THE EFFECT OF GAMMA IRRADIATION ON *SALMONELLA* SPECIES IN SMOKED WHITEFISH

by

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ABSTRACT

Salmonella give, *Salmonella typhimurium* and *Salmonella java* were artificially inoculated into freshwater whitefish (*Coregonus clupeaformis*) at three different stages of the smoking process. The effect of each step on the survival of the species and the gamma irradiation dosage required for complete destruction was determined.

When the inoculation took place prior to freezing, all three species survived the freezing at 0°F for 48 hours, brining at 60° Sal for 25 minutes and smoking at temperatures up to 167°F. The irradiation dosage required for complete destruction of the species was found to be 0.4 M rad for *S. give*, 0.3 M rad for *S. typhimurium* and 0.2 M rad for *S. java*.

When samples were inoculated after freezing and prior to brining, all species survived the brining and smoking. A dosage of 0.4 M rad was found to be necessary for the destruction of *S. give*, while 0.3 M rad was sufficient for the destruction of *S. typhimurium* and *S. java*.

At the third stage, the samples were inoculated after smoking. A higher dosage of gamma irradiation was necessary for the destruction of all three species. *S. give* required a dosage of 0.5 M rad, while *S. java* and *S. typhimurium* required 0.35 and 0.4 M rad respectively.

Although a definite variation in sensitivity to gamma radiation was shown with the three species studied, it would

be safe to say that a level of 0.5 M rad of gamma irradiation is sufficient for their destruction in smoked whitefish.

It also became evident that under the conditions used in this study, S. give is the most heat resistant species.

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INTRODUCTION

Salmonella food poisoning resulting from the consumption of smoked whitefish has been well known as far back as 1934 (Kleeman *et al.*, 1942). The same authors reported an epidemic in 1940 involving 47 patients who had eaten smoked whitefish. Olitzky *et al* (1956) reported another outbreak during the 1955 Memorial Day holiday in which 37 cases were recognized. And finally, the most recent outbreak which was reported by Gangarosa *et al* (1968) occurred during May 26 - June 2, 1966 when approximately 300 people became infected.

It is well established that hot smoked whitefish can be consumed without further cooking. However, it could be assumed that all fish may not have received a uniform temperature treatment while being smoked, due to different proximities to the source of heat. This, therefore, could lead to survival of some non-pathogenic as well as pathogenic micro-organisms such as *Salmonella* and *Shigella*. At the moment, little is known concerning the effect of smoking on the survival or destruction of *Salmonella* organisms in whitefish.

Gamma irradiation is well known for its radappertization (sterilization) and radicidation (pasteurization) effect, and application of irradiation treatment as a means to destroy *Salmonella* in smoked whitefish appears to be feasible.

The present investigation was undertaken to establish the most suitable level of gamma irradiation which, combined with smoking, would free the whitefish from selected species of Salmonella.

REVIEW OF LITERATURE

The Organism

Salmonella was first discovered by Drs. Salmon and Smith (1885), and was believed to be the causative agent of hog cholera (cholera-suis). The genus name was given to the group in honor of Dr. D. E. Salmon and the first organism of the group isolated was called Salmonella cholera suis.

In 1893 S. abortus-equi was isolated from vagina of aborting mare by Kilbourne (1889) and S. gallinarum, the causative agent of "fowl typhoid" was discovered by Klein. Then Loeffler (1892) isolated the "mouse typhoid bacillus" which was later called S. typhimurium. In 1906 Rettger isolated S. pullorum, the causative agent of pullorum disease in young chicks.

To date, greater than 1,200 known strains of the genus Salmonella have been identified and all of which are capable of causing infection in both man and animal. The organism is shown to be Gram-negative rods which are either motile by means of peritrichous flagella or non-motile. Bio-chemically

they attack numerous carbohydrates by producing acid or acid and gas. However, they do not attack lactose, sucrose and salicin. In 1955, White and Kauffman established the present method of antigenic analysis of Salmonella group by using specific diagnostic antisera, the "O" antigen (body antigen) and "H" antigen (flagella antigen) can be determined.

The Source and Vectors of Salmonella

Salmonella is a widespread group of organisms which are found everywhere that man and beast exist. The medical and veterinary medical literature during the past few years have shown that a wide variety of birds, reptiles, domestic and wild animals, garden snakes, pet turtles, frogs, and finally, domestic pets have been found to harbor and pass Salmonella.

Edwards et al (1948) reported that domestic poultry probably is the largest single reservoir of these organisms. More than two-thirds of approximately 20,000 cultures isolated from animals between 1934 and 1963 were derived from the domestic fowl (C.D.C. Annual Report 1963). Yamamoto et al (1962) found that 32.5% of 123 flocks and 19.4% of 314 breeder turkeys were positive for Salmonella in a three-year testing program for S. typhimurium.

The Salmonella in egg problem came to focus during the Second World War when the American spray-dried eggs imported

to Britain were found to be contaminated with Salmonella. Solowey et al (1947) reported that Salmonella were found in 35% of 5,000 samples of spray-dried whole eggs. Hinshaw (1959) concluded that contamination and infection of the egg is mainly from infected intestinal contents coming in contact with the shell during expulsion from the body. Haines et al (1940) and Stokes et al (1956) reported that under the proper temperature and humidity the Salmonella can penetrate through the egg shell. Yamamoto (1961) reported that the ovarium transmission of Salmonella can also occur and he was able to isolate S. typhimurium from the oviduct of turkey hens.

The most common seratypes isolated from eggs and egg products were S. infantis (12%), S. montevideo (12%), S. heidelberg (11.3%), S. siegburg (6.1%) and S. oranienburg (5.6%)(C.D.C. Annual Report 1966).

Cattle and swine have also been reported to be carriers of Salmonella and could pass on the organism through their by-products. In the case of swine, various workers have shown that the rate of infection increases as the animal leaves the farm. This was shown by Shotts et al (1961) when Salmonella was isolated from 9% of the samples obtained at the barn, 26% in samples taken at the abattoir before slaughter and 80% in samples taken after slaughter. Galton et al (1954) reported that Salmonella were found in 50% of

fresh pork sausage and 12.5% of smoked sausage.

A survey conducted by Galton et al (1954) showed that S. give and S. bareilly were isolated from some of the Florida dairy cattle. However, Salmonella became a major problem in the U. S. during the latter part of 1950 and early part of 1960, when 40 cases were reported in Florida of which the S. typhimurium was the predominant species. Salmonella were also isolated from young calves in the State of Michigan where 78 cattle were found to be infected (Moore et al 1962). In England, Smith (1951) isolated S. typhimurium from the feces of healthy cattle and S. dublin from sick cattle. Taylor (1962) reported isolation of 27 types of Salmonella from cows and calves between 1956 and 1960 with S. dublin being the most common one, followed by S. typhimurium. It is well known that the transmission of infection to man from infected cattle occurs through the consumption of contaminated beef and beef products and improperly pasteurized milk and milk products.

Animal feed has also been recognized as one of the main sources of Salmonella. According to C.D.C. Surveillance Report (1966), animal feed has contributed to 16.5% of the non-human Salmonellosis. Erwin (1955) examined 206 poultry feed samples and isolated S. oranienburg from 3 samples. Watkins et al (1959) reported that 18.5% of 200 samples of poultry feed and animal by-products were positive for Salmonella serotypes. Morehouse (1961) reported that among

the most common serotypes isolated from animal by-products were S. senftenberg, S. typhimurium, S. infantis, S. cubana, S. oranienburg and S. montevideo.

Most recently, insects such as flies, roaches, ticks and fleas have also been found to be carriers of Salmonella. McNeil (1944) isolated S. typhimurium from flies which were trapped in turkey ranches. Greenberg et al (1963) reported that in a Mexican abattoir, up to 66% of the flies were positive for Salmonella. Welch et al (1941) made a study on Salmonella incidence in rat population and found that the incidence ranged from 0.7% to 13%. They also reported that the organism remained viable for 148 days in rat feces.

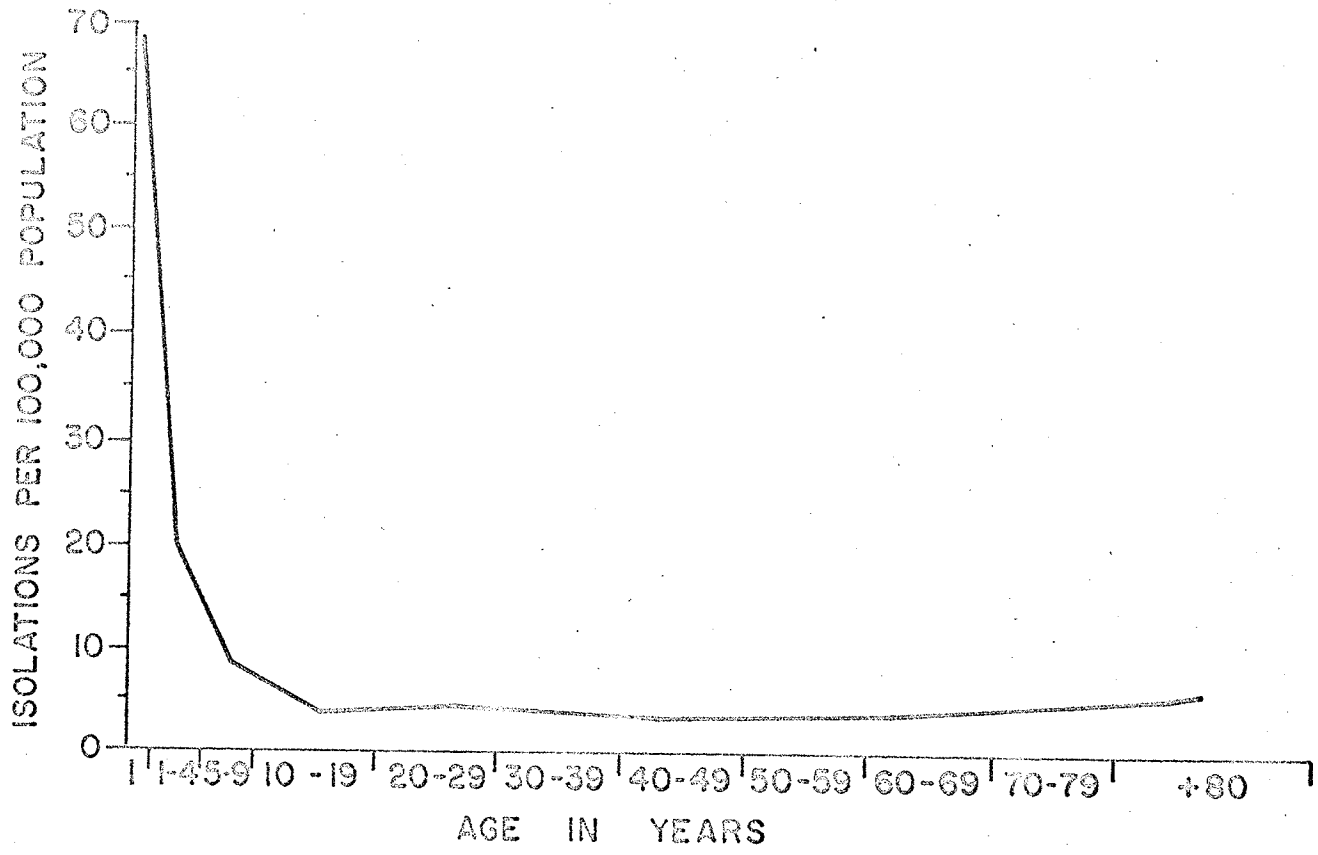
Pathogenicity of Salmonella

The human Salmonellosis is known to have high incidence and low mortality. According to the U. S. Department of Health, Education and Welfare (1964 and 1965 reports), the Salmonellosis is widespread and occurs in both sexes with equal frequency. However, the majority of cases are in children, especially those under one year of age. The infection rate decreases quite rapidly and remains almost on a plateau until the age of 49. Then a slight increase will take place during the age of 75-80 (Fig. I).

McCullough and Eisele (1951) evaluated the pathogenicity of several serotypes of Salmonella on healthy volunteers.

