# BACTERIAL DECOMPOSITION OF SALMON[1]

ALBERT C. HUNTER

*From the Microbiological Laboratory[2] of the Bureau of Chemistry, United States Department of Agriculture*

Received for publication December 30, 1919

A study of the decomposition of the food fishes presents an interesting field for both the chemist and the bacteriologist. While the chemistry of fish decomposition has been investigated to some extent, comparatively little has been reported regarding the bacteriology of the problem. Browne (1917) reported the results obtained from his work on the decomposition of various fish during storage in ice stating that autolysis, rather than bacterial action, seems to play the most important part in the initial stages of decomposition. The part played by bacteria in the decomposition of sardines has been studied and reported by Obst (1919). The bacteriology of canned or preserved fish has received the attention of several workers. The question of whether or not the *Bacterium coli* is an inhabitant of the intestines of fish has been investigated by Browne, (1917), Eyre (1904), Houston (1903–04), Amyot (1901), and others but the papers of Browne and of Obst already mentioned seem to be the only studies made of the part played by bacteria in the actual decomposition of the flesh of fish before preserving.

The examination of a large amount of canned salmon at the Bureau of Chemistry during the past year has led to an extensive study of the raw fish from the time it is caught until it is put into the cans. The object of the investigation, from the bacteriological viewpoint, was to determine whether or not bacteria

[1] Presented before American Society of Bacteriologists, December 30, 1919.

[2] Published by permission of the Secretary of Agriculture. In carrying on this investigation valuable criticism and suggestions have been given by Dr. Charles Thom of this laboratory.

were responsible for the decomposition of the salmon and, if such was found to be the case, to establish a correlation between the physical appearance of the fish and the presence of the bacteria after holding the salmon for known lengths of time under known conditions. This necessitated the determination of total counts, and later of the kinds of bacteria, present in the muscular tissue of the salmon and also an investigation as to how these bacteria gain access to the flesh.

Four species of salmon were used in the investigation, namely, the sockeye or red salmon (*Oncorhynchus nerka*), the humpback or pink salmon (*O. gorbuscha*), the silver or coho salmon (*O. kisutch*) and the chum or dog salmon (*O. keta*). All the fish used were caught in fish traps near the San Juan Islands in Puget Sound. The fish were handled as they are handled commercially by the fishermen in that locality and accurate data were kept as to the methods of handling, the length of time out of water, the temperature at which they were held, etc.

The method of procedure was usually as follows: A trip was made to the trap late in the afternoon and the night was spent on the fishing boat alongside the trap. Early the next morning the trap was lifted and emptied of fish. One or two fish were selected for immediate examination and the others were placed in the hold of the boat to be transported to the fish house. On arrival at the fish house the desired number of salmon were placed in a large box in a corner of the fish house. A thermograph was kept with the fish throughout the whole period. Each morning one fish was removed from the box and examined. The general appearance of the fish was noted and recorded. When the odor and appearance of the salmon indicated that the fish were in an advanced stage of decomposition the experiment was terminated and a new experiment begun. Whenever it was found impossible to visit the trap on the fishing boat the fish were received at the fish house and the data in regard to the time and place of the catch were obtained from the fisherman. The fish usually arrived about eight hours after they were taken from the water. In the case of sockeye and humpback salmon three separate catches were allowed to decompose and were studied. The first

lot in each case was a preliminary experiment and the bacteriological examination was not usually conducted on more than the first one or two days. Only one lot of silver and chum salmon was examined. The temperature on the days on which the fish were held never fell below 50° F. nor rose above 70° F.

In the bacteriological examination of the fish total counts of bacteria were made from the muscle tissue of the back and belly of the fish and agar slant cultures were made from various parts and organs of the fish including the mouth, gills, stomach, ceca, intestines, heart, liver and kidney. Cultures in lactose broth fermentation tubes were made from the stomach, ceca, and intestines.

