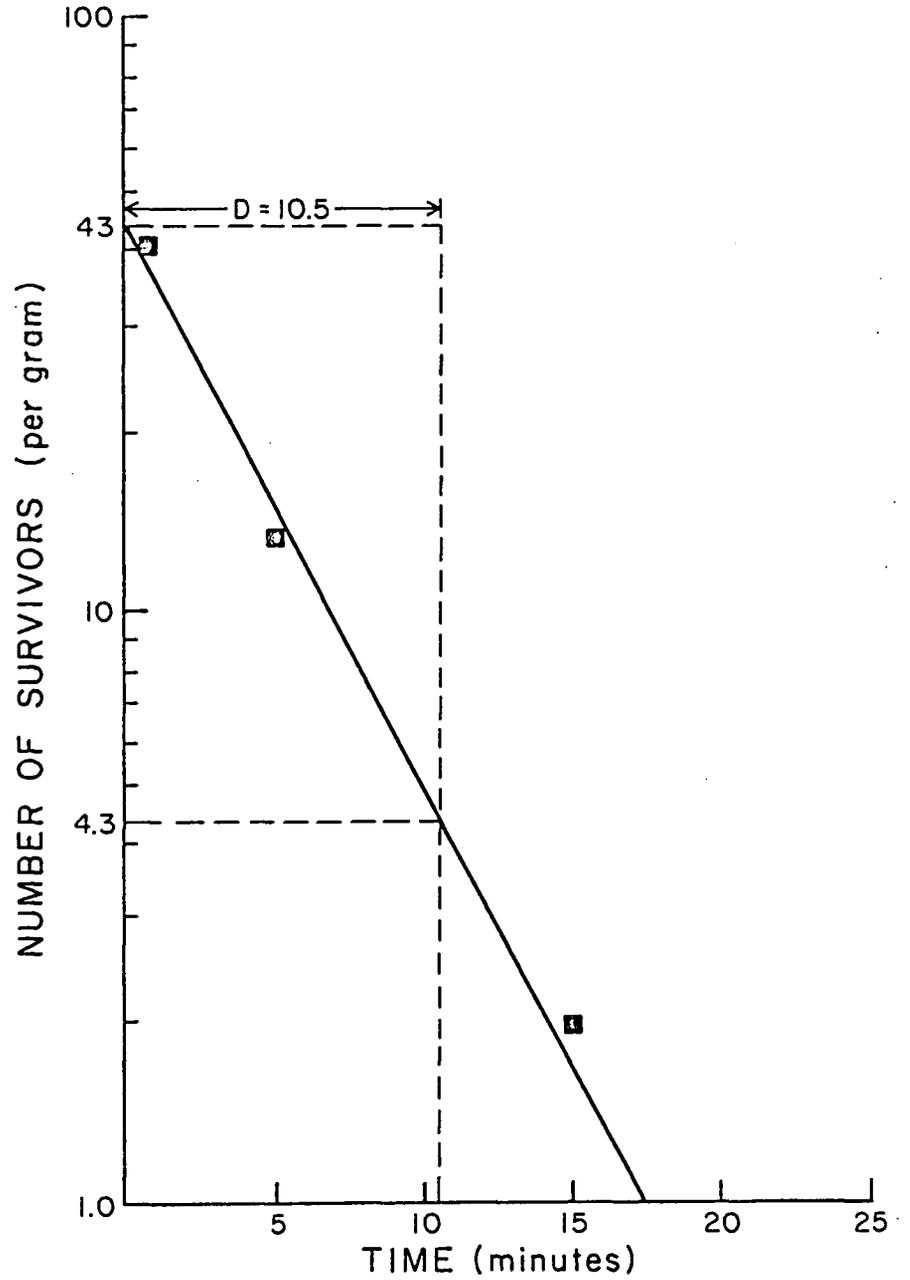


Figure 9. Death rate curve of *B. cereus* isolate in pumpkin pie at 108°C.