FIGURE I

RATE OF HUMAN ISOLATIONS OF *SALMONELLA*
BY AGE GROUP


ISOLATIONS PER 100,000 POPULATION
70
60
50
40
30
20
10
0
1
1-4
5-9
10 - 19
20-29
30-39
40-49
50-59
60-69
70-79
+80
AGE IN YEARS

They showed that there was a wide difference between the number of organisms required to create illness. For example, S. meleagridis required a dosage of approximately 10^6 to cause illness; while for S. anatum, the range was from 587,000 to 860,000 cells. They also showed that S. bareilly induced illness with 125,000 cells and S. newport with 152,000 cells. It was also shown that there is a great difference between the strains of each species. One strain of S. meleagridis required 24 million cells and S. anatum, 44.5 - 67.2 million cells in order to create illness.

The Outbreaks

Galton et al (1964) reported that the increase in the number of reported human Salmonellosis outbreaks is due to improved methods and facilities for detection of Salmonella, increase in general awareness of the disease and improved reporting systems. This therefore has resulted in a very sharp decrease in typhoid fever incidences (3,268 cases in 1946 as compared to 608 cases in 1962) and an increase in Salmonellosis (727 cases in 1946 as compared to 9,680 in 1962)(Table I).

Dauer (1952) reported 9 outbreaks of Salmonellosis 1938 to 1951, and in 1961 he reported an average of 23 outbreaks involving 100,000 individuals; 65 (3%) were water borne. From this study, Dauer concluded that the actual

TABLE I

REPORTED CASES OF TYPHOID FEVER AND OF OTHER SALMONELLOSIS. UNITED STATES - 1946 - 1962

Year	Typhoid Fever	Salmonellosis
1946	3,268	723
1947	3,075	951
1948	2,840	882
1949	2,795	1,243
1950	2,484	1,233
1951	2,128	1,733
1952	2,341	2,596
1953	2,252	3,946
1954	2,169	5,375
1955	1,704	5,447
1956	1,700	6,704
1957	1,231	6,693
1958	1,043	6,363
1959	859	6,606
1960	816	6,929
1961	814	8,542
1962	608	9,680

number of food borne disease outbreaks was probably 10-20 times larger than the number reported. Feig (1950) reported that from 926 outbreaks of gastroenteritis occurring 1945 to 1947, 72 of them involved Salmonella. Edwards (1958) reported that the incidence of Salmonellosis in England was 28 times that of the United States.

In April 1962, a program of Surveillance of Salmonellosis in the United States was jointly established by the Communicable Disease Centre of the Public Health Service and the Epidemiological Association of State and Territorial Laboratory Directors. The unit reported a total of 21,113 human isolations of Salmonella in the U. S. (C. D. C. Annual Report 1964). At the same time a total of 5,461 Salmonella isolations were reported from non-human sources (Fig. II and III). It is rather interesting to note, that of these, 55.2% were from poultry and wild fowl and 9.2% from eggs and egg products. Among the most common Salmonella isolated from human food products were S. heidelberg, S. tennessee, S. thompson, S. typhimurium, S. montevideo and S. infantis.

According to Taylor (1960), S. typhimurium was the most common type in England and Wales between 1956-1960 accounting for 75% to 80% of the outbreaks. This was followed by S. thompson, S. newport and S. heidelberg. In Canada up to the end of 1962, a total of 101 different types of

Figure II

REPORTED HUMAN ISOLATIONS OF SALMONELLA
UNITED STATES, 1963 - 1966

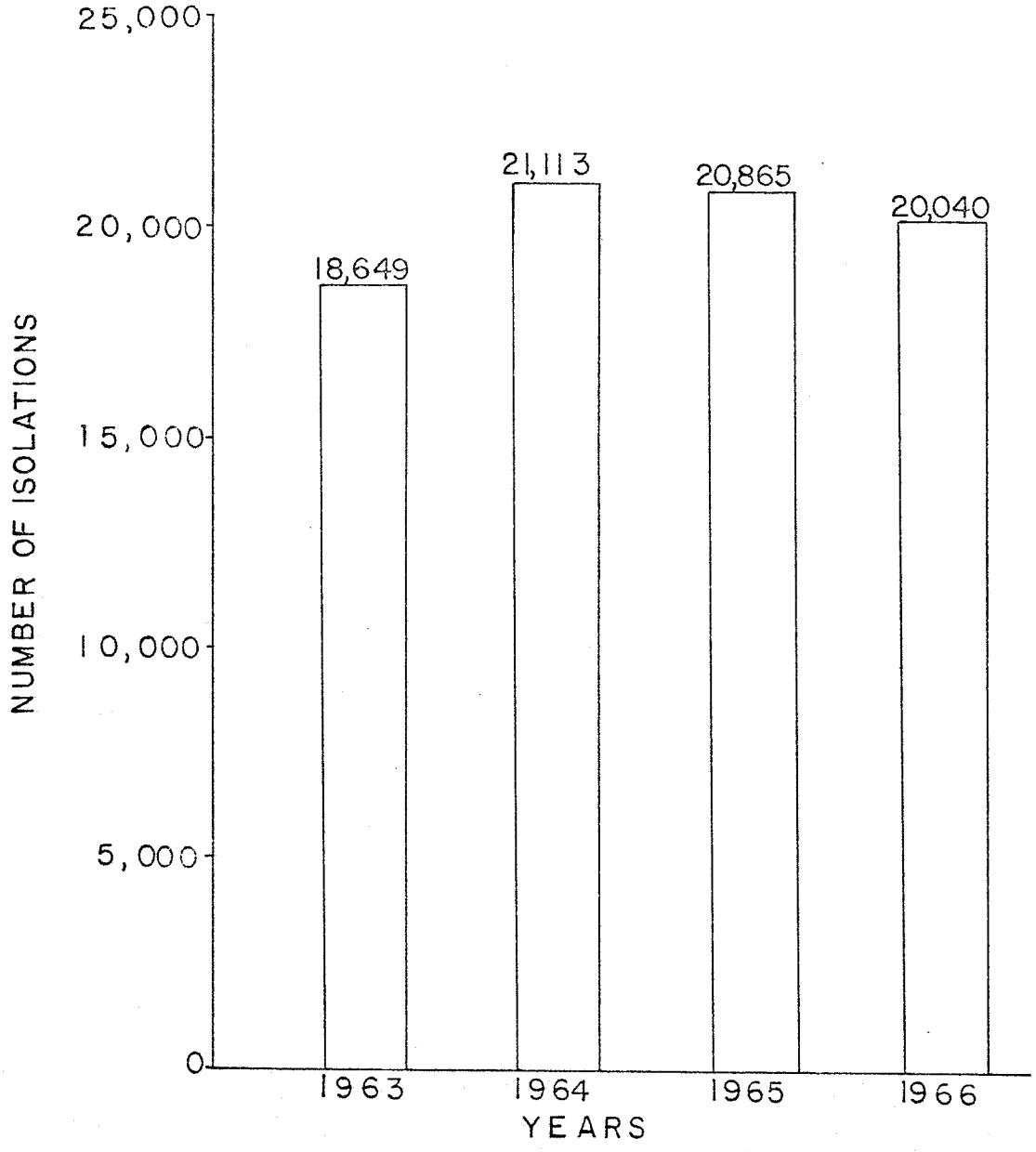
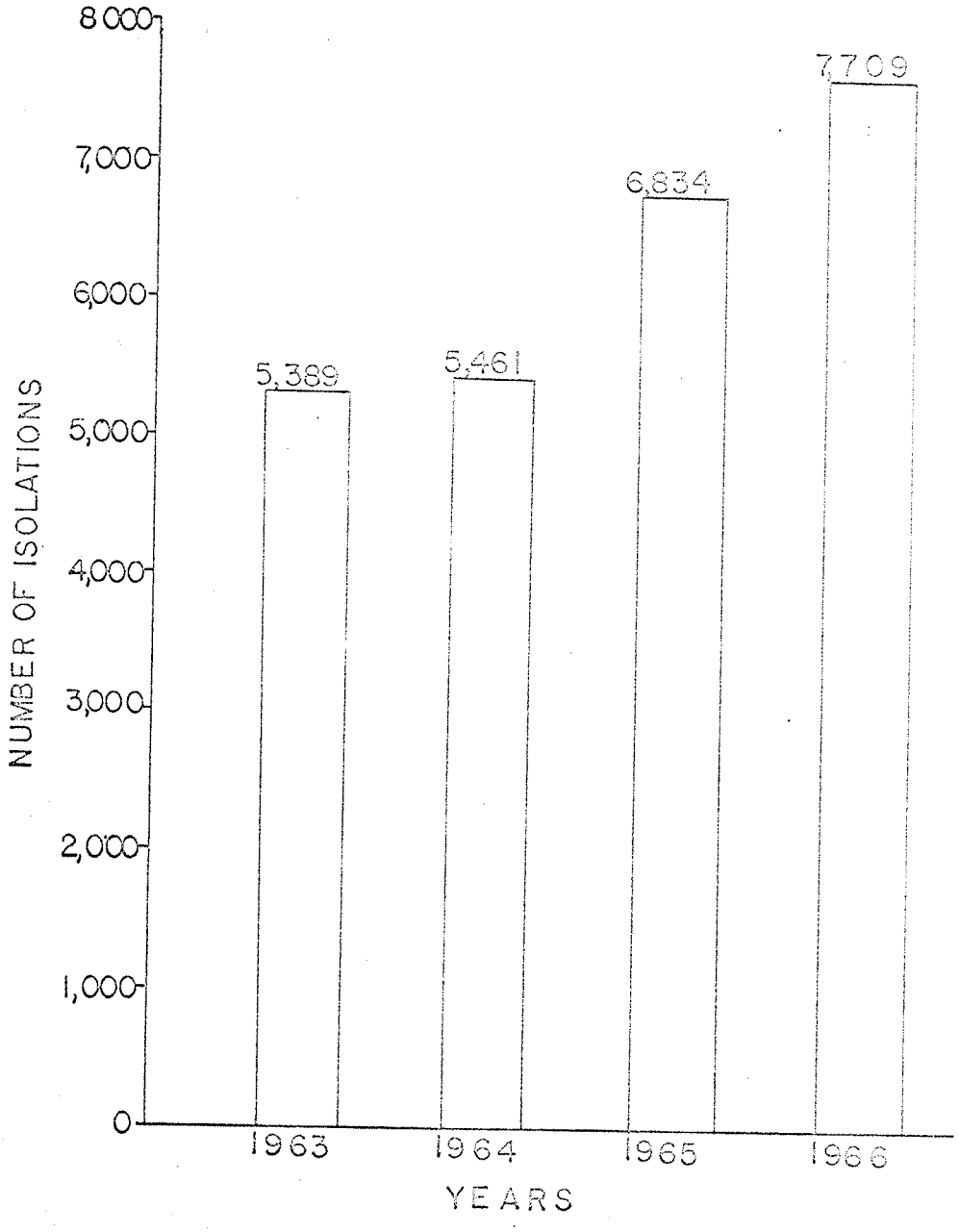


Figure III

REPORTED NONHUMAN ISOLATIONS OF SALMONELLA
UNITED STATES, 1963 - 1964



Salmonella were identified (American Journal of Medical Science Report). The most common ones isolated from man were S. typhimurium, S. heidelberg, S. thompson and S. paratyphi B.

Kleeman et al (1942) reported the following outbreaks which were due to the consumption of smoked fish:
(a) a total of 32 residents of Brooklyn and Queens and 2 from Newark, N. J., became ill during April 1934, with symptoms of acute food poisoning, vomiting, abdominal pain and diarrhea. All patients were found to have consumed smoked whitefish which was purchased from a wholesale smoked fish establishment in Brooklyn, N. Y. This outbreak resulted in the death of a 49 year old woman two days after the first symptoms appeared. However, the remaining patients recovered after varying periods of time.

