A STUDY OF THE MICROBIOLOGICAL QUALITY OF HADDOCK FILLETS AND SHUCKED, SOFT-SHELLED CLAMS PROCESSED AND MARKETED IN THE GREATER BOSTON AREA[1]

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SUMMARY

Data obtained during the months of July and August of 1961 on the microbiological quality of haddock fillets and shucked, soft-shelled clams processed and marketed in the greater Boston area have been compiled and critically evaluated for commercial and sanitary significance. Differences were observed in the aerobic-facultative microbial counts of haddock fillets processed at different times of day in a given company. Soft-shelled clams dug from unpolluted beds in the vicinity of Boston, when shucked in a commercial plant, were found to have counts which were about one hundred times lower than clams shipped in from the Maryland area and subsequently shucked in the local marketing area. This points out the desirability of processing shellfish (by radiation treatment or otherwise) at a point close to the source of supply. Anaerobic (clostridia) counts were somewhat higher in shucked clams than in haddock fillets although high counts (clostridia) were not encountered with either product.

As part of a comprehensive program of research being conducted by the Department of Nutrition and Food Science of the Massachusetts Institute of Technology, Cambridge, Massachusetts, for the advancement of the fisheries industries of the northeastern Atlantic states, a study was initiated to evaluate the microbiological quality of haddock fillets and shucked, soft-shelled clams processed and marketed in the greater Boston area. The objective of this study was two-fold. First, to obtain a current, representative picture of the levels of microbial populations in these commercially important sea foods at the point of processing and distribution for a variety of recommendational purposes; and, second, to obtain the best possible sources of supply of these sea foods for a program of radiation-pasteurization intended to indicate the feasibility of expanding the inland markets for these sea foods by significantly extending their storage life at refrigeration temperatures above freezing. The sanitation implications of the findings from this survey are discussed in this paper.

METHODS

Sampling Procedure

Haddock Fillets. Three representative haddock fillets were taken from the end of the processing line in each of three plants three times daily. The fillets were placed in clean polyethylene film envelopes which were carefully sealed and placed in crushed ice in an insulated container for transportation back to the laboratory. The three plants participating in the survey were typical of large-, medium-, and small-scale fish-filleting establishments in the greater Boston area.

Shucked, Soft-Shelled Clams. Half-pint samples of shucked, soft-shelled clams, packaged in plastic-lined cardboard containers, were obtained each day from three clam-shucking establishments in the general vicinity of Boston. These samples were drawn from the shucking line three times daily, and the containers placed in polyethylene film envelopes which were sealed and packed in ice for storage until called for the following morning. The concerns selected for the survey were typical of shucking establishments in this general area.

Sample Preparation

Haddock Fillets. The sealed polyethylene film envelopes containing the three haddock fillets were carefully opened and the fillets placed on sterile aluminum foil sheets. Sections were aseptically cut with sterile surgical scissors from the three fillets, and weighed out to a total of 50 g in the covers of sterile, refrigerated, stainless steel Oster Blendor cups. These sections were then aseptically transferred into the body of the blendor cup. The cover and cutting-transfer utensils were rinsed three times with sterile, refrigerated diluent into the body of the blendor cup. A total of 450 ml of diluent was added to the 50 g of diced haddock fillets.

Shucked, Soft-Shelled Clams. The containers of clams were removed from the polyethylene envelopes, and the contents of each (clams and liquor) thoroughly mixed with sterile spoons. Fifty grams of clams plus liquor were aseptically weighed out in blendor cup covers, as previously described, and the contents transferred and rinsed three times into the body of the cups with sterile, refrigerated diluent. A total of 450 ml of diluent was added to the 50 g of clams and liquor.

Both haddock fillet and shucked, soft-shelled clam

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samples were blended for three minutes at 15,000 rpm, and then allowed to stand for ten minutes at 5 C to permit the foam to break and the emulsion to become more homogeneous in consistency.