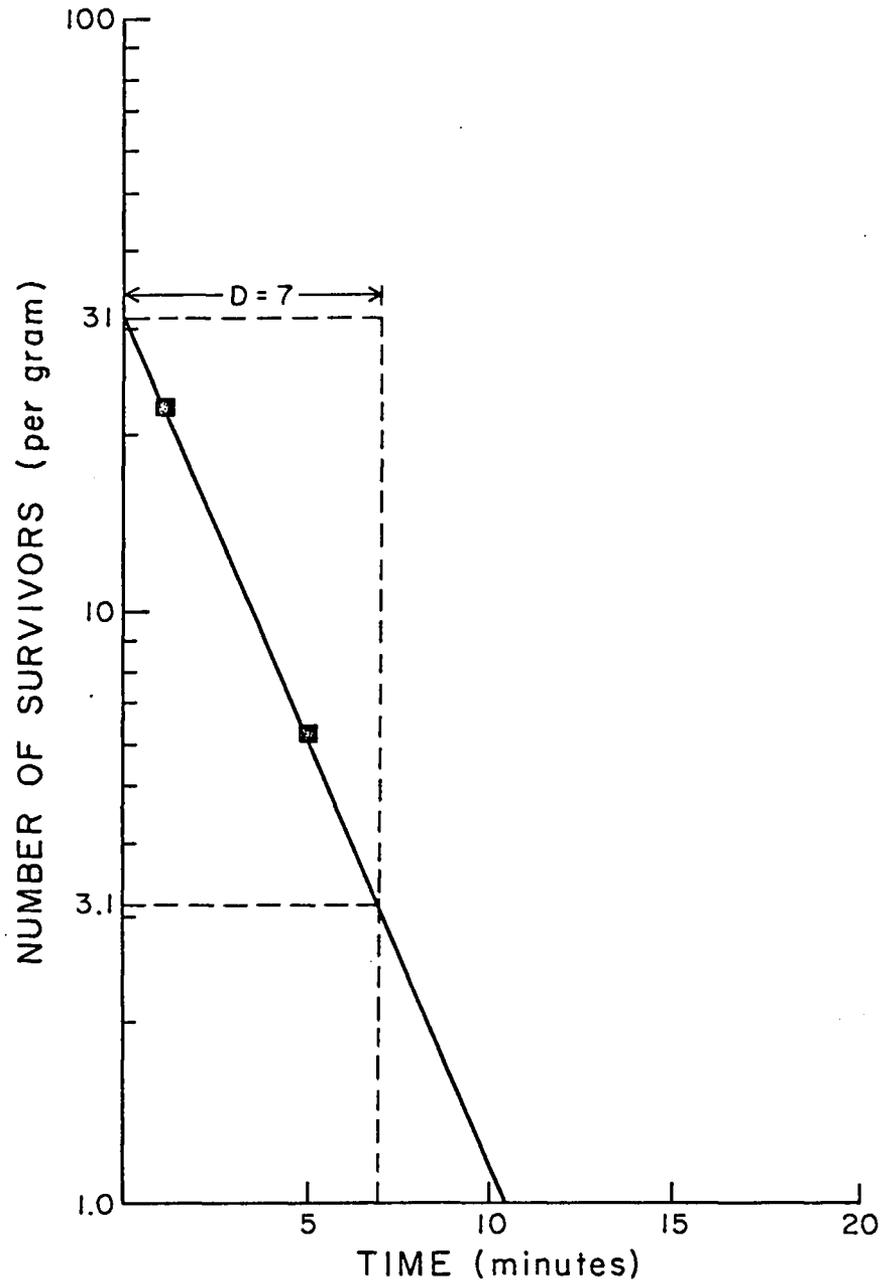


Figure 10. Death rate curve of *B. cereus* isolate in pumpkin pie at 124°C.

surviving spores recovered from any thermal injury.

Unlike the case with coliforms and S. aureus, B. cereus contamination was probably due to pre-processing contamination. It has been documented that B. cereus occurs in milk (Davies and Wilkinson, 1973; Stone and Rowlands, 1952) and spices (Powers, et. al., 1976; Kim and Goepfert, 1971b), both of which are in pumpkin pie.