(b) on July 16, 1940, several cases of acute gastroenteritis were reported to the Department of Health in Brooklyn, N. Y. which resulted in the infection of 47 cases in 18 separate families. Symptoms in all patients were abdominal pain, diarrhea and high temperatures. This outbreak resulted in the death of a 5 year old boy and a 51 year old man. Through a series of examinations by various health officers, it was discovered that the families affected had eaten smoked whitefish. It was also reported that vats used for washing,

soaking and brining fish drained directly into a sewer line that was connected to a common toilet drain which in turn emptied into a nearby ditch. Samples taken from the ditch water were found to be positive for S. typhimurium. The same organism was also isolated from stool samples of 14 consumers, 3 retail food handlers, 1 wholesaler and 5 whitefish.

Olitzky et al (1956) reported that during the Memorial Day weekend, May 28 - 30, 1955, 3 outbreaks of gastroenteritis occurred in Philadelphia. Smoked fish was found to be the vehicle for transmission of the Salmonella organism. Stool samples taken from the patients were positive for S. newport. This organism was also isolated from the remaining smoked fish which had been refrigerated. Some authors reported another outbreak involving 11 people who had eaten smoked whitefish at a buffet supper. Further studies proved that one of the female employees working at the packaging table was the carrier of S. newport.

The most recent outbreak of Salmonellosis due to the consumption of smoked whitefish was reported by Gangarosa et al (1968). During May 26 - June 2, 1966, more than 300 people were involved in an epidemic of febrile gastroenteritis after ingestion of smoked whitefish contaminated with S. java. Further investigations revealed that frozen whitefish arriving from Canada were also contaminated with

S. java. It was finally concluded that the water and ice used at the source could have been contaminated with S. java which resulted in the contamination of plant employees and eventually the final product.

Effect of Cold Temperature and Freezing on Salmonella

The survival of Salmonella in frozen food products can be traced as far back as 1926 when Prucha and Brannon isolated Salmonella from ice cream. Orr and Moore (1953) reported that S. gallinarum can survive in distilled water which has been frozen and thawed, and refrozen for as long as 43 days. Woodburn and Strong (1960) froze S. typhimurium in five simple food substrate (0.0003M Phosphate buffer, corn syrup, waxy rice flour, sodium alginate and egg white) at -11°C, -21°C and -30°C. Samples were drawn after 24 hours, 1 week, 4 weeks and 10 weeks for comparison with original count. They reported that S. typhimurium survived for 10 weeks at all three temperatures in all substrates.

Wallace and Tanner (1935) reported that Salmonella inoculated into cherries and frozen at 0°F and -40°F survived after three months of storage. Orr and Moore (1953) also reported that S. gallinarum survived temperatures of -20°C for 148 days in a naturally infected liver.

Refrigeration of food contaminated with Salmonella does not destroy the organism but merely prevents their

multiplication. Angelotti et al (1961) demonstrated that the Salmonella in custard decreased as the temperature was reduced from 10°C to 4°C. However, working with chicken a-la-king, they found that Salmonella multiplied at temperatures of 7°C and above. They concluded that the growth of Salmonella in perishable food is inhibited at temperatures of 5°C and below. Raj and Liston (1961) reported that S. typhimurium can survive, and to some extent increase in number at temperature of -18°C when artificially inoculated into frozen fish homogenate.

Effect of Heat on Salmonella

It has been well established that most strains of Salmonella are destroyed at a temperature of 55°C for 1 hour or 60°C for 15-20 minutes. All are killed by autoclaving at 121°C for 20 minutes.

Tittsler (1930) reported that eggs artificially inoculated with S. pullorum must be boiled for 5 minutes to obtain complete sterilization. Angelotti et al (1961a) demonstrated that 10^6 Salmonella organisms per gram of chicken a-la-king can be completely destroyed by exposing the product to temperatures of 65.6°C for 12 minutes.

Weidman et al (1956) and Beloian and Schlosser (1963) reported that an internal temperature of 71.1°C is required for complete destruction of Salmonella organisms. However,

certain strains of Salmonella require much higher heat for their destruction than others. Anellis (1954) worked with S. senftenberg (775W) and reported that this strain had a F_{140} as high as 88.75 minutes. Thomas et al (1966) worked with S. senftenberg (rough and smooth strain) in four different media. They reported that different temperatures were required in different foods for complete destruction. For example, 775 WR in 0.5% NaCl had D_{150} of 0.837 minutes in skim milk, 1.208 minutes in beef bouillon 0.648 and, finally, in green pea soup 1.001 minutes. At the same time, the D_{150} for strain 775 WS in 0.5% NaCl was 0.519 minutes, in skim milk 0.655, and green pea soup 0.584 minutes. The resistance of S. senftenberg was also reported by Osborne et al (1954) who concluded that temperatures of 140°F for 83-95 minutes is required for the complete destruction of this strain from eggs.

Effect of Irradiation on Salmonella

Heat treatment has been used and is still being used for the destruction of Salmonella organisms in a wide variety of food products. Gamma irradiation is proposed as an alternative process for the destruction of Salmonella and other micro-organisms. It has offered important advantages for some products. One of the main advantage of radiation pasteurization (radicidation) is that it can be applied

without removing the food from its container. Also, when food is required to be marketed in the raw form, radicidation would have certain advantage over heat pasteurization.

Brooks et al (1959) studied the effect of gamma irradiation on frozen whole eggs, horse meat and desiccated coconut and reported that Salmonella present in these products can be eliminated by means of gamma irradiation. Ley and Freeman (1963) worked with five different serotypes: S. typhimurium, S. senftenberg 775 W, S. paratyphi B., S. gallinarum and S. meleagridis. They reported that S. typhimurium to be the most resistant one in the variety of media used (desiccated coconut, frozen horse meat, bone meal, frozen whole eggs and liquid eggs). A dosage of 0.5 M rad was required for a 10^7 reduction in frozen eggs and 0.64 M rads for a 10^5 reduction in frozen horse meat. In desiccated coconut, a dosage of 0.45 M rad was required for a 10^3 reduction and 0.5 to 0.75 M rad was effective for the complete destruction of Salmonella in bone meal. Thornley (1963b) reported that S. typhimurium was the most resistant species studied.

Dyer et al (1966) working with S. typhi, S. paratyphi B., S. wichita and S. pollorum in Hartsell's broth, reported that the minimal lethal dose for the first three species was 5×10^5 rad. However, for S. pallorum, the minimum lethal dose was 3×10^5 rad.

Comer et al (1963) studied the radiation sensitivity of 18 Salmonella species in frozen whole eggs. The dosage required for 10 fold reduction of these species ranged from 0.36 M rad to 0.54 M rad with S. give being the most resistant and S. senftenberg the least resistant. They concluded that the level of 0.54 M rad was quite safe in the case of frozen whole eggs.

Ingram et al (1961) studied the effect of gamma irradiation on S. typhimurium, S. gallinarum and S. senftenberg in liquid and frozen whole eggs. They reported that S. typhimurium was the most resistant, requiring a dosage of 0.42 M rad for a 10^7 fold reduction of original population.

According to Erdman et al (1961) the nature of the suspending medium, the chemical structure of the food and spices added can greatly influence the relative radiation sensitivity of a specific culture. Mossel and Degroot (1965) inoculated samples of fish meal with ten different serotypes and reported that the dosage of 0.8 M rad is sufficient to destroy the organisms. Ley (1966) reported that the D value for S. typhimurium was 75 K rads in frozen albumen, 174 K rads in fish meal, and 67.9 K rads in frozen whole egg. However, the D value for the same organism in frozen phosphate buffer was 39.1 K rads.

Commercial Smoking Process

It is well known that the discovery of the use of smoke as a method of preservation of flesh food such as meat and fish was accidental. It originated from the drying process when heat was used. However, when its flavor and length of preservation were noticed, the smoking of meat and fish as a method of preservation became common.

Smoked fish has been a common diet for those in coastal and isolated interior regions of North America. Their use, however, has been declining and they are used most commonly as an appetizer, despite the fact that the value and quality has increased.

The principle of smoking fish is the removal of moisture by salting (brining) and the achievement of certain desirable flavor characteristics to the product. However, a few investigators have claimed that wood smoke has a bactericidal effect. Rector (1925) claimed that the preliminary salt treatment is important in preservation, and, at the same time, makes the fish firmer by drawing out large quantities of water.

Shewan (1953) claimed that the preservation effect of salt in smoking fish is due to:

(a) the restriction of microbiological activity as a result of desiccation of tissues;

(b) the direct action of NaCl on the putrifactive micro-organisms present;

(c) the interference of NaCl with the rapid action of the proteolytic enzymes;

(d) the removal of oxygen and sensitization of micro-organisms to CO_2.

Tressler (1923) reported that the preservative action of wood smoke is largely due to the presence of a number of organic compounds such as xylenols, creosol, methyl esters of higher phenols, formaldehyde, acetic acid, acetone and methyl (wood) alcohol.

Commercial smoking of fish usually involves brining, drying, treatment with smoke and heat treatment. The fish used for this purpose was usually frozen at temperatures ranging from $0^{\circ}F$ to $-20^{\circ}F$, then thawed in large thawing tanks. The scales are removed and the gut cavity washed to remove excess blood. The smoking procedure used by various commercial plants is often different, each having their own technique obtained through years of experience. Studies carried out on procedures used by five smoking plants in the Winnipeg area revealed that all plants generally followed a similar pattern. The procedure employed is as follows:

After thawing, scaling and cleaning, the fish is cut into sections of $1\frac{1}{2}$" to 3" wide or used as a whole fish.

Brining or salting is performed by placing the fish in tanks containing a mixture of salt and water. The concentration of brine from plant to plant ranges from as low as 30° Sal to as high as 50° Sal. Brining time depends upon the concentration of the brine, lasting from 3-4 hours to overnight. The fish is then coated with vegetable dye which gives a bright red to organge color to the product. Each piece is hung on a metal bar and left to air dry for 4-5 hours.

There are two common methods of smoking: (a) cold smoke, in which the temperature ranges from 77° to 90°F and the end product remains uncooked, and (b) hot smoke, in which the temperature ranges from 90°F to 215°F and the end product is cooked and ready for consumption.

The length of smoking depends upon the type of smoking desired. In cold smoking a period of 4-5 hours is usually sufficient. In hot smoking, it takes 3-4 hours during which the temperature is kept below 90°F and an additional 3-4 hours, with temperature raised to 215°F in order to cook the product.

The finished product is usually left in the smoking chamber overnight, then wrapped and sent to the market. A good percentage of smoked whitefish is sold in the fresh state, however, some have also been frozen. Both procedures of smoking are used extensively in Canada and the U. S., although hot smoke is more common when whitefish is being

smoked.

Media Used for Recovery of Salmonella

(a) Pre-enrichment Media: The use of a pre-enrichment for isolation of Salmonella from food products has been reported by many investigators. Taylor and Silliker (1961) and Taylor et al (1963) reported that the use of a pre-enrichment medium for isolation of Salmonella from dry food products and products containing high number of Gram-negative micro-organisms is quite essential. Byrne et al (1955) showed that a higher number of Salmonella is recovered from inactivated dried yeast when the yeast suspension is incubated at 30°C for 24 hours before being transferred into a selective enrichment medium. Jameson (1963) reported that higher Salmonella recovery is obtained when nutrient broth is used as a pre-enrichment medium. Hobbs (1963) indicated that when nutrient broth was used as a non-selective enrichment for dried whole eggs, much superior results were obtained.

(b) Enrichment Media: Various selective enrichment media have been applied for isolation of Salmonella from various food products. Byrne et al (1955) reported that Selenite-F with cystine gave much superior Salmonella recovery from egg albumin when compared with Selenite-F and Tetrathionate broth. Taylor (1961) showed that Brilliant

Green Tetrathionate enrichment broth provided better recovery from dried albumin when compared with Selenite-cystine broth. Georgala and Boothroyd (1964) used Selenite-F enrichment incubated at 3°C, 43°C and 45°C for isolation of Salmonella from meat and meat products. They reported that incubation of Selenite-F enrichment at 43°C instead of 37°C improved the selectivity of the medium.

(c) Selective Plating Media: A wide variety of selective plating media has been employed for isolation of Salmonella. The most common one used by various workers are Bismuth Sulfite Agar (BSA) (Wilson and Blair 1927, 1931), Salmonella Shigella Agar (SS) (Leifson 1935), Brilliant Green Agar (BGA) (Kristensen et al 1925), and finally, Brilliant Green Sulfa Agar (BGS) (Galton et al 1954).