Aerobic-Facultative Pour Plate Procedure

1. Ten ml of the blended, emulsified samples were aseptically pipetted from the blendor cups into 90-ml refrigerated dilution blanks; and, thereafter, diluted further for plating. Three decimal dilutions were employed for both haddock fillets and shucked, soft-shelled clams to insure obtaining plates which would yield statistically desirable microbial colony populations. Generally, 10^3, 10^4 and 10^5 dilutions were used for haddock fillets, and 10^4, 10^5 and 10^6 dilutions for shucked clams. These three sample dilutions were plated out in triplicate.

2. Approximately 10 ml of agar Medium B (DW)[1], tempered to 45 C, was aseptically poured into the Petri dishes containing the sample inocula, and thoroughly mixed. This medium was determined, by a previous investigation *(4)*, to yield maximum recovery of microorganisms from both sea foods.

3. The cooled, solidified cultures were incubated for five days at 20 C and the macrocolonies which developed were counted and tabulated.

Anaerobic (clostridia) Tube Preparation

1. One ml of the 10^2 dilution of both haddock fillet and shucked, soft-shelled clam samples were aseptically transferred, in duplicate, into sterile oval culture tubes (Corning No. 9200) containing sterile solutions of sodium sulfite, Polymixin B sulfate, and sodium sulfadiazine in the concentrations recommended by Angelotti and Hall *(2)*.

2. Seven ml of the basal SPS agar[2], tempered to 45 C, was immediately aseptically pipetted into the tubes; mixing the inocula, reagents, and agar thoroughly. The tubes were then quickly placed in a 20 C water bath to rapidly solidify the agar mixture.

3. Following solidification, 5 ml of BBL thioglycollate supplement plus 1.5% agar was aseptically pipetted over the first agar to help establish and maintain anaerobic conditions in that medium.

4. The cooled, solidified tubes were incubated for 40 hours at 30 C, and the black macrocolony zones that developed were counted and tabulated (presumptive count for clostridia).

5. Confirmation of the black colonies as clostridia was accompished by removing the agar from the tubes, picking the black colonies, performing morphological tests to determine shape and spore formation and analyzing for the presence of catalase.

RESULTS AND DISCUSSION

Data from the microbiological survey (aerobic and anaerobic clostridia counts) of products from haddock and clam processing plants were analyzed statistically by the analysis of variance method (three periods considered, AM, M, PM) and by Duncan's multiple range test (two periods considered, AM vs. M, AM vs. PM, M vs. PM).

Aerobic-Facultative Macrocolony Counts

Haddock-Fillets. It is a well known fact that prior to filleting only small numbers of bacteria, if any, are present in the flesh of fish such as haddock *(3, 5)*. Contamination of such products with bacteria occurs, therefore, mostly during filleting and subsequent handling prior to packaging.

An examination of Tables 1 and 3 indicates that in any of the three plants A, B or C the average contamination of fillets with aerobic-facultative bacteria at any time of day would provide counts near 400,000 per g or higher. This may be contrasted with the Canadian requirement of 250,000 per g for freshly frozen fillets and the statement that in inspected plants in Canada average contamination of freshly frozen fillets has been lowered to about 70,000 per g *(1)*.

Statistical analyses of aerobic-facultative counts on products from haddock filleting plants indicated that in plants A and B there was a gradual build-up of contamination on equipment, subsequently transferred to the product, as the day progressed. This eventually resulted in significantly higher counts in product produced in the afternoon as compared to

[1]*Medium B (DW)*

Ingredients	*g per liter*
BBL Trypticase	15.0
BBL Phytone	5.0
Bacto-Yeast Extract	5.0
NaCl	4.0
Na_2SO_3	0.2
1-Cystine	0.7
Bacto-Dextrose	5.5
Bacto-Agar	15.0
Distilled Water	1.0 liter

Final pH 7.0 ± 0.1

[2]*Medium SPS*

Ingredients	*g per liter*
Bacto-tryptone	15.0
Bacto-Yeast Extract	10.0
Iron Citrate	0.5
Bacto-Agar	15.0

Final pH 7.0 ± 0.1

Add the following millipore-filtered solutions:

	Final concentration in medium
Fresh Sodium Sulfite	0.05%
Polymixin B Sulfate	10 PPM
Sodium Sulfadiazine	0.12 mg per ml sodium

