A BACTERIOLOGICAL STUDY OF FRESH MUSSELS

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Methods of handling and shipping fresh mussels were discussed at a recent (1943) meeting of sanitarians of the U. S. Fish and Wildlife Service and the Office of Coordinator of Fisheries. With the exception of the work of Loosanoff (1943A), there had been no study to indicate the conditions under which quality could be maintained. In order to answer some of the more pertinent and practical questions, therefore, a study was made at the Service's College Park, Maryland, Laboratory, of the bacteriology of fresh mussels, in the shell, packed in different types of containers and held over a period of time.

Two types of quantitative bacteriological determinations were employed: The coliform content and the standard plate count. The former is generally considered to indicate the degree of fecal pollution, since the coliform group of bacteria is the best index available at the present time for this type of contamination. The plate count, by itself, has little significance, but when changes in count occur under various conditions, the direction of the change usually indicates alterations in the quality of the product. When the counts increase, a possibility of subsequent spoilage and decreased wholesomeness is indicated. The highest quality shellfish, from a sanitation viewpoint, are those which are entirely free of coliform bacteria and which yield low plate counts.

SOURCE OF MUSSELS: The mussels used in these experiments were from two sources; namely, Indian River, Milford, Connecticut, and Long Island Sound, about one mile from Point No Point, Connecticut (Loosanoff (1943B)). In the former area, the samples were taken from a bar 30 to 40 feet from shore. They were collected at low tide by means of a hand rake. In the latter area, a dredge was used for the collection of the specimens.

PRELIMINARY TREATMENT: After washing the mussels in river or sea water, they were brought to the laboratory and immediately packed in the experimental containers, or stored in tanks of sea water until used (Loosanoff (1942)). If the latter was done, the period of storage was never more than two days. Three types of containers were used in this study: Wooden barrels, which held two bushels of mussels apiece; splint hampers, each of which held one bushel; and splint baskets, which also held one bushel each. The barrels were of two types, regular and ventilated, the latter with one row of $\frac{1}{2}$-inch vent holes around the center and another row 6 inches from the bottom of the barrel.

Shortages of barrels required their re-use after thorough cleaning, but new baskets and hampers were used for each of the four experiments conducted. These were thoroughly scrubbed and rinsed and permitted to dry for two days before being filled with mussels.

The barrels containing mussels were kept well surrounded with ice during each experimental run. Similar treatment was given the specimens packed in the hampers

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and baskets, except that an equivalent pack was kept without icing. All the containers were stored on a screened porch and kept covered with a tarpaulin to prevent contamination by flies and to shield the samples from direct sunlight.

The total period of storage for each experimental pack was four days, the samples being removed for bacteriological examination at the end of each 24 hours. The average air temperature during the period of the study was 60.2° F.

Sampling involved the removal of sufficient mussels from each pack so that 10 closed, living specimens could be obtained for examination. Top and bottom samples were taken from the barrels. To obtain initial bacterial counts, three samples of 10 specimens each were taken from the freshly collected mussels in every instance. These were packed in metal containers, refrigerated at the time of collection, and examined at the laboratory as soon as possible.

BACTERIOLOGICAL METHODS: Prior to the opening of the mussels, the specimens were first rinsed in running cold water and thoroughly scrubbed with a stiff brush. The byssus, or beard, was then removed, and each specimen that lost shell liquor in this process was discarded.

Following this, the specimens were immersed for 10 minutes in a hypochlorite solution containing 10 p.p.m. of available chlorine, and all animals that gaped or floated at the end of this time were discarded. Upon removal of the chlorine solution, the mussels were placed upon paper towels and allowed to drain dry. Those that gaped during this process were also discarded. The specimens were then opened with a sterile knife, and the meats and liquor of 10 mussels introduced into 100 ml. of sterile phosphate buffer solution. This mixture was shaken vigorously 25 times, and the supernatant was used as a source of inoculum.

Two types of quantitative determinations were made. The first was the standard plate count using nutrient agar. The plates were poured in triplicate at each of three dilutions, and the bacterial count per animal was determined from the plates having between 30 and 300 colonies after 48 hours incubation at 37° C. The second was the coliform content determination by the use of lactose broth. Three sets of five tubes each were seeded with 10, 1.0, and 0.1 ml. portions of the inoculum, respectively. Incubation was at 37° C. for 48 hours, the tubes being examined for gas production after 24 and 48 hours. Positive presumptive tests were partially confirmed with brilliant green lactose bile (2 percent) and the confirmations were completed by the usual methods (Standard Methods of Water Analysis, 1936). Hoskins' (1939) tables were employed for determining the Most Probable Number (M.P.N.) of coliform bacteria.

GENERAL OBSERVATIONS: Although no records were kept to show the incidence of weak and gaping mussels under the conditions of storage, certain general observations were made. These are listed below to show the changes which occurred in the various containers.