Scope of Investigation

The scope of this study was to ascertain the effect of smoking and the effect of gamma irradiation on the destruction of selected species of Salmonella organisms in smoked whitefish. Specifically, the following aspects were investigated.

1. The survival of Salmonella organisms following smoking and irradiation treatments on samples of whitefish which were contaminated prior to entering the plant.

2. The survival of Salmonella organisms following smoking and irradiation on samples of whitefish which were contaminated during the brining operation.

3. The survival of Salmonella organisms following irradiation in samples of whitefish which were contaminated after smoking and during the packaging operation.

4. Establishment of the level of irradiation required to destroy the Salmonella organisms from each of the above mentioned steps.

The procedures being used in this study were designed to closely match those of normal commercial operations.

MATERIALS AND METHOD

The Brine Maker

The brine used in this study was prepared in a brine maker which was designed by A. W. Lantz (1966)(Fig. IV). It was made of a 5-gallon capacity polyethylene container with a plastic bowl connected to the outlet valve, which is placed on top of a fine mesh screen at the bottom of the container. The bowl was covered by approximately 3 inches of large glass beads, 3 inches of fine glass beads, 4 inches of fine sand and finally, 8 inches of coarse salt. Water was added from the top of the container and after a period of 30 minutes, the outlet faucet was opened and adjusted so that one drop of saturated brine fell every second into a plastic container. The brine obtained by this method was saturated and the salinometer reading was 100 sal. The brine was then kept at 60°F and diluted to the desired concentration before use.

The Smoker

The smoke house used in this study was also designed by A. W. Lantz (1964) and was constructed with few minor modifications (Fig. V). The galvanized sheet metal smoker consisted of a stack which was attached to a 60-foot chimney, a transition section or hood, a smoke chamber where the fish were suspended, a duct and finally a smoke producer

Figure IV
THE BRINE MAKER

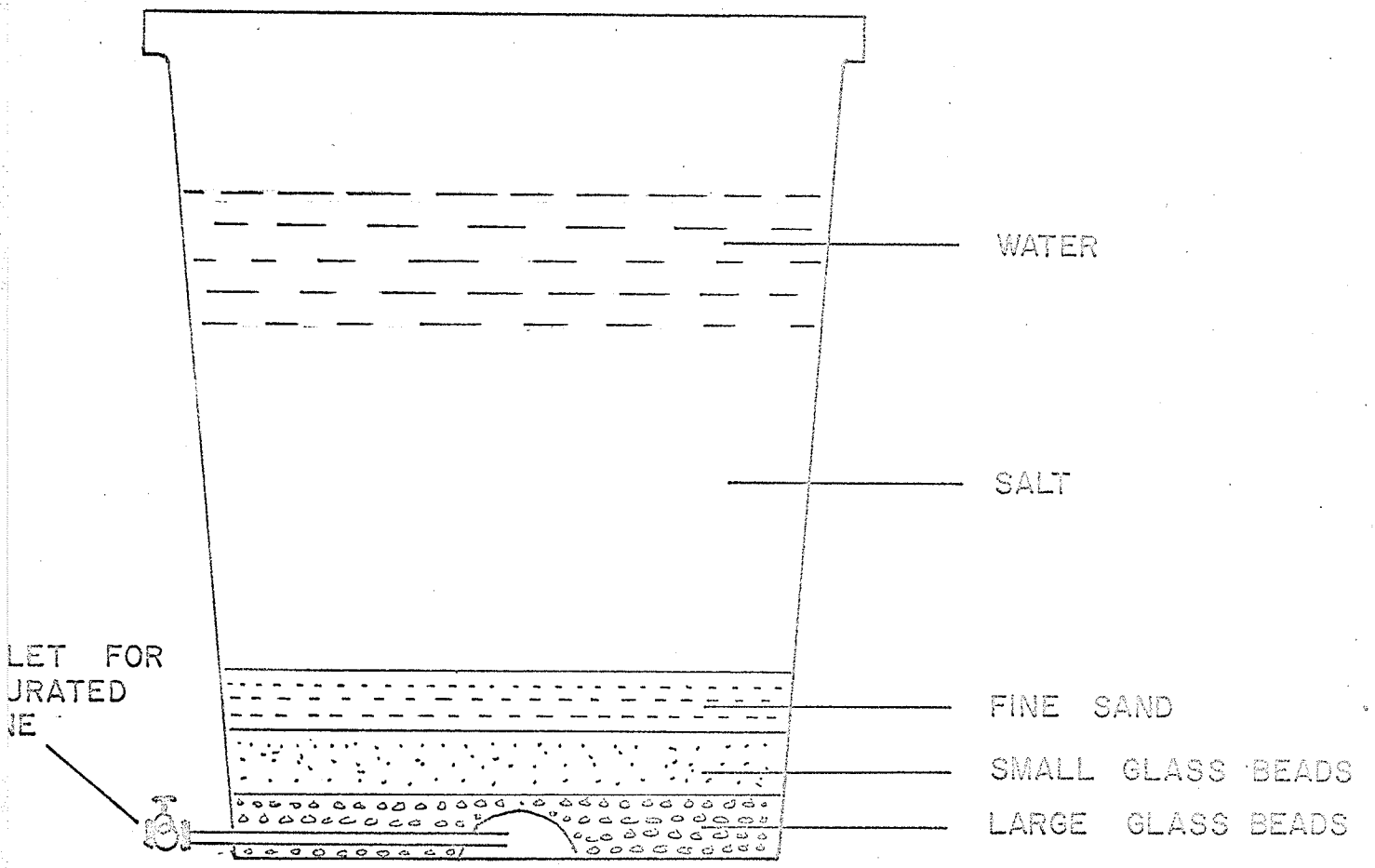
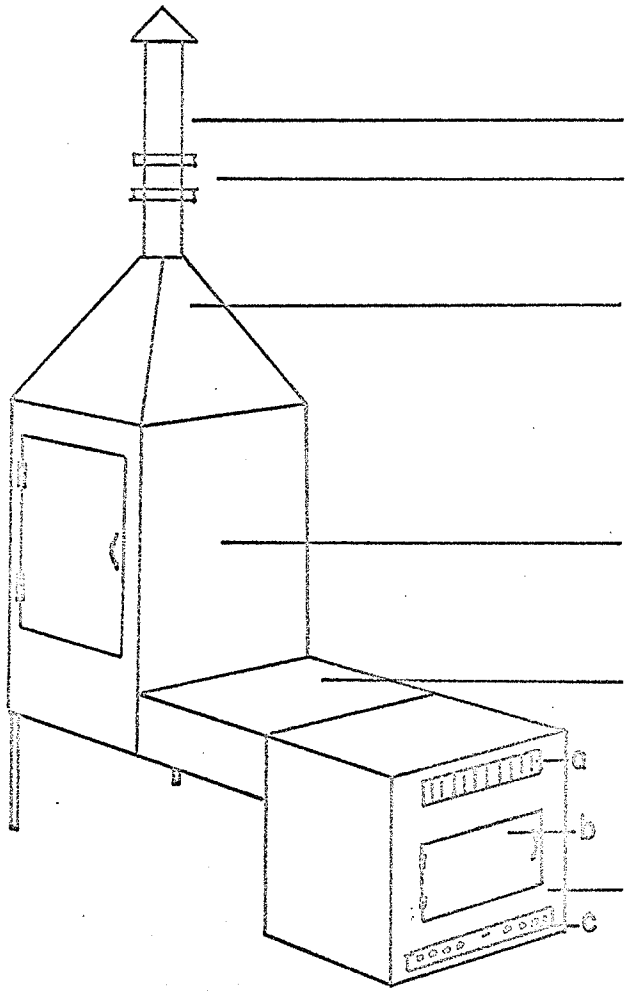


Figure V

THE SMOKEHOUSE



STACK

HEATING ELEMENTS

TRANSITION SECTION OR HOOD

SMOKE CHAMBER

DUCT

SMOKE PRODUCER

a. shutter vent
b. door
c. sliding shutter vent

which consisted of two adapters for controlling output of smoke and heat. In addition, two heating elements capable of producing heat up to 550°F were installed in the stack in order to destroy any Salmonella which may escape through the chimney during the smoking process.

The Irradiation Unit

The irradiation unit used in this study was a Co60 Gammacell Model 220 designed and constructed by the Atomic Energy of Canada Ltd. This unit contained a chamber of 6" in diameter by 8 1/8" in height. The radiation source is Co^{60} with a half-life of 5.3 years which radioactivity will decrease about 1% per month. The unit has an energy source of 1.17 and 1.33 Mev and the ability to penetrate 40 cm if the material is irradiated from both sides (El-Bisi 1968). Due to decaying factors of the source, the exposure time for specific dose levels were calculated monthly by using the appropriate decay factors.

The Salmonella Species

The cultures of Salmonella used in this study were:

1. S. give 9268 which has been reported by Comer et al (1963) to be the most radiation resistant strain.

2. S. java 10721 which was reported by Gangaroza et al (1968) to be the causative agent of Salmonellosis outbreak due to the consumption of smoked whitefish in three states of New York, New Jersey and Pennsylvania.

3. S. typhimurium 13311 which was reported by Bowmer (1965) to be the most common strain associated with food poisoning in the U. S. A.

All cultures were obtained from the American Type Culture Collection, Rockville, Maryland, U. S. A.

Preparation of Cultures

Various methods for the preparation of the Salmonella cultures have been reported. Shiflett et al (1967) used Brain Heart Infusion (Difco) for 6 hours at 40°C while Erdman et al (1961) used nutrient broth (Difco) containing 0.3% yeast extract incubated at 37°C for 24 hours.

In this study, each tube of freeze-dried stock cultures of Salmonella was broken asceptically and the content was mixed with 3 ml of sterile 0.1% peptone water, 0.5 ml of this mixture was then transferred to 6 tubes, 2 having 5 ml of Brain Heart Infusion (Difco), 2 with 5 ml of nutrient broth (Difco) and finally 2 tubes having 5 ml of trypticase soy broth. One tube from each of 3 media were incubated at 37°C for 24 hours while the others were incubated at 41°C for 18 hours. After these periods, 1 ml from each tube was transferred into 6 flasks each containing 200 ml of the previously mentioned media, placed on a reciprocal shaker and incubated at 37° and 41°C, respectively for 36 hours. Total bacterial counts from each media revealed that nutrient broth at 37°C resulted in much better growth

than the others (Appendix I). Therefore, this medium was used throughout the entire study.

The Comparison Study of Various Media Used for Recovery of Salmonella

Due to various methods reported by different workers for the isolation and identification of Salmonella species from various food products, a preliminary experiment was undertaken in order to select and establish the most suitable procedure to be used in this study.

In this study, three non-selective pre-enrichment media, lactose broth (Difco), nutrient broth (Difco) and Brain Heart Infusion (Difco), were used by transferring 25 gm of the sample into three jars containing 100 ml of the media. After blending for 2 minutes, the jars were incubated at 37°C for 2, 4, 8 and 12 hours before transferring into enrichment medium.

In the preliminary studies, a comparison was also made in various enrichment media and temperature of incubation using Selenite-F, Selenite Cystine and Tetrathionate broth (Difco). This was accomplished by adding 125 ml of each enrichment media into three jars containing different pre-enrichments and incubating at 37°C and 41°C for 24 and 48 hours respectively.

Four different selective media (BSA, SS, BGA and BGS) were used in the preliminary study by transferring 0.5 ml of the culture on each plate and spreading with a bent glass rod and incubating at 37°C for 24 hours.

The results obtained from this comparison study (Appendix II) revealed that pre-enrichment of the cultures has great significance for recovery of Salmonella. Pre-enrichment in B.H.I. for 4 hours at 37°C gave much superior results to that of lactose and nutrient broth. At the same time, enriching in Tetrathionate broth at 41°C for 24 hours made it superior to Selenite-F and Selenite Cystine broth. For the selective plating media, both Brilliant Green Sulfa and Salmonella Shigella Agar produced equally good isolations of Salmonella. However, the former was used since it is known to be more selective for Salmonella species and supressed the growth of coliform organisms (Galton et al 1954). Therefore, these selected media were used in this study for enumerating the Salmonella species.