TABLE 1. AEROBIC-FACULTATIVE MACROCOLONY COUNTS FROM HADDOCK FILLET AND SHUCKED, SOFT-SHELLED CLAM SAMPLES OBTAINED FROM COMMERCIAL PROCESSING ESTABLISHMENTS IN THE GREATER BOSTON AREA (INCUBATION—5 DAYS AT 20 C —AVERAGE OF THREE REPLICATE PLATES-AGAR B(DW)

Haddock fillets (X 10^{-4}/gram)									Shucked, soft-shelled clams (X 10^{-5}/gram)								
Company A			Company B			Company C			Company D			Company E			Company F (X 10^{-3}/gram)		
AM	12N	PM	AM	12N	PM	AM	12N	PM	AM	12N	PM	AM	12N	PM	AM	12N	PM
20	13	200	10	18	4	38	28	22	59	28	65	55	10	5	49	25	22
9	19	24	4	5	36	18	13	130	39	47	9	47	30	55	55	35	13
41	110	74	40	43	160	420	61	190	15	13	16	31	4	7	79	110	40
53	24	24	77	16	67	430	83	61	31	13	27	16	8	1	80	74	37
18	17	200	17	48	44	58	220	90	37	55	250	5	42	64	8	210	35
26	60	21	39	38	13	150	120	76	34	59	210	24	10	11	39	82	120
73	53	140	36	88	100	330	63	270	27	98	38	26	14	11	54	170	78
15	56	210	46	33	77	100	34	150	9	35	43	11	90	110	92	24	81
24	32	20	21	68	2	48	33	45	65	38	12	3	10	6	43	11	42
110	69	3	15	73	37	120	72	69	32	46	9	45	130	110	64	100	51
27	21	200	45	21	53	200	140	10	52	25	25	83	170	230	71	26	29
79	36	27	22	14	53	150	83	170	26	13	200	56	69	74	59	56	74
31	58	87	44	55	140	74	110	170	8	22	27	50	43	41	47	10	53
43	57	18	27	35	29	34	26	34	52	38	65	3	80	160	38	140	150
23	42	370	45	29	70	320	53	90	23	9	16	84	14	43	50	26	44
90	33	200	35	36	45	170	35	120	43	43	46	86	11	6	41	110	22
25	50	67	40	23	67	190	40	78	12	25	12	28	22	15	69	36	55
54	26	28	30	73	20	130	330	270	28	9	12	34	7	5	11	17	13
46	41	44	30	40	37	360	32	36	23	14	25	11	5	4	16	16	32
23	23	58	35	35	59	98	53	88	65	35	7	15	24	6	64	42	43
62	100	120	80	52	57	81	62	140	39	16	27	32	6	5	39	48	38
40	98	64	82	36	60	60	70	280	10	14	180	11	6	6	73	45	47
20	21	26	41	45	100	220	97	38	11	32	34	6	10	25	78	20	57
21	31	21	27	50	340	44	45	69	67	12	17	10	18	10	26	69	75
27	31	44	24	28	170	71	100	120	9	30	10	44	7	97	70	21	19
55	34	43	49	16	41	140	45	77	71	6	26	10	9	7	68	83	76
39	79	54	52	33	42	59	94	49	5	33	7	88	21	8	120	64	76
49	21	50	62	92	74	63	110	130	7	9	86	8	59	6	110	39	20
Mean count																	
Std. deviation	40.8	44.8	87.0	38.4	40.8	71.3	149.1	80.4	109.7	32.1	29.2	53.6	32.9	33.2	40.3	57.6	61.0	51.5 = M
95% confi-	24.4	25.8	86.9	19.6	22.1	67.1	119.7	65.3	74.4	27.0	41.1	289.7	26.4	50.3	31.6	26.3	50.1	31.6
dence limits	9.4	10.0	32.6	7.6	8.6	26.2	46.3	25.2	28.7	10.4	20.1	112.0	10.2	19.5	12.3	10.6	20.5	12.9