Pathogenicity of the B. cereus isolates was determined by the mouse lethality test. Results are shown in Table VI. Death of mice took place rapidly, within 2-3 h, for the broth culture. The filtrate seemed to take longer, ranging from 5-11.5 h, for death to occur. Symptoms of the dying mice were similar to those described by Burdon, et al. (1967) --listlessness, ruffled fur, ears back, paralysis of the hind quarter, and prior to death, labored breathing and convulsions.

With both the filtrate and the broth, mice injected with product from store D died faster than the others. Perhaps this is a more virulent strain. The reason for the failure of the positive filtrate control to produce death is not known. Repeated effort could not induce death. The explanation could lie in the type of toxin produced in that death did occur with the broth preparation. B. cereus produces a wide range of extra-cellular metabolites largely during the exponential growth phase. Some of these include proteases,  $\beta$ lactamases, peptide antibiotics, phospholipases, and hemolysins (Gilbert, 1979). Spira and Goepfert (1975) reported that B. cereus produced an enterotoxin which was synthesized and released during the exponential growth phase. Another possible factor for the filtrate preparation not being able to produce death could be that the culture had been maintained for years at the Microbiology Department at

Table VI. Mouse Lethality of B. cereus Isolates from Pumpkin Pie.

Store	Treatment	Initial inoculum (per gram)	Number of deaths	Time to death (h)
A	Broth	$1 \times 10^9$	4/4	2.5-3
D	Broth	$1 \times 10^8$	4/4	2
Positive Control	Broth	$1 \times 10^8$	4/4	2.5-3
Negative Control	Broth	-	0/4	-
A	Filtrate	$1 \times 10^9$	3/4	7.5-11.5
D	Filtrate	$1 \times 10^8$	4/4	5
Positive Control	Filtrate	$1 \times 10^8$	0/4	-
Negative Control	Filtrate	-	0/4	-

Oregon State University and its pathogenicity had not previously been tested. Another reason could be that the culture is a deviant strain which is common with this organism (Goepfert, et. al., 1972).

Toxin synthesis for B. cereus is not clearly understood. Frequent transfer of the organism seems to be necessary to prevent transfer of certain biochemical properties. Burdon, et. al. (1967) found that cultures not activated by frequent transfers resulted in no death or illness in mice even at levels of  $2.3 \times 10^9$  organisms/g. In this study the initial count was  $1.0 \times 10^8$  to  $1.0 \times 10^9$  organisms/g -- well above the LD<sub>50</sub> range reported by Lamanna and Jones (1963) to be  $1.05 \times 10^7$  to  $3.2 \times 10^7$  organisms/g. The main interest in this study was to determine if the isolates were capable of inducing illness and not necessarily characterize the toxin. Work should be continued in this area to learn more about the characteristics of the isolates.

### Summary

It should be noted that three out of the four stores sampled sold pumpkin pies that contained either S. aureus or B. cereus. Both of these pathogens could cause foodborne illness given the proper conditions to grow and produce enterotoxin. Store C's product was obviously mishandled after being baked as evidenced by the coliform and S. aureus counts showing up at just 24 h when incubated at 25°C. Given the current distribution practices of leaving the pies at room temperature for 2-5 days, it appears that if contaminated, these bakery products would certainly pose a public health risk.

## Growth of Pathogens

Bakery products are subjected to various temperatures before they are eaten, ranging from being stored in the refrigerator to sitting in the grocery bag in the car on a hot summer day for a few hours. Pumpkin pies were inoculated with pathogens and incubated at 4, 25, and 35°C to simulate potential food handling practices. Results are shown in Tables VII, VIII and Figure 11.

### Refrigeration Conditions

Table VII indicates that refrigeration controls the growth of the three pathogens tested. Pumpkin pie was unable to support the growth of S. aureus, Salmonella typhimurium, and B. cereus for up to two weeks when held at 4°C.

### Room Temperature Conditions

Figure 11 shows the growth curve of the pathogens tested when pumpkin pies were stored at 25°C for 84 h.