The Inoculation of Samples and Application of Preferred Enumeration Technique

As stated in the Introduction, the main object of this investigation was to establish a level of gamma irradiation which would eliminate various Salmonella species from whitefish, which is artificially contaminated during

various stages of smoking. For this purpose, therefore, the following three stages were individually examined.

(a) Fish Inoculated with Salmonella Species Prior to the Freezing, Brining, Smoking and Irradiation Treatment.

A total of 5 dressed head-on Lake Winnipeg whitefish were obtained from a commercial source. Each fish was classified as medium in quality. Scales, slime and excess blood in the body cavity were removed from each fish and then cut into approximately 6 steaks, two inches wide.

Prior to inoculation with Salmonella, the total microbial flora and MPN (most probable number) coliforms of each fish was analysed by placing 50 grams of the fish into a sterile stainless steel blender jar, adding 450 ml of sterile 0.1% peptone water and blending for two minutes on a Waring Blender. The serial dilutions were prepared in bottles containing 0.1% sterile peptone water and duplicate plates were poured from respective dilutions using Trypticase Glucose Extract Agar (Difco) and incubated at 25°C for 72 hours. The M.P.N. coliform test was performed using double and single strength of lactose broth (Difco) and incubated at 35°C for 48 hours.

Inoculation of the samples was performed by placing six steaks in a stainless steel beaker containing 200 ml of approximately 2 x 10^7 S. give per ml of nutrient broth; six

into a beaker containing 200 ml of approximately 2 x 10^7 S. java per ml of nutrient broth and, finally, six into a beaker containing 200 ml of approximately 2 x 10^7 S. typhimurium per ml of nutrient broth. All steaks were soaked in the appropriate cultures for 15 minutes, then removed aseptically and placed in sterile stainless steel trays and kept at $0^{\circ}F$ for 48 hours.

The original number of each Salmonella species inoculated was obtained by blending 25 gm of the sample with 125 ml of nutrient broth (Difco), incubated for four hours and then adding 100 ml of Tetrathionate broth (Difco) and incubating for an additional 18 hours at $41^{\circ}C$. The serial dilution was then prepared in bottles containing 0.1% of sterile peptone water, and 1 ml of each dilution was transferred on two plates of Brilliant Green Sulfa Agar (Difco)(Osborne and Stokes 1955), spread by means of a bent glass rod (hockey stick shape) and incubated at $37^{\circ}C$ for 24 hours.

The typical colonies on B.G.S. plates were picked from each plate and inoculated in Triple Sugar Iron Agar (T.S.I.)(Difco) by streaking the slant and stabbing the butt. All tubes were then incubated for 24 hours at $37^{\circ}C$. The tubes producing black discoloration were read and the number of Salmonella per gm of the product was recorded.

After a period of 48 hours, all samples were removed

from the freezer and thawed at room temperature. The Salmonella survival of each species was calculated using the previous method of enumeration.

The thawed fish steaks were then placed in three containers, each having 400 ml of 60° sal brine and brined for 25 minutes at room temperature. The survival of Salmonella after brining was also determined and the remaining samples were placed in the refrigerator overnight.

The smoking procedures was carried out by hanging the steaks in the smoking chamber of the smoker. The samples were exposed to 3-4 hours of cold smoke by using hardwood shavings during which the temperature did not exceed 90°F. For the last 4-5 hours of smoking, the temperature was raised by burning hard wood chunks and bringing the temperature to 155-185°F. During the entire smoking process, the temperature of the flesh and the smoke chamber was measured by means of a thermo couple. (Table II)

When the smoking process was completed, the samples were left in the chamber until cooled off, then transferred aseptically into cryovac bags and sealed. The effect of smoking on all three species of Salmonella was determined using the enumeration technique described earlier.

The irradiation of samples was performed by treating them with 0.075, 0.1, 0.2, 0.3 and 0.4 M rads. The irradiated samples were then taken to the laboratory for bacteriological

TABLE II

TEMPERATURE OF SMOKE CHAMBER AND FISH SAMPLES DURING THE SMOKING

Time	Chamber Temp. ^{o}F	Flesh Temp. ^{o}F
9:30 a.m.	35	-
10:30 a.m.	57	-
11:30 a.m.	69	-
12:30 p.m.	88	52
1:30 p.m.	105	69
2:30 p.m.	131	92
3:30 p.m.	158	130
4:30 p.m.	169	138
5:30 p.m.	181	152
6:30 p.m.	184	167
7:30 p.m.	181	167
8:30 p.m.	174	158

Average of five determinations (Appendix III).

examination and enumeration of the Salmonella.

(b) Fish Sections Inoculated with Salmonella Species Prior to Brining, Smoking and Irradiation Treatment.

The steaks were frozen at 0°F for 48 hours, thawed at room temperature, and inoculated with cultures of Salmonella using the procedure described earlier. The brining of samples was carried out by placing them in three different containers of 60° sal brine for a period of 25 minutes. All samples were then removed asceptically, placed on stainless steel trays, and kept in the refrigerator overnight. The effect of brining on each species was determined. The following day, all samples were placed in the smoker for a period of approximately 8 hours. The temperature during the smoking procedure was kept close to that of previous section by opening and closing the adapters in the smoke chamber. However, this was found to be an extensively difficult operation, and differences in temperatures as much as 3-8° could not be avoided. The effect of smoking on each species was determined and the remaining samples were individually placed in cryovac bags and sealed. The radiation treatment was carried out using dosages of 0.1, 0.2, 0.3 and 0.4 M rads. This was immediately followed by detection of Salmonella survival using the enumeration procedure described under Section (a).

(c) Fish Sections Inoculated with Salmonella Species after Smoking and before Receiving Irradiation Treatment.

At this stage, the inoculation of samples with the three species of Salmonella was performed after the freezing, brining and smoking processes were completed. The inoculation procedure used was the same as explained under Section (a), however, the samples were drained on sterile stainless steel screens for 30 minutes prior to packaging. Each section was placed individually in cryovac bags and sealed. The samples were then treated with 0.1, 0.15, 0.25, 0.3, 0.35, 0.4, 0.45, and 0.5 M rads, and the survival of each species of Salmonella was determined immediately after irradiation.

All samples were kept refrigerated for a period of two weeks after being treated with various dosages of gamma irradiation. At four-day intervals, a check for Salmonella recovery was made by taking 25 grams of the sample and using the enumerating technique described previously.

RESULTS

1. Fish Inoculated with Salmonella Species Prior to the Freezing, Brining, Smoking and Irradiation Treatment.

The bacterial population as determined by the total bacterial counts and by the MPN coliform counts of fish samples

prior to inoculation with Salmonella species were found to be quite low (Table III). The total bacterial counts decreased quite rapidly following freezing, brining and smoking processes. The coliform counts decreased even more dramatically after freezing and no coliforms were detected after treating the sample with 60° sal brine. The complete destruction of natural flora became evident after treatment of samples with 0.075 M rad. When the same samples were inoculated with approximately 2 x 10^9 organisms per gram of fish with each species of Salmonella and frozen at 0°F for 48 hours, there was approximately a twofold reduction in the numbers of S. java and S. typhimurium and a onefold reduction in the case of S. give. Only a onefold reduction in counts became evident when samples with S. java and S. typhimurium were treated with brine, however, there was a twofold reduction of S. give after the same treatment. A higher recovery of S. give as compared to the other two test organisms, was noticed when the samples were smoked for approximately 8 hours. Furthermore, the data in Table III indicates that of the three species of Salmonella, S. give appears to be more resistant to the freezing, brining and smoking processes.

The survival of each species of Salmonella after exposure to various dosages of gamma irradiation is shown in Figure VI. A rapid decline in the number of survival of all

TABLE III

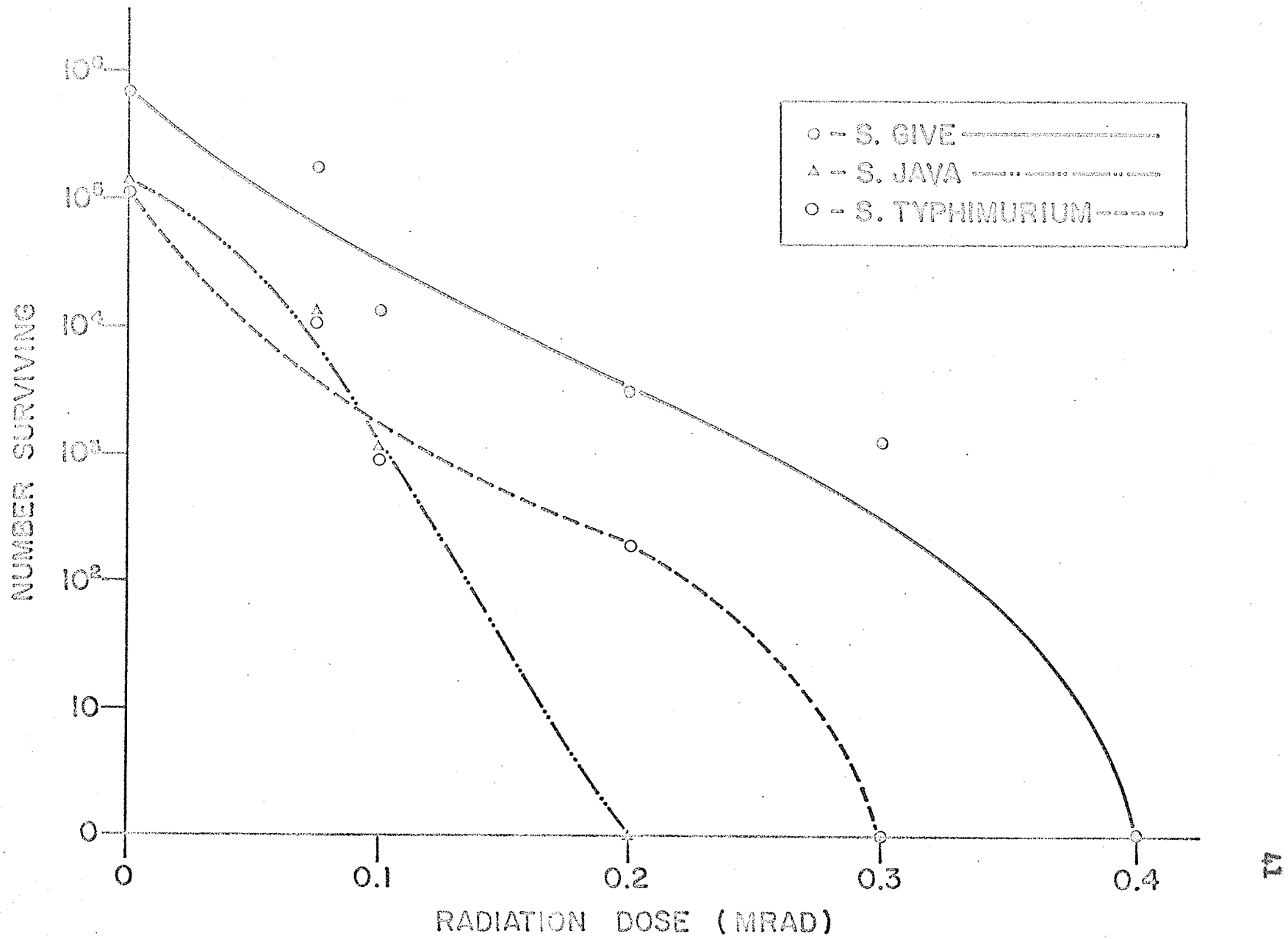
THE EFFECT OF FREEZING, BRINING, SMOKING AND VARIOUS DOSAGES OF GAMMA IRRADIATION ON SALMONELLA INOCULATED WHITEFISH AND OTHER MICROFLORA

Organism	Original Count/gm	Survival after Freezing/gm	Survival after Brining/gm	Survival after Smoking/gm	Survival of Irradiation/gm				
					0.075 M rad	0.1 M rad	0.2 M rad	0.3 M rad	0.4 rad
Natural flora	5,000	2,300	280	200	0				
MPN coliform	92	11	0	0					
S. give	2.08×10^9	1.17×10^8	9.16×10^6	6.92×10^5	283,000	11,960	5,180	1,660	0
S. java	1.94×10^9	8.65×10^7	1.6×10^6	1.77×10^5	16,000	844	0	0	
S. typimurium	2.06×10^9	6.12×10^7	1.78×10^6	1.47×10^5	14,000	700	161	0	

Average of five determinations (Appendix IV).