TABLE 2. MACROCOLONY COUNTS OF CLOSTRIDIA FROM HADDOCK FILLET AND SHUCKED, SOFT-SHELLED CLAM SAMPLES OBTAINED FROM COMMERCIAL PROCESSING ESTABLISHMENTS IN THE GREATER BOSTON AREA (INCUBATION-40 HOURS AT 30 C — AVERAGE OF DUPLICATE ANAEROBIC TUBES-SPS AGAR)

	Haddock fillets (X 10^{-2} per gram)									Shucked, soft-shelled clams (X 10^{-2} per gram)								
	Company A			Company B			Company C			Company D			Company E			Company F		
	AM	12N	PM	AM	12N	PM	AM	12N	PM	AM	12N	PM	AM	12N	PM	AM	12N	PM
	1	0	1	0	0	1	1	1	0	4	0	4	2	3	2	0	1	0
	3	4	3	0	0	0	1	1	1	0	5	2	0	1	0	2	1	1
	3	3	3	2	0	0	0	0	1	0	3	3	0	1	1	1	1	1
	3	2	2	1	1	0	1	2	0	5	4	1	1	0	0	2	3	0
	3	2	4	0	0	0	0	4	0	6	1	0	0	1	1	1	1	1
	1	2	1	1	1	0	1	0	0	4	6	1	0	1	0	1	1	2
	2	1	1	1	0	1	0	2	0	2	4	2	2	0	1	1	1	0
	0	0	1	1	0	1	1	1	0	3	2	2	3	0	0	0	2	1
	1	0	0	0	1	0	1	0	0	0	2	1	0	0	2	2	1	0
	1	2	0	0	0	3	2	0	1	0	7	4	2	2	3	1	0	0
	1	0	1	0	0	1	1	0	1	0	0	2	2	4	7	0	1	0
	0	1	3	0	1	0	1	0	1	2	1	1	2	2	2	3	0	0
	1	1	2	0	1	0	3	0	3	3	1	2	2	0	0	0	3	2
	4	2	0	0	0	1	0	1	1	1	0	4	6	2	0	2	0	0
	1	1	2	1	3	2	3	2	3	7	3	1	3	1	1	2	0	1
	1	3	1	0	1	0	2	0	0	2	1	1	0	0	2	0	2	1
	1	0	1	0	2	0	1	0	1	2	3	1	4	0	0	0	2	1
	1	1	1	0	0	0	3	0	3	1	2	3	2	2	0	2	0	0
	1	0	0	0	1	0	1	1	4	2	1	1	2	2	1	0	1	3
	0	0	2	0	1	0	0	1	2	1	0	1	1	3	3	2	1	2
	2	2	2	0	0	1	2	2	3	2	1	0	2	0	0	1	2	1
	0	2	3	0	1	0	3	2	0	3	1	0	1	0	1	1	0	1
	0	1	1	0	0	0	1	0	1	3	0	1	2	0	0	2	0	0
	0	1	0	1	1	2	1	1	2	8	4	3	0	6	4	0	2	2
	0	0	2	2	7	0	0	1	0	7	8	3	5	2	2	2	2	0
	2	0	2	1	6	5	2	3	5	2	1	1	4	2	4	1	2	3
	3	2	5	0	3	4	1	1	0	2	0	1	6	5	1	1	1	1
	1	1	2	1	1	0	0	2	0	1	0	0	4	1	2	2	0	3
Mean count	1.32	1.21	1.64	0.43	1.14	0.79	1.18	1.00	1.18	2.60	2.18	1.64	2.07	1.46	1.73	1.14	1.10	0.96 = $\bar{x}$

TABLE 3. SUMMARY OF RESULTS OF STATISTICAL ANALYSES — AEROBIC COUNTS ON HADDOCK AND CLAMS — WITHIN PLANT

Plant	Product	Period compared	Value of F	Level of significance[a]
A	haddock	AM, M, PM	27.3	1%
"	"	AM vs. M	—	>5%
"	"	AM vs. PM	—	1%
"	"	M vs. PM	—	1%
B	haddock	AM, M, PM	5.3	1%
"	"	AM vs. M	—	>5%
"	"	AM vs. PM	—	1%
"	"	M vs. PM	—	1%
C	haddock	AM, M, PM	4.1	5%
"	"	AM vs. M	—	1%
"	"	AM vs. PM	—	>5%
"	"	M vs. PM	—	>5%
D	clams	AM, M, PM	2.7	>5%
E	clams	AM, M, PM	0.27	>5%
F	clams	AM, M, PM	0.46	>5%

[a]When three periods compared (AM, M, PM) analyses made by analysis of variance. When two periods compared Duncan's multiple range test used.