Since the work was necessarily often done in the field at considerable distances from the laboratory, some difficulty was experienced in the plating of the muscular tissue for total count but the technic used, in general, worked very well. The body of the fish was thoroughly washed with alcohol and the alcohol burned off. With instruments sterilized by flaming in alcohol a small flap of skin just posterior to the dorsal fin was carefully lifted and pinned back. A piece of muscle weighing approximately one gram was transferred to a sterile flask of known weight. The flasks used were of thick-walled, heavy glass in order that they might not break under the vigorous shaking necessary to break up the tissue. Known amounts of sterile, broken glass and sterile NaCl solution were added and the whole vigorously shaken until the tissue was thoroughly broken up. This suspension of tissue was diluted and plated according to the usual methods. The flask containing the remaining suspension was tightly stoppered and saved until the laboratory could be reached when it was weighed and the exact amount of original tissue computed. The sample of flesh from the belly was taken in the same manner just posterior to the ventral fin. Glucose agar was used and all incubations were made at room temperature.

The results of the experiments in determining total count are given in tables 1, 2, 3, and 4.

TABLE 1

| LENGTH OF TIME OUT OF WATER | PART OF FISH EXAMINED | TOTAL COUNT OF BACTERIA FROM FLESH OF SOCKEYE SALMON | | |
|---|---|---|---|---|
| | | First series | Second series | Third series |
| *hours* | | *per gram* | *per gram* | *per gram* |
| Within 2 | Back | Sterile | Sterile | Sterile |
| | Belly | Sterile | Sterile | Sterile |
| 24 | Back | 665 | 4,000 | 1,100 |
| | Belly | 5,750 | 8,000 | 8,000 |
| 48 | Back | | 27,000 | 2,500 |
| | Belly | | 36,000 | 15,000 |
| 72 | Back | | 8,000,000 | 410,000 |
| | Belly | | 12,750,000 | 920,000 |
| 96 | Back | | 50,000,000 | 900,000 |
| | Belly | | 155,000,000 | 6,400,000 |

TABLE 2

| LENGTH OF TIME OUT OF WATER | PART OF FISH EXAMINED | TOTAL COUNT OF BACTERIA FROM FLESH OF HUMPBACK SALMON | |
|---|---|---|---|
| | | First* series | Second† series |
| *hours* | | *per gram* | *per gram* |
| Within 2 | Back | Sterile | Sterile |
| | Belly | Sterile | Sterile |
| 24 | Back | Sterile | 120 |
| | Belly | Sterile | 1,420 |
| 48 | Back | 2,000 | 1,750 |
| | Belly | 37,000 | 1,700 |
| 72 | Back | 7,000 | 660,000 |
| | Belly | 50,000 | 1,600,000 |
| 96 | Back | 15,000 | 3,100,000 |
| | Belly | 60,000 | 3,500,000 |

* This lot of fish was thoroughly washed on arrival at the fish house. All blood and slime was removed.

† This lot of fish was left unwashed.

TABLE 3

| LENGTH OF TIME OUT OF WATER | PART OF FISH EXAMINED | TOTAL COUNTS OF BACTERIA FROM FLESH OF SILVER SALMON | |
|---|---|---|---|
| | | Washed fish | Unwashed fish |
| *hours* | | *per gram* | *per gram* |
| Within 2 | Back | Sterile | Sterile |
| | Belly | Sterile | Sterile |
| 24 | Back | Sterile | 4,700 |
| | Belly | 200 | 71,000 |
| 48 | Back | 5,000 | 250,000 |
| | Belly | 6,500 | 480,000 |
| 72 | Back | 220,000 | 470,000 |
| | Belly | 2,500,000 | 2,200,000 |
| 96 | Back | 510,000 | 4,400,000 |
| | Belly | 2,800,000 | 11,600,000 |

