MICROBIOLOGICAL STUDY OF SURIMI PRODUCTION: PHASE II

FINAL REPORT

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Summary

Two Alaska shore-based surimi processing plants were studied to determine the source of microorganisms, the processing steps that were responsible for increases or decreases in the microbial population, and the factors that contributed to these changes. The growth kinetics of some of the bacterial isolates were also determined. Conclusions from this study were: (1) in both plants, the bacterial number usually increased at each pollock processing step with the highest counts detected in surimi, (2) in one plant, the main bacterial constituents were Flavobacterium and Pseudomonas, followed by Moraxella, Aeromonas, Lactobacillus, Serratia and Acinetobacter, (3) in another plant, the main bacterial constituents were Flavobacterium and Arthrobacter/ Corynebacterium, followed by Pseudomonas and Acinetobacter, (4) most processing steps had operated at temperatures below 5°C, except for the refiner and the former which registered 11°C and 10°C, respectively, (5) species of Pseudomonas, Aeromonas and Serratia were capable of rapid growth at temperatures as low as 8°C, with respective generation times of 3.0, 3.1 and 3.6 hours, (6) freezing and thawing of surimi did not change the microflora composition, (7) certain bacteria characteristically associated with a plant were also found in surimi produced there, and (8) a potential source of coliforms and related bacteria was attributed to the harbor.

Materials and Methods

Alaska pollock (Theragra chalcogramma), from the Gulf of Alaska, were off-loaded from the fishholds by pneumatic pumps into either a holding tank or totes and then were transported into the plant by a conveyor. The fish were hand-sorted by size and were headed, gutted and split into fillets either by machine or by hand. The fillets were mechanically skinned, rinsed with potable water and conveyed to the mincing operation. The minced fish from either a single or a pair of mechanical mincers was washed in a series of tanks. When a pair of mincers were in operation, the minced fish was combined in a drain tank prior to washing. The washed mince was dewatered by passing through twin rotary screens. The drained mince was then refined and dehydrated via two screw presses. Cryoprotectants were added to the dehydrated mince in the mixing bowl and the surimi was formed into blocks prior to freezing.

In-line samples, each approximately 150 g, were aseptically collected from the following processing points: after mincing, from the drain tank after the minces were combined, after washing, after refining, after dehydrating, and after the addition of cryoprotectants.

Whole fish were randomly sampled from totes that were holding the unloaded fish. A swab method was used for sampling fish skin. The sampling areas were standardized at the location below

incubation at 35°C for 24 to 48 hours (Mehlman, 1984). The identification of the confirmed coliforms was completed by the API 20E system (Analytab Products, Plainview, NY).

Additional differential or selective media used in conjunction with this study to identify bacteria were: esculin, Tween 20, Tween 40, MUG, 3.0% NaCl and 7.5% NaCl. Esculin agar (Smibert and Krieg, 1981) was prepared by including 0.1 g of esculin and 0.5 g of ferric citrate per liter of plate count agar (PCA) which was supplemented with 0.5% (w/v) NaCl. Esculinase activity was detected as a smokey-brown zone surrounding the colonies. Tween 20 and Tween 40 were each included, final concentration of 1.0% (v/v), in PCA supplemented with 0.1 g of $CaCl_2$ per liter (Smibert and Krieg, 1981). Lipase activity on Tween agar was detected as a precipitate surrounding the colonies. MUG (4-methyl-umbelliferyl-B-D-glucuronide) agar (Petzel and Hartman, 1986) was prepared by including 100 mg of MUG per liter of PCA. Positive B-glucuronidase activity was detected as blue fluorescent colonies under a hand-held, shortwave (366 nm) ultraviolet light. Salt agar was prepared by including either 30 g or 75 g of NaCl per liter of PCA. Growth in the presence of salt (either 3% or 7.5% by weight) was determined as colony formation after incubation at 25°C for 48 to 72 hours.

Fresh surimi that had been tested for microbial content was stored in sterile Whirl-pack bags (Nasco, Ft. Atkinson, WI) at -20°C for up to four months. Frozen samples were tempered at

and continuously shaken at 150 rpm. Duplicate flasks were simultaneously incubated at 25°C and shaken at 150 rpm in an environmental incubator shaker (Model G24, New Brunswick Sci. Co.). The incubation temperature of 25°C was maintained by a Lauda recirculating water bath (Brinkmann Instruments, Inc., Westbury, NY) interconnected to the incubator. Bacterial growth was measured spectrophotometrically (Spectronic 20, Bausch and Lomb, Rochester, NY) by optical density at 560 nm, against a water blank. Spectrophotometric readings were recorded before and after inoculation and during subsequent incubation of the cultures. The specific growth rate (k) was calculated from the equation (Stolp, 1988):

$$k = \frac{2.303(\log X_2 - \text{Log } X_1)}{T_2 - T_1}$$

where X refers to the bacterial population at two points during the logarithmic growth phase and T refers to the time period when the two points were selected. The generation time (g_t), the amount of time required for the bacterial population to double, was calculated from the equation (Stolp, 1988):

$g_t = 0.693/k$ where k refers to the specific growth rate.

All chemicals were of reagent grade and were purchased from either Sigma, Fisher, EM Science, or Baker. Microbiological media was purchased from Difco Labs, Detroit, MI.