24 hours: The mussels at the top of all containers were comparatively dry. Those in the bottoms of the barrels were moist, but this may have been due to water from the preliminary rinsing, since no open or gaping mussels were observed.

48 hours: Some of the mussels showed signs of weakness as evidenced by the ease of opening. The un-iced mussels in the hampers and baskets showed a slight loss of shell liquor, and those in the bottom of the barrels were more moist than on the previous day.

72 hours: The mussels in all of the containers showed a loss of shell liquor. There were open and gaping animals in all splint containers and throughout the barrels. There seemed to be less liquor loss among those in the bottoms, both of the barrels and the iced containers, than in the tops of the barrels and un-iced containers.

96 hours: The incidence of open and gaping mussels was increased over that of the previous day. The amount of shell liquor was less in all specimens as noted in sampling, the un-iced specimens containing the least amount of liquor. Those mussels at the bottom of the barrels were very wet, and when the barrels had been emptied, two to three quarts of liquid remained in the bottom of each.

BACTERIOLOGICAL FINDINGS: The bacteriological data are summarized in Table 1. Initially, all of the specimens showed plate counts well under 100 per mussel, the

Table 1 - Standard Plate Count and M. P. N. of Coliform Bacteria per Mussel During Storage in Various Containers

Type of Container	Conditions of Storage	Average Standard Plate Count Bacteria per Mussel — Hours Stored					Average M. P. N. of Coliform Bacteria per Mussel — Hours Stored				
		0*	24	48	72	96	0**	24	48	72	96
Splint Basket	Iced	61	79	69	110	248	189	162	166	217	251
Splint Basket	No ice	61	61	73	122	130	189	157	215	267	583
Splint Hamper	Iced	61	174	150	450	399	189	275	356	234	837
Splint Hamper	No ice	61	98	80	129	152	189	162	329	587	737
	All iced										
Barrel, top	Not ventilated	61	378	546	474	603	189	306	376	794	905
Barrel, bottom	Not ventilated	61	244	234	509	697	189	603	800	1310	1347
Barrel, top	Ventilated	61	180	285	661	516	189	160	360	1227	1190
Barrel, bottom	Ventilated	61	124	198	651	757	189	215	619	1109	1763

*The range of initial standard plate counts per mussel was from 10 to 96, averaging 61.
**The range of initial Most Probable Number of coliform bacteria per mussel was from 10 to 413, averaging 189.

highest being 96, the lowest 10, and the average of all samples, 61. The coliform scores were of the same order of magnitude, with the exception of the last batch procured. This group had been collected immediately after a severe storm had disrupted the mussel beds. The M.P.N. of coliform bacteria per animal, immediately after the storm, was 413, but the average of all of the other samples was 48. The lowest initial coliform score was 10, and the average for all samples, including those taken after the storm, was 189 per animal.

In the case of the splint containers, most of the iced samples yielded consistently higher standard plate counts than did the un-iced. On the other hand, the coliform score was generally lower in the case of the iced specimens in splint containers. In both cases there appeared to be a marked increase in the plate count after 72 hours of storage, regardless of the storage temperature. The coliform content increased appreciably after 48 hours of storage in the majority of instances.

Most of the plate counts and coliform scores were higher for the mussels in the barrels than in the smaller containers. The final bacterial content of the mussels at the bottom of the barrels was greater than at the top, and the ventilation had no apparent effect. Ventilation also failed to influence the bacterial content of the mussels at the top of the barrel, the coliform scores being greater in the ventilated container than in the unventilated one after 72 hours of storage.

DISCUSSION: Loosanoff (1943A) has studied the viability of mussels at various temperatures: When mortality is used as an index of keeping quality, his data indicate that at 30° F. mussels may be kept 30 days; at 40° F., 12 days; at 50° F., 8 days; at 60° F., 6 days; and at 70° F., 4 days. From a study of the bacteriological findings of the experiments reported herein, however, three days seem to be the longest safe period of storage, regardless of temperature.

The present studies clearly show that packing in small containers yields a better product from a practical bacteriological standpoint. It is further indicated that coliform scores can best be kept low by restricting the storage period to 48 hours.

Observations of the commercial practices indicate that the difficulties which have been experienced with this shellfish are probably due to two factors. The first is storage for too long a period, the time between harvesting and marketing usually being three to four days. (This probably does not occur in the canning industry, where the fresh mussels are not stored for any considerable time.) The second factor is the failure to exercise ordinary care with regard to general sanitation and cleanliness in permitting exposure to filth, sunshine, etc. Contamination and a period of incubation will unfailingly result in mussels of poor quality bacteriologically.

With reasonable care in handling, and prompt delivery to the consumer, mussels are a wholesome source of food.

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