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FOREWORD

ON JUNE 15, 1925, a mother and daughter of Solon, Ohio, developed fatal Type A botulism following the ingestion of canned sardines in tomato sauce.[1] In October, two additional fatalities as a result of eating sardines of the same pack were reported in Los Angeles, California. Federal and State authorities immediately launched a searching investigation in order to prevent the recurrence of similar food poisoning. The stocks of this particular sardine pack remaining on the open market were seized, examined, and destroyed. According to reports from the United States Bureau of Chemistry, a large part of the pack consisted of "flippers"[2] or "springers."[3] Some two hundred cans were carefully cultured and studied. Four of nine cans which were actual "swells"[4] contained the botulinus poison. Examination by the Hooper Foundation of fifty cases of this particular pack yielded five cans which were "hard swells." The contents, when fed, killed guinea pigs, and Type A (4) and Type B (1) Cl. botulinum were isolated.

Since the sardines originated in California, it became the duty of the California State Department of Public Health to provide protective measures against botulism in commercially canned fish products. The State Legislature had earlier passed Act 428, and the Governor on May 23, 1925, had approved the creation of a division of cannery inspection to regulate the conduct of canneries and to provide rules governing the proper sanitation of packing plants and the preservation of agricultural products according to

[1] Hayhurst, Emery R., Am. Jour. Pub. Health, 1926: 228.

[2] A "flipper" is a can which is either under very mild or no positive internal pressure. It may be perfectly normal in appearance; but the end if struck sharply against some solid object will become distended. The end of the can may be pushed back, with a gentle pressure, to its normal position.

[3] The term "springer" may be applied to a can the ends of which are more or less bulged, but in which the internal pressure is not sufficient to prevent pushing in the ends to their normal position. A springy motion of the can ends is encountered. Springers are characterized by complete loss of vacuum within the can. They may be caused by overfilling, sealing at too low a temperature, rough handling resulting in dented cans, or faulty sealing operations. Swelled cans pass through the springer stage.

[4] A "swelled" can is one the ends of which are bulged or distended by the formation of gas within the can through chemical or microbial processes. The degree of internal pressure exerted against the ends designates whether the can is a "soft" or "hard swell."

scientifically established principles. After the Solon and Los Angeles group intoxications following the eating of California canned pilchards, Act 428 was amended in 1927, and fish and fish products, meat and meat products, are now subject to the rules and regulations of the cannery inspection service. For five years (1920 to 1925) the George Williams Hooper Foundation of the University of California, in coöperation with the National Canners' Association and the American Can Company, was entrusted with the investigation and studies which preceded the formulation in 1924 and 1927 of the rules and regulations required by the State Cannery Division. At the request of Dr. George Ebright, President, and Dr. Walter M. Dickie, Secretary, of the California State Board of Health, the Foundation was invited in 1926 to plan and execute a detailed survey of the fish-packing plants and to undertake laboratory studies in order to determine practical processing procedures which would with certainty prevent botulism and put in the hands of the consumer a can remaining flat under all conditions of trade. Through the personal efforts of the late Mr. R. I. Bentley, Mr. Sam Hornstein, and Mr. I. Zellerbach, the California State Fish and Game Commission agreed to finance the entire research program for three years. The Commission later extended its support and granted to the Hooper Foundation a liberal annual donation from 1926 to 1933 inclusive. Without this assistance the work could not have been carried out. It is indeed a privilege to acknowledge again the liberality of the State Fish and Game Commission.

Obviously an investigation with the diversified and all-embracing scope of a seasonal packing industry could not be conducted by a few workers alone. Various agencies and organizations, Federal and State bureaus, unstintingly placed at the disposal of the Foundation their personnel, equipment, and resources. Acknowledgment is due to the United States Bureau of Chemistry, which delegated to the survey of the sardine- and tuna-canning plants, Dr. A. C. Hunter; Mr. Frank E. Gorell, Secretary of the National Canners' Association, for his continued assistance from his offices; the American Can Company, in particular the Research Division under Dr. F. F. Fitzgerald, who assigned Dr. C. O. Ball and Mr. C. T. Parks as active coöperators in the plant studies; the officers of the California Fish Packers' Association, for their valuable

services as a means of communication with the industry, and Mr. Milton P. Duffy, of the Cannery Division of the State Department of Public Health, for his coöperation during the tedious and frequently discouraging investigations.