Staphylococcus aureus. The growth curve (Figure 11) for S. aureus in pumpkin pie stored at 25°C follows the typical growth curve. The lag phase was short, lasting less than 12 h; the exponential phase ending at 42 h, and the constant growth period continuing throughout the remainder of the experiment, up to 3.5 days. It is likely that if carried out longer, the death phase would have been more evident. It should be noted that it took less than 24 h for the count to reach 500,000/g -- the number estimated to produce enough toxin to elicit food poisoning symptoms.

Table VII. Growth of Pathogens<sup>1</sup> in Pumpkin Pie Held at 4°C.

Time	<u>S. aureus</u> (MPN/gram)	<u>Salmonella</u> (per gram)	<u>B. cereus</u> (per gram)
0	210	240	150
1 week	<30	<4 <sup>2</sup>	<100
2 week	<30	<4 <sup>2</sup>	<100

<sup>1</sup> Counts based on the average of duplicate samples.

<sup>2</sup> Count based on 4 for one sample, and 3 for the duplicate count.

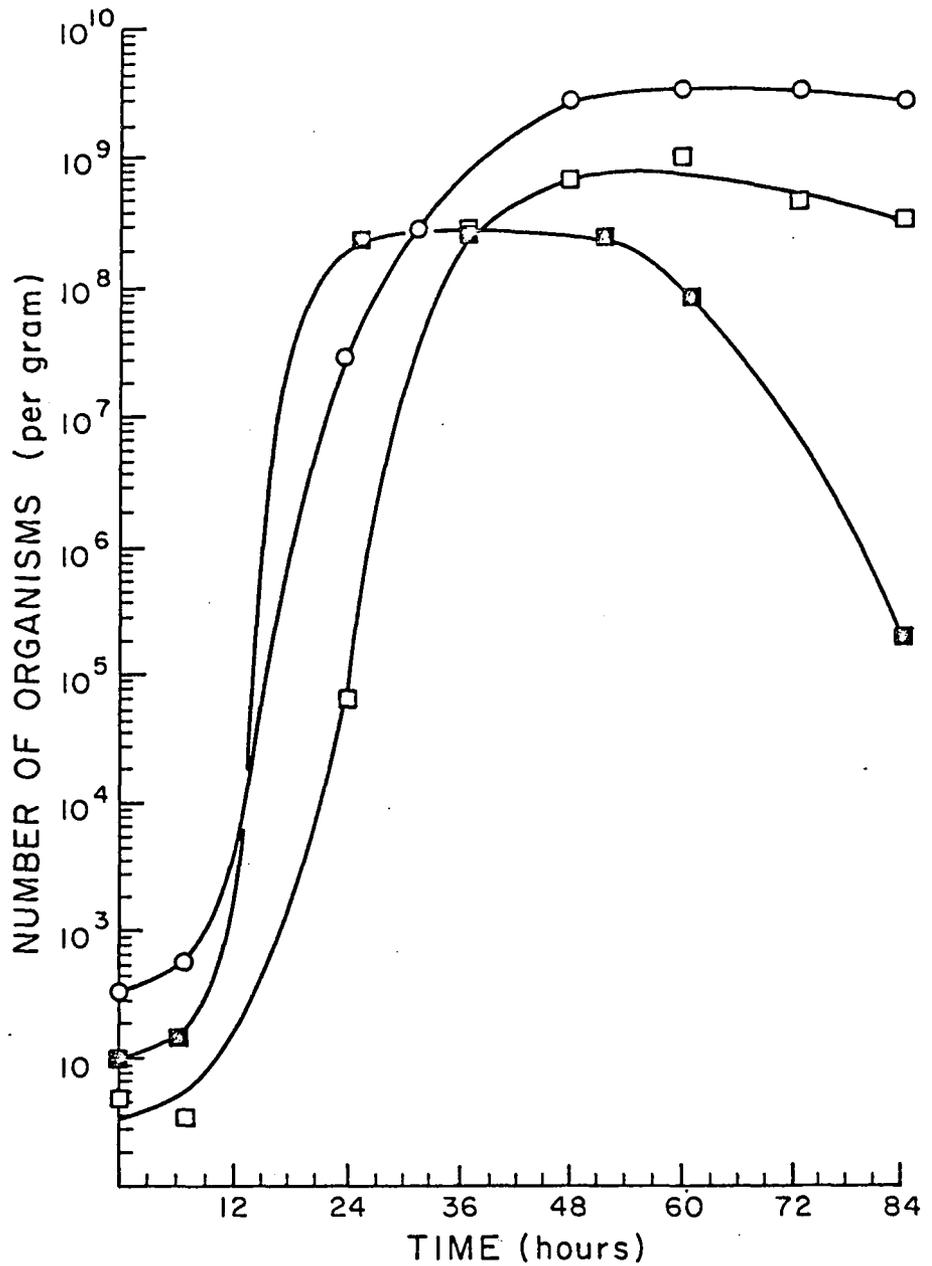


Figure 11. Growth curve of pathogens in pumpkin pie stored at 25°C. ○ - *S. aureus*, □ - *S. typhimurium*, \* - *B. cereus*.

Salmonella. When pies were inoculated with Salmonella typhimurium and stored at 25<sup>0</sup>C, the organism followed a pattern similar to that of S. aureus (Figure 11). The lag phase lasted 12 h and the exponential phase 24 h. The counts leveled off after 36 h. Again, there was no evidence of die off but if incubated longer, death would have occurred. It has been stated in the literature that one needs to ingest several million to several billion Salmonella/g to cause illness (Jay, 1978). In this case, it took only 32.5 h to reach the one million/g point when pies were stored at 25<sup>0</sup>C.

Bacillus cereus. Like S. aureus and Salmonella, B. cereus followed the typical growth curve when inoculated into pumpkin pie and held at 25<sup>0</sup>C. (Figure 11). Unlike S. aureus and Salmonella though, B. cereus showed a die off after 48 h. The constant growth period lasted for only 24 h. Small pinpoint colonies were observed on some KG agar agar plates. On further