TABLE 4

| LENGTH OF TIME OUT OF WATER | PART OF FISH EXAMINED | TOTAL COUNTS OF BACTERIA FROM FLESH OF CHUM SALMON | |
|---|---|---|---|
| | | Washed fish | Unwashed fish |
| *hours* | | *per gram* | *per gram* |
| Within 2 | Back | Sterile | Sterile |
| | Belly | Sterile | Sterile |
| 24 | Back | 860 | 750 |
| | Belly | 2,100 | 18,000 |
| 48 | Back | 2,600 | 2,200 |
| | Belly | 130,000 | 28,000 |
| 72 | Back | 480,000 | 340,000 |
| | Belly | 500,000 | 2,300,000 |
| 96 | Back | 620,000 | 1,250,000 |
| | Belly | 1,180,000 | 3,410,000 |

Cultures from the mouth and gills of the fish were taken by simply inserting a sterile loop into these parts and then smearing the adhering mucus over the agar slant. After the material from the back and belly of the fish had been collected and the cultures from the mouth taken, the body cavity was carefully opened and the body wall cut in such a way that it might be pinned back, exposing the viscera. Organs from which cultures were to be taken were seared with a hot instrument and then cut slightly with sterile scissors. The sterile loop was inserted through the small opening and some of the blood and mucus transferred to the agar slant.

Since all the salmon examined were caught during their spawning migration, there was never any food found in the stomach. On rare occasions a small amount of partly digested food would be found in the intestine but for the most part the whole digestive tract appeared to contain nothing but mucus.

In handling one lot of humpback salmon the fish were washed thoroughly with running water, cleaning the bodies entirely of blood and slime. It was noticed that these salmon did not decompose as rapidly as had previous lots and in the subsequent experiments particular attention was given to the effect of washing fish as soon as they were brought ashore.

Examination of the mouths and gills of 41 salmon of various species has shown that microorganisms are always present even when the fish are examined immediately on being taken from the trap. Yeasts, bacilli of various kinds and cocci were usually found in large numbers.

Examination of the stomachs of 36 salmon has shown that no microorganisms are present in this organ during the first twenty-four hours, provided there is no feed in the stomach. In 3 out of 6 lots of fish examined, bacteria were found in the stomach after the fish had been held forty-eight hours and in one other lot the bacteria were recovered from the stomach after holding seventy-two hours.

In examining the ceca of salmon from 7 different lots, bacteria were found in but one lot when the fish were examined immediately on being caught. One lot showed bacteria in the ceca after

twenty-four hours, while in four other lots bacteria were not recovered from this part of the digestive tract until the salmon had been held seventy-two hours.

Living bacteria were found in the intestine of a salmon from one lot immediately on being caught. The intestine in this case contained some partly digested food. In 3 other lots bacteria were not found in the intestine until the fish had been out of water forty-eight, seventy-two and ninety-six hours respectively. In one lot no bacteria were found after the fish had been held for ninety-six hours.

In examining the heart's blood of 4 lots of salmon no bacteria were obtained when the fish was first caught. In 2 lots living bacteria were found in the heart's blood after twenty-four hours, in one lot after forty-eight hours and in the other lot after the fish had been held seventy-two hours.

Blood in the large vessels among the viscera was examined and no bacteria were found in the blood stream when the fish were fresh. In one lot bacteria were obtained from the blood stream after twenty-four hours and in the other lots they were found after forty-eight hours. It was noted that even at the end of ninety-six hours the blood in these vessels had not coagulated.

The kidneys of 6 lots of salmon were examined and in 3 lots no bacteria were found after ninety-six hours. In the other 3 lots living microorganisms were found after the fish had been held forty-eight hours.

The livers of 4 lots of salmon were examined and were always found to be sterile.

Direct smears from the parts examined and smears from the cultures obtained have shown that the organisms obtained from the viscera and the blood stream are at least morphologically similar to those present in the mouth and gills during the first twenty-four hours. These organisms have been isolated and are now being made the subject of a further study, the results of which will be presented in a later paper.