From the beginning Mr. O. W. Lang, as Research Associate, has been in charge of the studies. His immediate assistants in the various parts of the problem have been R. S. Fellers, C. M. Judah, S. J. Dean, Jr., H. P. Stevens, J. B. Blair, M. E. Stockle, and D. Barry. Although the technical studies were in part completed in 1930, the compilation and the analysis of the data consumed the greater part of 1931, 1932, and 1933.

The benefits of this study have already been recorded. No cases of botulism from the eating of California canned fish or fish products have been reported since 1926.

K. F. MEYER,
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University of California.

San Francisco, August, 1933.

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General Introduction

There are many factors in the marine packing industry upon which depend the quality, sterility, danger of spoilage, and marketability of the product. Few of these factors have been seriously studied. Present methods of packing the various sea foods are entirely pragmatic, being based on the experience of packers and modified by certain economic factors. These economic factors are of vital importance, yet they have often had, under certain operating conditions, a detrimental effect upon the packing methods.

The specific study of the bacterial flora of marine packing plants for the purpose of ascertaining the stages in the canning process wherein the greatest bacterial contamination occurs, has not heretofore been attempted. The solution of this problem will in large measure determine the modern approach to sanitary methods as well as to the desired goal of marketable products of assured quality and safety. Upon information concerning the stages in the canning process in which the danger of pollution is greatest, rests the selection of the most efficient and most economical means of its prevention. With these points in mind, data have been obtained on the following canned marine products: tuna, sardines, abalone, barracuda (kamaboko or tempra), bonita, Pacific mackerel, shad, squid, and yellow tail.

Tuna Canneries

The investigation of the number and types of microörganisms commonly associated with the packing of tuna was begun in the 1927 canning season, and was continued through the season of 1928 as a check on the initial observations and in order to ascertain whether or not conditions had altered. A laboratory was installed on the premises of the Coast Fishing Company in Wilmington, California, making possible more complete access to canning operations and a very prompt laboratory examination of the samples taken in the different packing plants, thus eliminating the probability of increased growth during transportation.

PRELIMINARY

The tuna packing process.—With the exception of some frozen albacore imported from Japan, all tuna canned in southern California are caught either in local or Lower California waters. The tuna from local waters are usually delivered the same day they are caught; the catches from Lower California are often in transit over a period of from one to five weeks, they are heavily iced, and the use of refrigerating systems is necessary. When received at the cannery docks, the fish are loaded onto conveyors and are received in the cannery in carts, usually of all-metal construction. After being weighed the fish are dumped on the floor.

The butchering operation consists of removing the entrails, after which the fish are racked in baskets and given a wash, usually with fresh water from a hose. They are then delivered to the tuna cookers, in which they are subjected to temperatures ranging from 214° to 220° F, for precooking times of from two to eight hours. Both time and temperature are chiefly dependent upon the size and species of fish. On completion of the precook the fish are removed from the cookers and allowed to cool for from eight to twelve hours, or until they are sufficiently firm to be handled without jeopardizing quality.

The cleaning operation consists of removing the head and skin, the latter being scraped from the flesh with small knives. The flesh is divided longitudinally into four sections, and the dark meat and all adhering particles of membrane are removed. The cleaned pieces are placed on wooden trays and cut into required sizes to fit the cans. This cutting takes place at tables some distance away from where the cleaning is done. The fish are packed by hand, automatically salted, the required amount of cottonseed or olive oil is added, and the cans are sealed, washed, and retorted.

Because of the danger of condensation, tuna is seldom "exhausted." The exhaust times and temperatures that are now employed can hardly be considered sufficient to produce the vacuum necessary to maintain the can ends in a concave position. The effectiveness of an exhaust as now given is therefore negligible.

EXPERIMENTAL

Bacterial flora as influenced by the canning process.—For the purposes of this investigation the canning of tuna may be divided

into three operations—butchering, cleaning, and packing. The investigation of the apparent probable foci of contamination was therefore first concerned with these three procedures. The main localities from which samples were taken in the butchering operation were the fish carts, surface slime on fish before washing, body cavity before washing, surface skin after washing, and body cavity after washing. Samples of deep flesh were also taken; of these samples only those were used which were not covered by bruises or broken skin.