examination, colonies were found to be gram positive cocci. Further identification was not performed. It has been reported that B. cereus stimulates the growth of S. aureus (McCoy and Faber, 1966). However, Troller and Frazier (1968) detected an inhibitory action. Bacillus species produces the antibiotic polymyxin which S. aureus is resistant to (Buchanan and Gibbons, 1974). If polymyxin is inhibitory to other organisms but not S. aureus, the S. aureus would grow due to decreased competition. It is interesting to note that the cocci colonies appeared at 60.5 and 84 h during 25<sup>0</sup>C incubation. This was during the stationary phase of S. aureus growth. It has been estimated that one million/g B. cereus are needed to cause illness. It took only 15 h to reach this level when stored at 25<sup>0</sup>C.

### Severe Abuse Conditions

Storing pies at 35<sup>0</sup>C for 48 h did not inhibit the growth of S. aureus or Salmonella typhimurium, as shown by Table VIII. However, it did inhibit the growth of B. cereus. Duplicating the experiment yielded similar results. It was stated earlier that this organism has been reported to survive up to 49<sup>0</sup>C (Goepfert, et. al., 1972). Jay (1978) states that B. cereus grows rapidly in the 30-40<sup>0</sup>C range. The reason for the lack of growth at 35<sup>0</sup>C is unknown. One explanation could be the age of the organism. The culture could have lost some of its characteristics due to being obtained from a classroom situation, as mentioned previously. It has been reported that B. cereus is a variable organism and deviant strains are not uncommon (Goepfert, et. al., 1972). Perhaps this is a deviant strain, or one that has the lower maximum growth temperature.

### Staphylococcal Enterotoxin

Results indicate that S. aureus grows well in pumpkin pie when stored at 25 and 35<sup>0</sup>C. The presence of enterotoxin is the important factor in determining whether the organism would render the product unsafe to eat. Table IX shows that enterotoxin is produced in pumpkin pie when held at 25 and 35<sup>0</sup>C for 72 and 48 h respectively.