TABLE 4. SUMMARY OF RESULTS OF STATISTICAL ANALYSES — ANAEROBIC (CLOSTRIDIA) COUNTS ON HADDOCK AND CLAMS WITHIN PLANT

Plant	Product	Period compared	Value of F	Level of significance[a]
A	haddock	AM, M, PM	0.92	>5%
B	haddock	AM, M, PM	2.6	>5%
C	haddock	AM, M, PM	0.31	>5%
D	clams	AM, M, PM	1.5	>5%
E	clams	AM, M, PM	1.4	>5%
F	clams	AM, M, PM	0.64	>5%

product filleted prior to the luncheon recess. The implication is that in these plants the noonday cleanup was inadequate, allowing bacterial contamination to build up on equipment especially during afternoon periods. In plant C the highest aerobic-facultative counts were obtained in the morning, the counts gradually diminishing to the noontime period and building up again in the afternoon. Obviously the night-time cleanup in this plant was totally inadequate, resulting in highly contaminated equipment when production was started in the morning. As the day wore on the equipment was actually being cleaned by the product until noontime. Thereafter bacterial increases on equipment again caught up, apparently because of poor noontime cleanup, and the counts on the product again increased.

No attempt has been made to campare the level of aerobic-facultative counts obtained on products produced in different haddock filleting plants. However, the 95% confidence limits have been listed for these counts and some evaluation of such differences may be made from these data.

Shucked, Soft-Shelled Clams. Tables 1 and 3 show that there were no significant differences between aerobic-facultative counts made on clams at any period of the day (AM, M, PM) in any particular plant (D, E and F). This is to be expected since the only implement used for removing clams from the shell is a small knife and the product is shucked directly into the final container. Plant sanitation did not, therefore, provide a problem in any of the three plants.

When aerobic-facultative counts of shucked clams produced in the three plants are compared, one plant to another (see Table 1), there is an obvious highly significant difference between the count on product produced in plants D and E (2,900,000 to 5,400,000 per g) and product produced in plant F (52,000 to 61,000 per g). Plants D and E were shucking clams transported from Maryland, in the shell, by truck. Plant F shucked only locally dug clams. Since there were no significant differences in the counts within plants, morning through afternoon, no plant sanitation problems are indicated and the obvious conclusion is that high or low counts are related to the condition of the raw material. It is possible that clams removed from Maryland waters might be more highly contaminated but it would not be expected that the aerobic-facultative count of such waters would be as high as 2.9 to 5.4 x 10^6 per g. It is probable that some increase in bacterial count is taking place during transportation of the clams in the "in the shell" condition. Regardless of where the increase in bacteria count occurs, shucked clams having the higher counts indicated can be expected to have a relatively short storage life at refrigerator temperatures above freezing. Some method of processing such as radiation-pasteurization, carried out after shucking the product near the source, should facilitate distribution of such products and provide for extended storage life at refrigerator temperatures above freezing.

Anaerobic (clostridia) Counts

Anaerobic clostridia counts and the summary of the statistical analyses of data are reported in Tables 2 and 4. No build-up of clostridial types during the day's production was indicated for any plant processing either haddock or clams. The total anaerobic clostridia counts for both products was also comparatively low.

CONCLUSIONS

1. A survey of the bacterial contamination of haddock fillets produced in three different plants has indicated the need for better cleaning and sanitizing of equipment. A better noonday cleanup was indicated as requisite in all plants.

2. Aerobic-facultative counts in shucked clams were

found to be approximately one hundred times higher in clams transported in the shell from Maryland and shucked in the Boston area as compared to clams dug locally and shucked in the Boston area.

3. Anaerobic clostridia counts made on both haddock fillets and shucked clams produced in the Boston area indicated low concentrations of organisms of this type.

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