The flora of material adhering to the sides of the fish carts in the form of slime and scum will in general serve as an index of contamination found in the boat holds and on the fish. Here, obviously, would seem to be the first portal of entry into the canning plant. After the cleaning procedure, it is not customary to wash the fish until a cooker is available to receive them. Since a time interval of from three to five hours may often elapse between precooks, a free opportunity is provided for multiplication of organisms while the fish are lying on the floor or racked in baskets.

Samples of the fish were taken immediately after precooking, and again when thoroughly cooled and ready for the cleaning operation. These samples consisted of the surface of the fish, flesh in the region of the dorsal fin, and flesh in the body cavity. Samples were also taken from the accumulations adhering to the baskets in which the tuna had been precooked. In the canning procedures, specimens were removed from the cleaned flesh ready to be packed, from the canning trays, and from packing tables. The packing trays and canning tables are used in common during the canning process; it therefore is reasonable to assume that the flora occurring in the one locality would be directly associated with that occurring in the other, and the results derived have accordingly been incorporated under one heading. In addition to these samples, cultures of cottonseed oil, olive oil, and washings from the empty container were made. Since it is reasonable to assume that at this stage the precooking will serve to retard a residual contamination on the fish, which has not been removed in washing, the samples taken in the cleaning and canning operations will show the progress of infection during these procedures. In order to determine whether the number of organisms contained in the cleaned fish increases or decreases during a canning day, samples from these

sources also were taken at successive intervals. Cultures from the packing tables were made in like manner and for the same purpose.

Procedure for taking samples.—Sections of fish or other material were removed with a sterile scalpel and forceps. Wherever samples of fish were removed, the skin was first seared with a heated blade and then transferred to a previously tared, sterile bottle containing a small quantity of broken glass. Three to five grams of all samples was taken for study. The net weight was ascertained and the required amount of sterile tap water to give a one in ten parts suspension was pipetted into the bottle. Thorough shaking was necessary in order to macerate the sample, after which dilutions from this stock suspension were made.

Qualitative and quantitative determinations were performed. Selective mediums were employed for the qualitative differentiation. The dilution method was used in connection with these selective mediums for determining the quantities of definite types of organisms. The types for which examinations were made were: mesophiles, halophiles, aërobic thermophiles, sulphide spoilage thermophiles, and anaërobic sporulating mesophiles and thermophiles. The mesophilic cultures were incubated for 60 hours at 37.5° C and the thermophiles for 48 hours at 55° C. All mesophile and thermophile anaërobic cultures were examined daily; no tubes were discarded as negative until after ten days' incubation, since after this duration the culture media becomes rather dehydrated. Representative plates from each culture were counted and the results expressed in numbers of viable organisms per gram of material. Typical and distinct colony types were isolated and planted on agar slants for further study. Their biochemical reactions and their reactions in fish protein were determined. All mesophilic anaërobes were tested for toxicity by inoculation of ten-day-old broth cultures into mice. In the event of mortality, the type of toxin was determined by specific antitoxin protection tests.

Cultural methods.—For *ordinary mesophiles* Standard Methods Agar for milk analysis, as recommended by the American Public Health Association (1923), was employed. At first it was thought that, since the investigation dealt with the bacterial action in connection with fish proteins, a nutrient medium containing fish protein extractives as a base should be used. After investigation of

the comparative growth-efficiency of nutrient fish-protein-base agar against Standard Methods Agar, it was found that no material advantage could be gained; therefore Standard Methods Agar was employed.

For the study of *aërobic thermophiles* a dextrose brom-cresol-purple yeast agar medium employed by Cameron (1930) was used. In testing for thermophiles producing hydrogen sulphide, the deep yeast agar tubes containing sodium sulphite and ferric chloride, recommended by Cameron (1928), were used.

The medium for *halophiles* consisted of peptic digest agar containing 10 per cent salt.

Both *mesophile* and *thermophile anaërobe* spore counts were made by the dilution method in a medium consisting of ground beef heart meat particles to which peptic digest broth had been added. After the tubes had been inoculated they were stratified with vaseline and heated at 80° C for a half-hour. The tubes representing tests for anaërobic thermophiles were treated in the same manner except that they were stratified with molten agar which was allowed to solidify prior to incubation.