### pH and Physical Characteristics

As discussed previously, the pH of the pies decreased in every instance when stored at 25<sup>0</sup>C for 72 h (Table V). Table X shows the decrease in pH

Table VIII. Growth of Pathogens<sup>1</sup> in Pumpkin Pie Held at 35°C.

Time (hours)	<u>S. aureus</u> (MPN/gram)	<u>Salmonella</u> (MPN/gram)	<u>B. cereus</u> (per gram)
0	200	190	100
48	$1.3 \times 10^8$	$9.3 \times 10^7$	<100

<sup>1</sup>Counts based on the average of duplicate samples.

Table IX. Presence of Staphylococcal Enterotoxin A in Pumpkin Pie Inoculated with S. aureus<sup>1</sup>.

Time (hours)	Temp (°C)	<u>S. aureus</u> (per gram)	Presence of Enterotoxin A
24	25	$1.4 \times 10^8$	+
48	25	$9.1 \times 10^8$	+
72	25	$5.1 \times 10^8$	+
48	35	$4.3 \times 10^8$	+

<sup>1</sup>Pies inoculated with  $1.1 \times 10^4$ /g S. aureus.

of the pie filling when inoculated with the organisms studied and stored at 25°C for 84 h. When the pies were stored at 4°C, however, no significant change in pH was noted after two weeks.

After 24 and 48 h when stored at 35 and 25°C respectively, more often than not, a foul odor was noted. Also, the filling appeared more liquidy, and occasionally, mold growth was evident. It is unlikely that the pies would be eaten under these circumstances, however, it should be noted that often the bacteria counts were more than a million per gram before a physical change was evident.

### Summary

Data indicates that if pumpkin pies were contaminated and stored at room temperature, a public health risk could exist. Pies inoculated with S. aureus, Salmonella typhimurium, and B. cereus supported the growth of these organisms. According to the data obtained, it would take only one day before becoming "unsafe" to eat if pies were contaminated with S. aureus or B. cereus, and 1.5 days if contaminated with Salmonella typhimurium. The current distribution practices indicate that pumpkin pies are often stored at room temperature for more than two days (Figures 2-6). Held under refrigeration conditions, none of the pathogens grew, and only S. aureus and Salmonella grew when subjected to severe abuse conditions.

The chances for contamination need to be examined. Normally, food can become contaminated in one of two ways, either pre or post-processing contamination. Both types of contamination were detected in this study. If contaminated before processing, many organisms would be destroyed

Table X. pH of Pumpkin Pie after Storing at 25<sup>0</sup>C for 84h.

Pathogen	Time (hours)	
	0	84
<u>S. aureus</u>	6.2	4.7
<u>Salmonella</u>	6.2	4.1
<u>B. cereus</u>	6.3	4.7

during the cooking process. However, not all bakery products are heated (i.e., cream filled pastries). The proper handling of bakery products before and after processing is imperative to decrease the risk to the public.

#### Effect of Potassium Sorbate

Although refrigeration effectively controls the growth of pathogens in pumpkin pie, the lack of cooler space in retail markets is a large problem. Potassium sorbate was tested for its effectiveness in inhibiting the pathogens used in this study.

Figure 12 shows the results of incorporating 0.25% potassium sorbate into the pumpkin pie formula. It was effective in inhibiting the growth of Salmonella typhimurium and B. cereus but not S. aureus. From the literature, it appears that potassium sorbate is effective against S. aureus when used in combination with other inhibitory substances such as NaCl, tertiary butylhydroquinone, butylated hydroxyanisole, and nitrite (Robach, et. al., 1980; Davidson, et. al., 1979; Pierson, et. al., 1979).

There was no taste difference apparent when potassium sorbate was incorporated into the formula. Table XI shows that the pH decreased slightly after 73 h when treated with potassium sorbate. Perhaps this is due to the effect of the sorbate, alone, as the pH decreased regardless of microbial growth.