FIGURE VI

The Effect of Low Level Gamma Irradiation on
Salmonella Species Inoculated into
Fish Prior to Freezing


○ – S. GIVE
△ – S. JAVA
○ – S. TYPHIMURIUM
NUMBER SURVIVING
10^6
10^5
10^4
10^3
10^2
10
0
0
0.1
0.2
0.3
0.4
RADIATION DOSE (MRAD)

three species became evident when the samples were treated with 0.075 and 0.1 M rad respectively. However, this decline became non-linear, in fact, a "tailing effect" was noted in the case of S. give and S. typhimurium when they were further treated with 0.2 and 0.3 M rad of irradiation. The variation in radiation resistance among the three cultures was evident. The dose level required for a 10^5 fold reduction of S. give was 0.4 M rad, while for a 10^5 fold reduction of S. java and S. typhimurium, it was 0.2 and 0.3 M rad respectively.

2. Fish Sections Inoculated with Salmonella Species Prior to Brining, Smoking and Irradiation Treatment.

The data in Table IV indicated that the original bacterial population and the MPN coliforms on non-inoculated samples were low. This was mainly attributed to the effect of freezing on those organisms since these samples were fish which were frozen and thawed. The existing coliforms were completely eliminated after treating with 60° sal brine, while the other flora were not completely destroyed. Furthermore, approximately 10% of the microbial flora survived the smoking process. When the samples were inoculated with approximately 2×10^9/gm of each Salmonella species, all three test organisms were reduced by two logs after brining. However, when the samples were treated with smoke for a period of 8 hours, a three log reduction became

TABLE IV

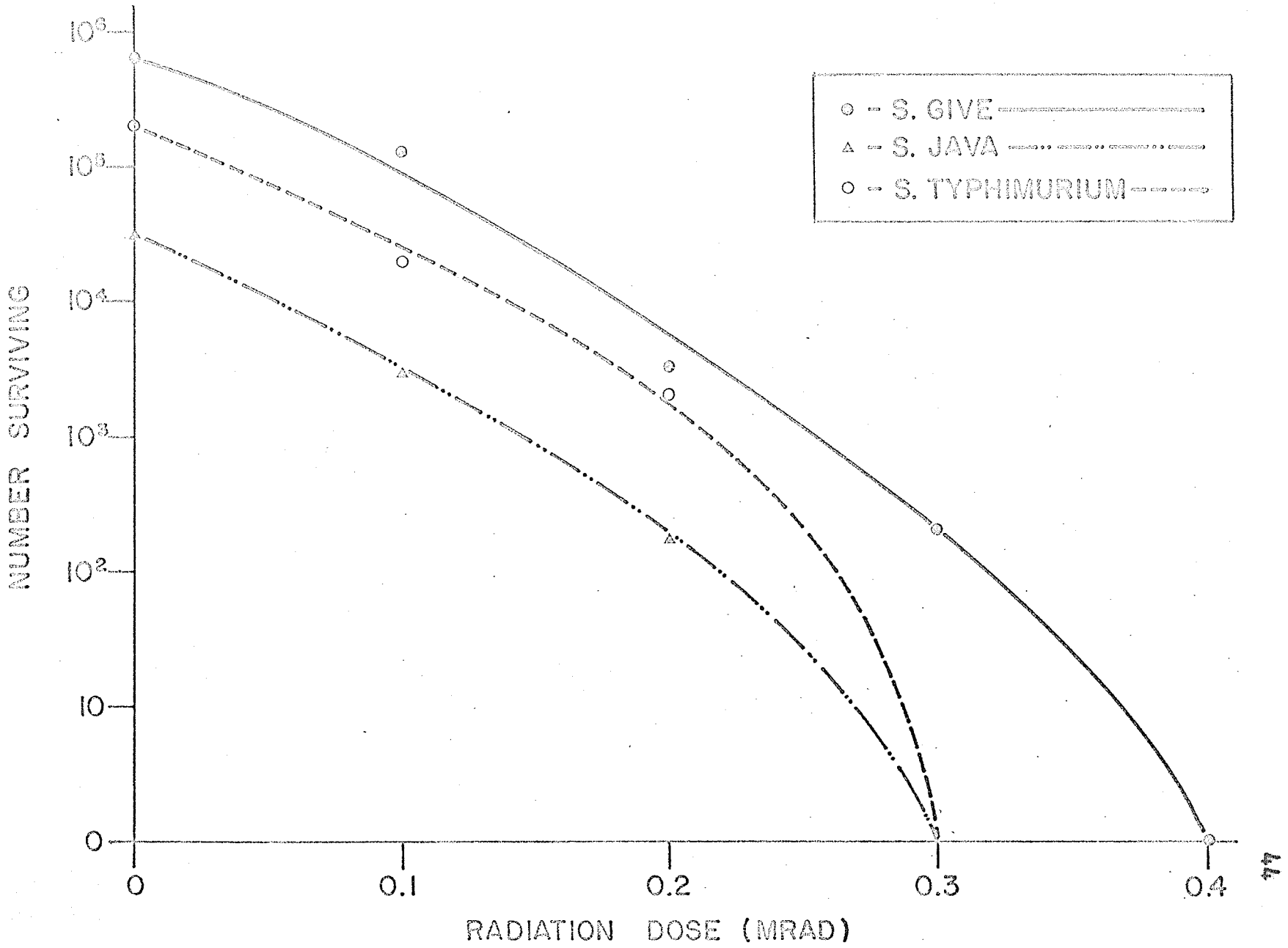
THE EFFECT OF BRINING, SMOKING AND VARIOUS DOSAGES OF GAMMA IRRADIATION ON *SALMONELLA* INOCULATED WHITEFISH AND OTHER MICROFLORA

Organism	Original Count/gm	Survival after Brining/gm	Survival after Smoking/gm	Survival after Irradiation/gm			
				0.1 M rad	0.2 M rad	0.3 M rad	0.4 M rad
tural flora	1,100	250	110	0			
coliform	36	0	0				
give	2.04×10^9	2.72×10^7	8.9×10^5	167,000	4,680	662	0
java	2.12×10^9	4.82×10^7	4.56×10^4	4,260	372	0	
S. typhimurium	2.38×10^9	1.68×10^7	3.48×10^5	29,600	3,660	0	

Average of five determinations (Appendix V).

FIGURE VII

The Effect of Low Level Gamma Irradiation on
Salmonella Species Inoculated into
Fish Prior to Brining


NUMBER SURVIVING
10^6
10^5
10^4
10^3
10^2
10
0
0
0.1
0.2
0.3
0.4
RADIATION DOSE (MRAD)
○ - S. GIVE
△ - S. JAVA
○ - S. TYPHIMURIUM

evident for S. java and approximately two log reduction in the case of S. give and S. typhimurium. Once again, S. give exhibited slightly more resistance towards the smoking process than the other two species.

The effect of gamma irradiation on the survival of the three species of Salmonella after smoking is shown in Figure VII. As was noted previously, the number of organisms decreased as the dosage was increased.

Comparatively, the most radiation-sensitive species at this stage were S. java and S. typhimurium which both required 0.3 M rad for approximately 10^4 and 10^5 reduction, while S. give again proved to be the most radiation-resistant, requiring a dosage of 0.4 M rad for a 10^5 reduction.

3. Fish Sections Inoculated with Salmonella Species after Smoking and before Receiving Irradiation Treatment.

The total natural flora isolated from the smoked samples prior to inoculation was found to be extremely low and there was no evidence of coliform present (Table V). The microbial flora were completely eliminated after treating the samples with 0.1 M rad of gamma irradiation.

Figure VIII illustrates the effect of various dosages of gamma irradiation on samples of smoked whitefish inoculated with approximately 2×10^9/gm of S. give, S. java, and S. typhimurium. It is interesting to note that there was a very rapid decline in the number of each species after

TABLE V

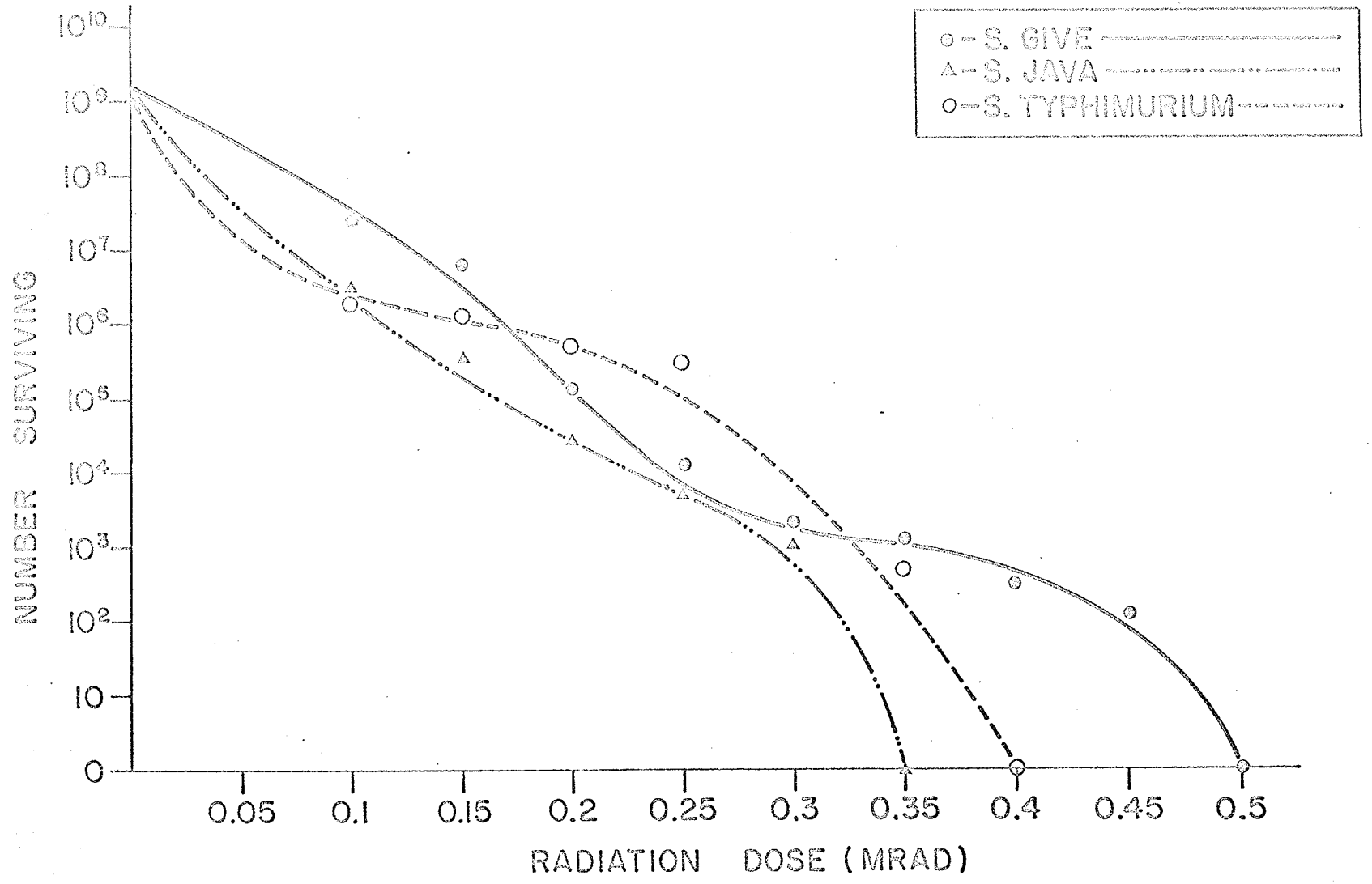
THE EFFECT OF VARIOUS DOSAGES OF GAMMA IRRADIATION ON *SALMONELLA* INOCULATED SMOKED WHITEFISH AND OTHER MICROFLORA

Organism	Original Count/gm	Survival after Irradiation/gm									
		0.1 M rad	0.15 M rad	0.2 M rad	0.25 M rad	0.3 M rad	0.35 M rad	0.4 M rad	0.45 M rad	0.5 M rad
Natural flora	560	-								
MPN coliform	-									
S. give	2.05×10^9	3.9×10^7	8.2×10^6	1.2×10^5	1.5×10^4	3.6×10^3	1,180	506	184	0
S. java	2.2×10^9	5.06×10^6	5.9×10^5	4.6×10^4	8.2×10^3	1,140	0			
S. typhimurium	2.1×10^9	3.82×10^7	1.56×10^6	6.6×10^5	4.6×10^5	2.9×10^3	630	0		

Average of five determinations (Appendix VI).