In order to acquire a typical cross-section of the general flora in relation to the canning procedures throughout the industry, and in order that the results obtained in one plant might be compared with those obtained in another, three canneries in the San Pedro district were chosen for the investigation.

Progressive pollution as indicated by Standard Methods Agar count.—The results of quantitative determinations on Standard Methods Agar are presented by canneries and the averages derived therefrom are shown in table 1. The counts derived from selective media are also shown in the table. The computations by canneries are as follows:

Organisms per gram sample

Fish carts:	
Plant A	1,700,000,000
Plant B	300,000,000
Plant C	100,000,000
Surface slime on fish before washing:	
Plant A	400,000,000
Plant B	54,000,000
Plant C	1,480,000,000
Surface skin after washing:	
Plant A	400,000,000
Plant B	1,100,000
Plant C	50,000,000
Body cavity after washing:	
Plant A	9,000,000
Plant B	272,000,000
Plant C	40,000,000

Body cavity of fish after butchering and before washing:	
Plant A	6,000,000
Plant B	10,000,000
Plant C	1,000,000
Dorsal flesh:	
Plant A	None
Plant B	200
Plant C	None

As previously stated, samples in connection with the precooking procedure were taken at two stages: immediately after having been precooked, and after cooling. It was the practice to take the after-cooling set just before the fish were cleaned. Cooling time ranged from twelve to sixteen hours. Samples which were taken immediately after the precooking operation yielded sterile cultures for every sample. The results of those determinations, made on samples taken after the fish had been allowed to cool, are as follows:

Organisms per gram sample

Surface of fish after cooling:	
Plant A	100,000
Plant B	10,000
Plant C	55,000
Dorsal flesh:	
Plant A	38,000
Plant B	56,000
Plant C	1,800
Flesh from body cavity:	
Plant A	2,000
Plant B	10,000
Plant C	12,000
Tuna baskets:	
Plant A	200
Plant B	10,000
Plant C	500,000

The results of examination of samples taken during the packing procedure are:

Cleaned flesh ready to can:	
Plant A	3,400,000
Plant B	1,000,000
Plant C	39,000,000
Can washings:	
Plant A	20,000
Plant B	10,000
Plant C	2,000
Packing tables:	
Plant A	150,000,000
Plant B	550,000,000
Plant C	3,360,000,000

Samples of cleaned flesh taken just prior to canning and samples removed from the packing tables at hourly intervals in the course of the packing day revealed no particular increase in numbers.

The examination of fifty samples of cottonseed oil and olive oil collected from the various canneries proved negative for every sample.

TABLE 1

A GENERAL AVERAGE OF ORGANISMS ISOLATED FROM CANNING OPERATIONS

Culture source	Organisms per gram of material				
	Standard Agar	Thermophiles	Halophiles	Anaërobic spores growing at 37.5° C	Anaërobic spores growing at 55° C
BUTCHERING OPERATION					
Fish carts	700,000,000	200	1,920,000	10	100
Surface slime on fish before washing	644,700,000	30	30,000	40*	10
Body cavity of fish before washing	5,700,000	10	10,000	10	Negative
Surface skin after washing	150,400,000	Negative	14,000	Negative	Negative
Body cavity after washing	107,000,000	Negative	10,000	Negative	Negative
Dorsal flesh	66	Negative	Negative	Negative	Negative
PRECOOKING OPERATION					
Surface of fish immediately after precook	Negative	Negative	Negative	Negative	Negative
Surface of fish after precook and after cooling	55,000	Negative	8,000	10	Negative
Dorsal flesh immediately after precook	Negative	Negative	Negative	Negative	Negative
Dorsal flesh after precook and after cooling	31,900	Negative	14,000	Negative	Negative
Body cavity immediately after precook	Negative	Negative	Negative	Negative	Negative
Body cavity after precook and after cooling	8,000	Negative	Negative	Negative	Negative
Baskets	107,060	Negative	Negative	10	Negative
CANNING OPERATION					
Cleaned fish ready for canning	14,460,000	6	1,035,000	10*	Negative
Packing tables	1,353,000,000	120	11,900,000	100	Negative
Can washings	10,730	Negative	Negative	Negative	Negative
Cottonseed and olive oil	.....	....	.....	Negative	Negative

* Type A toxin of Cl. botulinum demonstrated.