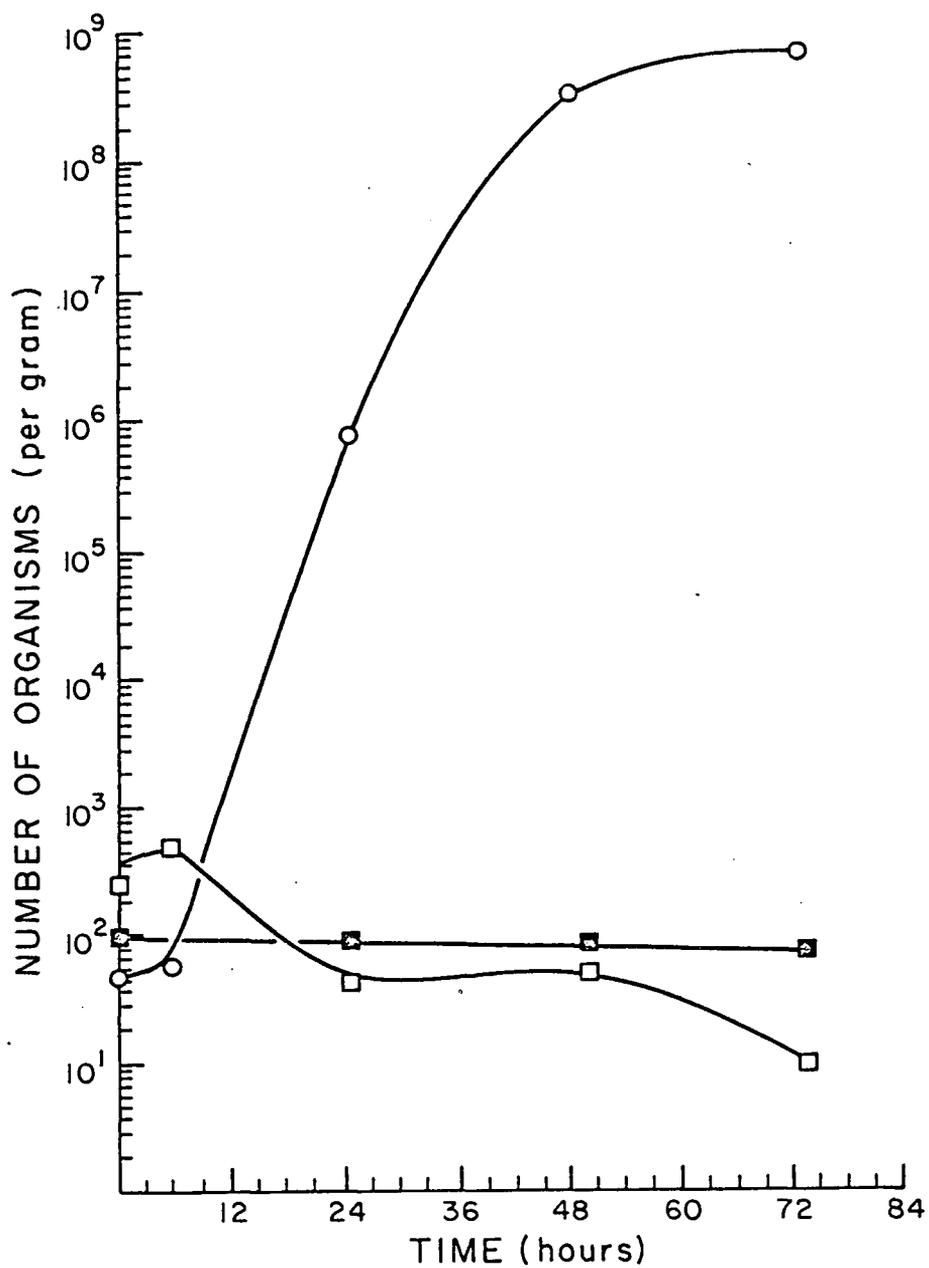


Figure 12. Growth curve of pathogens in pumpkin pie treated with 0.25% potassium sorbate and held at 25°C. ○ - *S. aureus*, □ - *S. typhimurium*, ■ - *B. cereus*.