FIGURE VIII

The Effect of Low Level Gamma Irradiation on
Salmonella Species Inoculated into
Fish after Smoking


○ – S. GIVE
△ – S. JAVA
○ – S. TYPHIMURIUM
NUMBER SURVIVING
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
10^{4}
10^{3}
10^{2}
10
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
0.5
RADIATION DOSE (MRAD)

treatment with 0.1 M rad. However, the decrease became more gradual as the dosage was increased and the "tailing effect" which was evident in Figure VI became evident for all three species. S. typhimurium exhibited a higher resistance when exposed to 0.2 and 0.25 M rad; however, a sharp decline in the number of survivors was shown following exposure to 0.3 and 0.4 M rads. To obtain a 10^9 reduction in the number of organisms per gram of product S. give required a dosage of 0.5 M rads, S. typhimurium a dosage of 0.4 M rad and S. java, only 0.35 M rad.

Studies on the presence of Salmonella following irradiation and storage at $5^{\circ}C$ failed to show any growth (recovery) up to period of two weeks.

DISCUSSION

In recent years several well documented episodes of Salmonella food poisoning due to contaminated smoked whitefish have been reported by Kleeman et al (1942), Olitzkey et al (1956) and Gangarose et al (1968). In all cases, the final product was found to be contaminated with Salmonella. This contamination could have occurred, during any one of the varuous stages of processing: (a) fish being caught from polluted water; (b) fish being contaminated during thawing and brining; (c) fish being handled by employees who are the carriers of Salmonella and (d) post-smoking contamination from unsanitary equipment.

This study examined the possibilities of contamination arising from these sources and the results confirmed previous findings that smoked whitefish can be a source of Salmonellosis if it has not been processed under sanitary plant and personnel conditions. It has also shown that the smoking procedure being used by the average commercial smoking plant may very well not be sufficient for the destruction of Salmonella organisms if the fish was contaminated.

According to results obtained from this study, a two-log reduction in S. java and S. typhimurium and one log reduction in S. give became evident after freezing the samples at $0^{\circ}F$ for 48 hours. This data corresponds with the findings

reported by Wallace and Tanner (1935) and Orr and Moore (1953) who showed the survival of several species of Salmonella after being frozen for a few days to several months. Such a decrease in the number of Salmonella organisms by freezing also agrees with the findings of many other investigators who have claimed that decline in bacterial population is quite rapid around 0°F and continuing storage at this temperature results in a marked decrease in the number of recoverable organisms.

In commercial practice, the fish are usually frozen at temperatures ranging from 0°F to -20°F and stored for periods up to one year. Assuming that the fish have been caught from highly polluted waters and are contaminated with a high number of Salmonella organisms, the chances for Salmonella survival after freezing would be quite good. Although there would be no increase in the number of cells during the storage, the surviving cells could conceivably reproduce while the fish are being thawed and subsequently kept under a favourable temperature and humidity. The surviving cells, could then contaminate plant equipment and lead to the infection of the plant employees.

The effect of salt on the survival of Salmonella species has not been investigated as fully as the effect of other physical processes. However, the bacteriocidal action of salt on the majority of micro-organisms is well established, and

salt has been used for preservation of a wide variety of food products.

A one log reduction of all three species of Salmonella used was evident after being treated with brine of 60° sal concentration for 25 minutes. The effects of salting was not as significant as it was expected to be, however, this could have been due to the short time allotted for salting and the protective action of fish tissue and the moisture content of the fish.

Generally speaking, most strains of Salmonella are known to be heat sensitive except for S. senftenberg 775W which has proven to be the most heat resistant. Various temperatures have been suggested for the destruction of Salmonella organisms in a wide variety of foods ranging from 150°F to 160°F for 12 to 15 minutes.

In this study, the internal temperature (flesh temperature) was raised as high as 167°F during the latter part of the smoking process. This resulted in a one log reduction when inoculation was performed prior to freezing the fish, and a two log reduction when inoculation was done prior to brining. The present data do not agree with those temperatures suggested by Weidman et al (1956) and by Beloian and Schlosser (1963) who reported that an internal temperature of 160°F would be sufficient for destruction of Salmonella. It also suggests that the temperature used for

elimination of Salmonella from one food product cannot be applied to the other, due to a wide variety of physical and chemical structural differences existing among food products.

A most significant finding was that of the resistance of S. give to heat as compared to the other two species. Unfortunately, no reference was available which could support this finding. However, the present study indicated that a higher temperature is required for the destruction of S. give as compared to S. typhimurium and S. java.

According to Comer et al (1963), S.give is the most radiation resistant strain. It requires a dosage of 0.54 M rad for a 10^7 fold reduction when artificially inoculated into frozen fried egg. However, Nickerson et al (1957) reported S. typhimurium to be the most resistant serotype examined in liquid and frozen egg white. Dyer et al (1965) showed that S. paratyphi B, which is closely related to S. java was the second most radiation resistant strain.

The results obtained from this study indicated that low levels of gamma irradiation would eliminate the three species of Salmonella used regardless of the source of contamination. S. give was shown to be the most resistant serotype requiring a dosage of 0.5 M rad for approximately 10^9 fold reduction. This confirmed the findings of Comer et al (1963) to some extent. However, this did not agree with the dosage level required for a "safe" reduction. Since they

obtained a 10^7 fold reduction with 0.54 M rad and the present results indicates a 10^9 fold reduction with only 0.5 M rad. This difference in resistance can be attributed to many conditions, of which the type of product being examined was the most important. The dosages required for a reduction of S. typhimurium and S. java were found to be 0.4 and 0.35 M rads respectively. These figures generally agreed with those reported by Nicherson et al (1957) and Dyer et al (1965).

However, when the samples were inoculated prior to freezing and brining, a much lower dosage was required for complete destruction ranging from 0.2 M rad for S. java to 0.4 M rad for S. give. This is mainly due to a lower number of organisms and possible damage done to their cellular structure by the various physical processes (freezing, brining and smoking).

The "tailing effect" reported by Dyer et al (1965) who worked with inoculated solid crab meat was also shown to some extent in this study, especially when the samples were inoculated after smoking (Figure VIII). However, this was not evident when the samples were inoculated prior to brining. This effect has not been adequately explained, but many suggest that the physical damages done to the cells and food constituents could have made them more sensitive to radiation. On the other hand, this "tailing effect" suggests

that within a population of the same species of micro organisms, there is a selected number of organisms which are slightly more resistant to gamma irradiation (Frazier, 1967).

It is interesting to note that there was a general reduction in the number of Salmonella species after treatment with the various processes. However, except for low level gamma irradiation, none of these processes eliminated the Salmonella from the end-product. The survival number after various treatments ranged from 1.47×10^5/gm to 8.9×10^5/gm. According to studies performed by McCullough and Eisele (1951), such a high number of Salmonella would be sufficient for the initiation of Salmonellosis in man. There has been little information as to the number of Salmonella organisms isolated from the smoked fish. However, in view of findings available from other food products such as frozen egg malange, dried egg, desiccated coconut, it seems that 10^5/gm of Salmonella would be a reasonable number which may be isolated from smoked fish. With this assumption, it is safe to say that if a smoked fish product carried such a high number of Salmonella per gram, it would create Salmonellosis upon consumption.

It should also be emphasized that the gamma irradiation possesses a distinct potential as a means of eliminating Salmonella from smoked whitefish regardless of the source of contamination. At the same time, the dosage required for this purpose is greatly dependent upon various conditions

such as the chemical composition of the media, temperature, availability of oxygen, and the presence of some additives such as spices. Erdman et al (1961) reported that the radiosensitivity of micro-organisms was decreased when they were inoculated in broth as compared with buffer. The effect of the complexity of the media on the radiosensitivity of Salmonella was also shown by Dyer et al (1965). They noticed that a high dosage was required for the destruction of three Salmonella serotypes when inoculated into solid crab meat. The dosage required was considerably decreased as the crab meat was diluted out. Therefore, to ensure adequate destruction of micro-organisms by irradiation, it is essential to perform acceptable irradiation dosages for each type of food based upon trials closely simulating practical operating conditions.

The use of low levels of gamma irradiation not only has been reported to eliminate the majority of non-spore formers, pathogenic and non-pathogenic micro-organisms, but also has extended the shelf life of a wide variety of fishery products. At the same time, high dosages of gamma irradiation have been reported to create some physical changes in the product.

Ostovar et al (1967) reported that a dosage of 0.3 M rad caused a noticeable change in color and texture of fresh whitefish flesh accompanied by a strong "burnt" odor which

after 8 days of storage at ice temperature gradually disappeared. Ingram and Rhodes (1962) reported that high dosages of gamma irradiation have created noticeable changes in odor, texture and appearance of some fish products such as herring, crab, cod and sole. Power et al (1964; 1964) have also reported these physical changes in haddock fillets and scallops. However, most of these off-flavors seems to disappear after a few days in storage.

In this study, the effect of various dosages of gamma irradiation on flavor, texture and odor of smoked whitefish was not examined, since the product under investigation was inoculated with three potential pathogens. However, a study of the effect of various dosages on the flavour of smoked whitefish is presently under investigation by the School of Home Economics, University of Manitoba. If the results of this study reveal that no major physical changes occur after treating the smoked whitefish to a dosage as high as 0.5 M rad, then it can be ensured that a smoked whitefish product which is contaminated with Salmonella as high as 10^9/gm would be safe for consumption.

In Conclusion, this investigation revealed the following:

1. Under normal commercial practice, smoked whitefish would be a source of Salmonellosis if the fish has been

contaminated prior to or after smoking processes.

2. The end product could be rendered free from Salmonella organisms by use of low level gamma irradiation.

3. Among the three species studied, S. give proved to be the most radiation resistant species, followed by S. typhimurium and S. java respectively.

4. S. give was also shown to be the most heat resistant of the three species.

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APPENDIX I

TOTAL BACTERIAL COUNTS OBTAINED FROM EACH *SALMONELLA* SPECIES USING DIFFERENT PRE-ENRICHMENT MEDIA AND TEMP.