In regard to the physical appearance of the fish it was noted that during the first forty-eight hours, at the temperatures on Puget Sound, no marked decomposition takes place. The eyes of the fish remain bright; the gills are red with no foul odors; the

flesh is firm and sweet and the viscera remain normal in appearance. The fish at the end of the seventy-two hour period were in such a state of decomposition that they were designated as "stale." The eyes became slightly sunken, the gills dark in color and either sour or foul in odor; the digestive tract was darkened and usually rather foul-smelling. The flesh of the salmon at the end of the seventy-two hour period was usually soft with some sour or putrid odor. At the end of the ninety-six hour period the salmon were markedly decomposed and beyond the state where they should be considered as fit for food. The eyes were deeply sunken, the gills blackened and exceedingly foul smelling, the skin dry and cracked and the flesh very soft and putrid. The viscera of such fish were always very much darkened in color and very foul. As will be seen in tables 1, 2, 3 and 4, the total count of bacteria in the flesh from such fish in one case was as high as 155,000,000 per gram.

A study of the data in the tables has demonstrated several interesting facts. The muscular tissue of freshly caught salmon is sterile. In studying the intestinal contents of fishes, Eyre used pieces of flesh of the fish as controls and found them sterile. The counts obtained on the flesh from the belly are always higher than those from the flesh of the back. Since some of the bacteria in the flesh undoubtedly get there through the skin, the fact that the skin of the belly is thinner and more easily broken may help to explain this higher count. The very high counts in the flesh from both the back and belly are sufficient to explain the softening and decomposition of the tissue. In most cases the fish which were washed upon arrival at the fish house had lower total counts than those held unwashed. The washed fish did not decompose as rapidly as did the unwashed fish.

Results of the examination of the various organs of the body would seem to indicate that the sources of infection as regards these organs, and to a considerable extent as regards the muscular tissue also, are the gills and mouth. It would appear that a great many organisms make their way through the blood channels to the viscera and the muscular tissue within forty-eight to seventy-two hours after the fish has been removed from the water. Rough handling of the fish will, however, break the

skin and allow many organisms present on the surface to penetrate the flesh.

In regard to the presence of *Bacterium coli* in the digestive tract it may be briefly stated that in no case was this organism isolated from the ceca or intestines of the salmon examined.

## SUMMARY

1. The muscular tissue of freshly caught salmon is sterile.

2. After ninety-six hours at temperatures between 50°F. and 70°F. the total count of bacteria in the muscular tissue has been found to be as high as 155,000,000 per gram. The high counts obtained are sufficient to explain the decomposition of the tissue.

3. Thoroughly washing the fish on arrival at the dock results in lower total counts. The washed fish decompose less rapidly than the unwashed fish.

4. The mouths and gills of salmon contain living microorganisms of various kinds even when fresh from the water.

5. The digestive tract of salmon is sterile when there is no food present. This is in agreement with the findings of Obst in studying sardines.

6. The various organs of the body become infected through the blood vessels usually within ninety-six hours after the fish are caught.

7. Salmon out of water more than forty-eight hours at temperatures between 50°F. and 70°F. are decomposed to such an extent that they are not desirable as food.

## REFERENCES

AMYOT, J. A. 1901 Is the colon bacillus a normal inhabitant of the intestines of fishes? Rpts. and Papers of the A. P. H. A., **27**, 400.

BROWNE, W. W. 1917 The presence of the B. coli and B. welchii groups in the intestinal tract of fish (Stenomus chrysops). J. Bact. **2**, 417.

BROWNE, W. W. 1918 Do bacteria play an important part in the initial stages of decomposition of fish during storage in ice? Abst. Bact., **2**, 6.

EYRE, J. W. H. 1904 On the distribution of B. coli in nature. Lancet, **166**, 648.

HOUSTON, A. C. 1903–1904 The bacteriological examination of the intestinal contents of certain sea-fowl and of fishes. Rpt. of Med. Officer of Local Govt. Board, Great Britain, p. 528.

OBST, M. M. 1919 A bacteriologic study of sardines. J. Infect. Dis., **24**, 158.