Table XI. pH of Pumpkin Pie Treated with Potassium Sorbate and Inoculated with Pathogens.

Time (hours)	<u>S. aureus</u>	<u>Salmonella</u>	<u>B. cereus</u>
0	6.2	6.2	6.4
73	5.6	5.0	5.7

## SUMMARY AND CONCLUSIONS

There are several bakery products which could be considered as "potentially hazardous" foods under the definition given in the model Retail Food Store Sanitation Ordinance. More specifically, lemon meringue pie, pumpkin pie, and cream filled pastries, according to this study. Under the definition, these products would need to be held under refrigeration, a condition not currently being practiced for the most part, in retail food stores.

The results of this study indicated the knowledge of bakers in regard to safe food handling practices is minimal. Little attention has been given to an education program for bakers. Perhaps the reason is due to the nature of the product they deal with. Bakery products as a whole are not considered as critical as meat from a spoilage standpoint. There is a definite need for an education program for bakers so that they may better understand the microbiological spoilage of products they prepare.

If bakers were educated in regards to proper food handling practices, they would still be working with a handicap. Few bakeries have adequate refrigeration space--not only in the preparation area but also the display areas. Future designs of bakeries need to account for larger refrigeration capacity.

Given the current distribution practices, along with the microbial quality of pumpkin pies, it appears that a public health risk is present. Table XII summarizes distribution practices found related to the microbial quality of retail pumpkin pies. As previously discussed, current pull date practices when compared with microbial quality leaves much to be desired.

Table XII. Current Handling Practices and Microbial Quality of Retail Pumpkin Pies Stored at 25°C.

Store	Pulldate (Days)	Display Practices	APC at Pulldate (per g)	Presence of pathogens at pulldate
A	3	Refrigeration	$4.2 \times 10^9$	+
B	2	Room temperature	$9.9 \times 10^6$	-
C	3	Room temperature	$1.9 \times 10^8$	+
D	5	Room temperature	$4.5 \times 10^5$ (3 days)	+

The fact that 75% of the products sampled were contaminated with pathogens places a strong emphasis on the need for proper storage of the product. If abused or allowed to remain at temperatures suitable for growth, a public health hazard could exist. Ingredient contamination along with post-processing contamination are the reasons for the presence of pathogens in this study. Baking was sufficient to destroy S. aureus and Salmonella but not B. cereus.

Another conclusion that can be made from the study is that pathogens, when present in pumpkin pie, grow when incubated at 25<sup>0</sup>C. S. aureus and Salmonella grew at 35<sup>0</sup>C but B. cereus did not. Refrigeration at 4<sup>0</sup>C controlled the growth of all three organisms. Potassium sorbate, at a concentration of 0.25% inhibited Salmonella and B. cereus but not S. aureus.

Further study could be conducted in many areas. The same study could be performed using other products and/or other microorganisms. A question left to be answered is, are there other inhibitory substances that could be used in pumpkin pie that would control the growth of all three pathogens used in this study? Sorbate alone was not effective, but perhaps in combination with other substances it could work. Perhaps by altering the pH or  $a_w$ , without imparting an undesirable flavor, growth could be controlled. This is a desirable area of study as if growth of pathogens could be controlled without refrigeration, cost savings of many dollars could result for the food retailer.

An area not considered in this study but which is important is that of handling practices of the consumer as soon as the food leaves the retail store. It would also be interesting to find out how many cases of food-borne illness were caused by abuse in the retail market--an area not

currently being reported by the Center for Disease Control.

Perhaps the greatest area that needs to be researched has to do with B. cereus. This organism is still a mystery. Why the organism elicits two kinds of illnesses and the mode of pathogenicity is of importance in learning to control this organism. In this study alone a few questions regarding B. cereus were evident (i.e., why the broth but not the filtrate culture caused death in mice, why no growth occurred at 35°C in pumpkin pie, to name a few). As is often the case with research, it seems that more questions were raised than answered in this study.

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