	Nutrient Broth		Brain Heart Infusion		Trypticase Soy Broth	
Organism	37° C	41° C	37° C	41° C	37° C	41° C
S. give	1.6×10^8	3.2×10^6	7.1×10^5	2.2×10^5	6.3×10^5	1.2×10^5
S. java	2.1×10^7	1.9×10^6	2.4×10^6	1.8×10^6	3×10^6	4.2×10^5
S. typhimurium	1.3×10^7	2.5×10^6	4.1×10^6	5.1×10^5	4.3×10^6	3.1×10^5

APPENDIX II

COMPARISON STUDY ON RECOVERY OF *SALMONELLA* SPECIES USING VARIOUS ENRICHMENT AND SELECTIVE MEDIA

Media		*S.give*	*S.java*	*S.typhimurium*
Pre-enrichment: BHI				
Enrichment: Tetrathionate				
Selective Media:	B.G.S.	3.1×10^8	4.4×10^9	3.1×10^9
	S.S.	2×10^8	2.9×10^8	5.2×10^8
	B.G.A.	2.5×10^7	1.2×10^8	3.9×10^7
	B.S.A.	1.2×10^5	1.9×10^6	9.2×10^5
Pre-enrichment: BHI				
Enrichment: Selenite-F				
Selective Media:	B.G.S.	4.9×10^6	4.6×10^7	3.9×10^8
	S.S.	3.2×10^7	6.1×10^7	4.9×10^7
	B.G.A.	1.9×10^5	4.2×10^6	7.1×10^6
	B.S.A.	6.2×10^3	3.9×10^4	4.1×10^5
Pre-enrichment: BHI				
Enrichment: Selenite Cystine				
Selective Media:	B.G.S.	6.3×10^7	7.2×10^8	9.1×10^9
	S.S.	4.1×10^6	4.9×10^6	4.8×10^8
	B.G.A.	3.8×10^6	3.8×10^5	3.1×10^8
	B.S.A.	2.9×10^5	4.9×10^5	1.9×10^7

BHI - Brain Heart Infusion B.G.S. - Brilliant Green Sulfa

S.S.- *Salmonella Shigella* B.G.A. - Brilliant Green Agar

B.S.A. - Bismuth Sulfite Agar

APPENDIX II (continued)

Media		*S.give*	*S.java*	*S. typhimurium*
Pre-enrichment: Lactose Broth				
Enrichment: Tetrathionate				
Selective Media:	B.G.S.	2.6×10^7	3.1×10^8	2.9×10^8
	S.S.	1.1×10^7	5.2×10^8	6.1×10^8
	B.G.A.	4.9×10^6	3.8×10^6	6.2×10^6
	B.S.A.	3.8×10^4	4.9×10^4	6.2×10^5
Pre-enrichment: Lactose Broth				
Enrichment: Selenite-F				
Selective Media:	B.G.S.	1.9×10^6	4.9×10^7	1.8×10^6
	S.S.	3.9×10^7	6.1×10^6	3.1×10^5
	B.G.A.	6.1×10^5	5.9×10^5	4.2×10^4
	B.S.A.	9.2×10^4	3.6×10^5	1.9×10^5
Pre-enrichment: Lactose Broth				
Enrichment: Selenite Cystine				
Selective Media:	B.G.S.	3.2×10^6	4.9×10^7	3.1×10^6
	S.S.	4.6×10^5	8.2×10^6	9.1×10^6
	B.G.A.	3.3×10^5	9.1×10^5	6.3×10^5
	B.S.A.	1.2×10^5	3.5×10^4	1.8×10^5

BHI - Brain Heart Infusion B.G.S. - Brilliant Green Sulfa

S.S.- *Salmonella Shigella* B.G.A. - Brilliant Green Agar

B.S.A. - Bismuth Sulfite Agar

APPENDIX III

TEMPERATURE (°F) OF SMOKE CHAMBER AND FISH SAMPLES DURING FIVE TRIALS OF SMOKING

Trials	1		2		3		4		5		Average	
Time	Chamber Temp.	Flesh Temp.	Chamber Temp.	Flesh Temp.	Chamber Temp.	Flesh Temp.	Chamber Temp.	Flesh Temp.	Chamber Temp.	Flesh Temp.	Chamber Temp.	Flesh Temp.
0930	37	--	34	--	35	--	35	--	34	--	35	--
1030	58	--	56	--	57	--	59	--	55	--	57	--
1130	69	--	69	--	67	--	68	--	69	--	69	--
1230	87	50	90	54	88	51	91	52	86	53	88	52
1330	102	68	106	71	108	73	106	68	103	66	105	69
1430	134	90	130	93	128	91	130	94	133	92	131	92
1530	156	127	161	131	158	130	156	134	159	128	158	130
1630	171	141	168	140	170	135	167	138	169	136	169	138
1730	180	156	178	150	183	151	181	154	183	149	181	152
1830	184	169	186	165	187	167	182	169	181	165	184	167
1930	178	167	181	170	180	166	182	167	184	165	181	167
2030	173	161	176	159	174	159	173	160	174	151	174	158

APPENDIX IV

THE EFFECT OF FREEZING, BRINING, SMOKING AND VARIOUS DOSAGES OF GAMMA IRRADIATION ON *SALMONELLA* INOCULATED WHITEFISH AND OTHER MICROFLORA

Trial	Original	After Freezing	After Brining	After Smoking	.075	0.1	0.2	0.3
. give								
1	2×10^9	1.8×10^8	2×10^7	4.2×10^5	250,000	11,200	2,500	800
2	2.2×10^9	2.3×10^8	1.9×10^6	3.8×10^5	245,000	10,500	3,700	1,200
3	2.4×10^9	1.2×10^7	3.1×10^6	2.9×10^5	180,000	8,200	5,500	1,600
4	1.8×10^9	2.5×10^7	2.8×10^6	2.1×10^6	500,000	2,100	9,100	2,800
5	2×10^9	1.4×10^8	1.8×10^7	2.7×10^5	190,000	8,900	5,600	1,900
Average	2.08×10^9	1.17×10^8	9.16×10^6	6.92×10^5	283,000	11,960	5,180	1,660
. java								
1	1.8×10^9	1.2×10^7	1.5×10^6	2.9×10^4	14,500	1,000	0	
2	2.1×10^9	1.8×10^8	2.6×10^6	5.6×10^5	29,000	1,100	0	
3	1.7×10^9	1.2×10^7	1.7×10^5	3.9×10^4	18,000	500	0	
4	1.9×10^9	2.1×10^8	3.8×10^6	2.6×10^5	11,000	420	0	
5	2.2×10^9	1.9×10^7	2.9×10^5	1.1×10^4	8,000	1,200	0	
Average	1.94×10^9	8.65×10^7	1.6×10^6	1.77×10^5	16,000	844		
. typhimurium								
1	2.2×10^9	1.7×10^7	1.9×10^5	1.2×10^4	9,000	500	190	
2	1.2×10^9	2.5×10^7	8.2×10^5	3.6×10^4	15,000	240	95	
3	1.8×10^9	4.8×10^7	4.1×10^6	2.5×10^5	12,000	1,100	200	
4	2.1×10^9	1.9×10^8	1.5×10^6	4.2×10^5	25,000	1,400	240	
5	2.3×10^9	2.6×10^7	6.2×10^5	2.1×10^4	11,000	290	82	
Average	2.06×10^9	6.12×10^7	1.78×10^6	1.47×10^5	14,000	700	161	

APPENDIX IV (continued)

Trial	Original	After Freezing	After Brining	After Smoking	.075	0.1	0.2	0.3
atural Flora								
	6,300	3,900	680	460				
	8,000	3,100	340	240				
	3,200	1,300	110	90				
	3,200	1,400	120	98				
	4,300	1,800	150	112				
Average	5,000	2,300	280	200				
PN Coliform								
	92	0	0	0				
	91	18	0	0				
	110	37	0	0				
	92	0	0	0				
	75	0	0	0				
Average	92	11						

APPENDIX V

THE EFFECT OF BRINING, SMOKING AND VARIOUS DOSAGES OF GAMMA IRRADIATION ON *SALMONELLA* INOCULATED WHITEFISH AND OTHER MICROFLORA

Trial	Original	After Brining	After Smoking	0.1	0.2	0.3	0.4
S. give							
1	2×10^9	1.6×10^7	2.8×10^5	210,000	4,500	500	0
2	2.4×10^9	5.2×10^7	5.3×10^5	290,000	6,200	1,100	0
3	2.1×10^9	2.5×10^7	8.9×10^5	130,000	4,400	700	0
4	1.9×10^9	1.8×10^7	6.2×10^6	110,000	5,100	620	0
5	1.8×10^9	2.5×10^7	9.1×10^6	98,000	3,200	390	0
Average	2.04×10^9	2.72×10^7	8.9×10^5	167,600	4,680	662	0
S. java							
1	2.1×10^9	2.4×10^7	5.3×10^4	5,200	320	0	
2	2.2×10^9	4.8×10^7	6.2×10^4	4,800	450	0	
3	2.1×10^9	3.9×10^7	2.8×10^4	1,900	300	0	
4	1.9×10^9	2.8×10^7	5.6×10^4	6,200	520	0	
5	2.3×10^9	5.2×10^7	2.9×10^4	3,200	270	0	
Average	2.12×10^9	4.82×10^7	4.56×10^4	4,260	372	0	
S. typhimurium							
1	2.3×10^9	1.8×10^6	2.9×10^5	32,000	2,500	0	
2	2.1×10^9	2.8×10^7	4.2×10^5	45,000	5,200	0	
3	1.9×10^9	5.2×10^6	3.2×10^5	18,000	1,300	0	
4	2.4×10^9	3.1×10^7	5.2×10^5	31,000	4,200	0	
5	3.2×10^9	1.8×10^7	1.9×10^5	22,000	5,100	0	
Average	2.38×10^9	1.68×10^7	3.48×10^5	29,600	3,660	0	

APPENDIX V (continued)

Trial	Original	After Brining	After Smoking	0.1	0.2	0.3	0.4
Natural Microflora							
	1,500	370	98	0			
	1,200	225	120	0			
	920	410	115	0			
	1,100	120	105	0			
	780	125	112	0			
Average	1,100	250	110				
MPN Coliform							
	18	0	0				
	0	0	0				
	36	0	0				
	72	0	0				
	54	0	0				
Average	36	0	0				

APPENDIX VI

THE EFFECT OF VARIOUS DOSAGES OF GAMMA IRRADIATION ON SALMONELLA INOCULATED SMOKED WHITEFISH AND OTHER MICROFLORA

Trial	Original	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45
S. give									
1	2.1×10^9	5.4×10^7	1.2×10^6	6.2×10^4	9.8×10^3	1.2×10^3	1,000	230	129
2	1.9×10^9	2.9×10^7	5.2×10^6	3.8×10^4	2.1×10^4	4.2×10^3	2,100	800	162
3	1.8×10^9	3.8×10^7	2.4×10^7	4.5×10^9	1.2×10^9	6.9×10^3	4,100	1,100	520
4	2.2×10^9	2.5×10^7	1.8×10^6	3.9×10^5	5.1×10^3	3.8×10^3	1,600	900	130
5	2.1×10^9	4.9×10^7	9.1×10^6	7.2×10^9	2.8×10^4	1.9×10^3	1,100	500	79
Average	2.05×10^9	3.9×10^7	8.2×10^6	1.2×10^5	1.5×10^4	3.6×10^3	1,180	506	184
S. java									
1	1.9×10^9	3.3×10^6	1.2×10^5	9.1×10^4	3.3×10^4	1,700	0		
2	2.8×10^9	4.2×10^6	3.1×10^5	4.8×10^4	2.1×10^3	1,500	0		
3	2.1×10^9	3.5×10^6	4.8×10^6	5.1×10^4	2.5×10^3	1,400	0		
4	1.8×10^9	9.2×10^6	8.1×10^5	3.1×10^4	1.9×10^3	1,100	0		
5	2.4×10^9	5.1×10^6	2.3×10^5	1.2×10^4	1.8×10^3	1,000	0		
Average	2.2×10^9	5.06×10^6	5.9×10^5	4.6×10^4	8.2×10^3	1,140			
S. typhimurium									
1	2.1×10^9	5.2×10^7	1.2×10^7	6.2×10^6	3.1×10^5	1.3×10^3	200		
2	1.8×10^9	5.1×10^7	2.1×10^7	5.8×10^6	5.1×10^5	4.1×10^3	920		
3	2.1×10^9	2.9×10^7	1.1×10^7	4.9×10^6	3.8×10^5	2.8×10^3	500		
4	2.4×10^9	4.1×10^7	2.2×10^7	7.2×10^6	4.2×10^5	1.9×10^3	720		
5	2.1×10^9	1.8×10^7	1.2×10^7	9.1×10^6	7.1×10^5	4.7×10^3	810		
Average	2.1×10^9	3.82×10^7	1.56×10^6	6.6×10^5	4.6×10^5	2.9×10^3	630		

APPENDIX VI (continued)

Trial	Original	0.1	0.15	0.2	0.25	0.3	0.35	0.4	0.45
Natural flora									
	830	0							
	410	0							
	630	0							
	380	0							
	550	0							
Average	560								