The taxonomic identities of microorganisms isolated from both plants would yield important information in determining if certain bacteria are characteristically associated with surimi processing. Flavobacterium species were the predominant bacteria in surimi produced at both plants (Table 9). The microflora of surimi produced at the new plant differed with that from the old plant by the absence of Arthrobacter/Corynebacterium species and the higher percentages of Pseudomonas and Acinetobacter species (Table 9). Flavobacterium and Pseudomonas species were predominant in minced fish from the new plant while Flavobacterium, Pseudomonas, Aeromonas and Lactobacillus species were predominant in surimi (Table 10). Flavobacterium, Arthrobacter/Corynebacterium and Pseudomonas species were predominant in minced fish and surimi from the old plant (Table 10). Thus, the Arthrobacter/ Corynebacterium species appeared to be characteristically associated with the old plant.

We investigated the causes for the differences in the microbial count and the microflora of the two plants by sampling additional sites such as pollock skin, fillets, water and fish waste. In the old plant, the microbial population of pollock skin was higher than that for the new plant (Table 11). Conversely, the microbial population in surimi was lower in the old plant than that for the new plant (Table 11). These data would indicate either microbial contamination or growth was apparently higher during processing at the new plant or washing

withstand dehydration and starvation (Lee and Kolbe, 1982) and these bacteria are characteristically found in fish slime (Chai and Levin, 1975). The low percentage of Flavobacterium species and high percentage of Moraxella species on pollock at the new plant could be due to differences in fish handling methods. Pollock were held in an icewater holding tank at the old plant whereas the fish at the new plant were held in totes prior to being transported into the plant. Moraxella species predominated the microflora of pollock skin at the new plant, yet these bacteria did not compete well since its numbers were reduced in the fillets and in surimi (Table 14). Arthrobacter, Flavobacterium, Acinetobacter and Pseudomonas species had been persistent and predominant bacteria of raw, iced shrimp while Moraxella species had been reduced during processing (Lee and Pfeifer, 1977). Pseudomonas species found on surimi in both plants were perhaps the result of both in-plant contamination and growth (Table 14). Pseudomonas species grow rapidly on proteinaceous foods that are held at refrigeration temperatures and these bacteria are the prime causes of seafood spoilage (Hobbs, 1983).

Temperatures were recorded at key surimi processing steps for use in the growth kinetics study. The temperatures ranged from 1.5°C to 4.0°C for the mincing and washing steps (Table 15 and 16). The refining step registered the highest temperature (11.5°C) as well as the most variability. For separate sections

We determined the recoverability of the bacterial flora after the surimi had undergone freezing and thawing. The identities of the bacteria and their respective proportions in the microflora of freeze-thaw surimi were similar to that of fresh surimi (Table 14 and 18). The high survival rate (45% to 67%) after freezing and thawing would indicate that the bacteria in surimi were well protected. The similarity between microbial flora in frozen and unfrozen surimi would indicate such protection was equally provided to all microbial genera.

Our final objective was to determine the possible origins of the microorganisms found during surimi processing. Two potential sources for microbial contamination were sampled: in-plant sites and environmental (harbor water) sites. Microorganisms were isolated from three separate sites in a surimi plant that was not in operation due to a pollock season closure (Table 19). Arthrobacter/Corynebacterium species were predominating microrganisms in dry areas of the plant whereas Pseudomonas species predominated in a wet area (Table 20). These bacteria also comprised the majority of the microorganisms found in surimi, thus the plant environment may be a contributing factor.

Harbor water was tested due to its proximity to fishing vessels and the fish processing plants. Bacteria were isolated from each of six locations along the harbor (Table 21). The identities of the bacteria at one location showed the prevalence of Moraxella, Flavobacterium and Arthrobacter/Corynebacterium

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Table 3. Total Coliform Counts[a] of Samples Obtained at Surimi Processing Steps of the New Plant

#	Mince 1	Mince 2	Drain	Wash	Refine	Dehydrate	Surimi
1	<3	<3	--[b]	<3	<3	23	23
2	<3	23	93	4	15	43	43
3	<3	<3	<3	<3	<3	<3	7
4	<3	--	21	9	240	460	>1100
5	4	9	14	23	75	210	460
6	<3	<3	23	<3	43	>1100	>1100
M	<3	<3	21	<3	29	127	252

[a] Total coliform counts reported as the most probable number (MPN) per gram.
[b] No sample taken.
# = Sampling day.
M = Median value for the six sampling days.

Table 4. Median Values of Total Coliform Counts at Two Surimi Plants[a]

Process	New Plant	Old Plant[b]
Mince 1[c]	<3	5
Mince 2[c]	<3	--
Drain[d]	21	--
Wash	<3	<3
Refine	29	31
Dehydrate	127	23
Surimi	252	43

[a] Total coliform counts reported as the most probable number (MPN) per gram.
[b] Data from Brown et.al., 1988.
[c] Two types of in-line mincers in the new plant were sampled.
[d] The drain tank containing the combined minces in the new plant was sampled.

Table 7. Total Coliform Counts of Samples Obtained at Surimi Processing Steps of the Old Plant[a]

#	Mince	Wash	Refine	Dehydrate	Surimi
1	43	<3	7	240	75
2	<3	<3	9	<3	43
M	22	<3	8	120	59

[a] Total coliform counts reported as the most probable number (MPN) per gram.
# = Sampling day.
M = Median value for the two sampling days.

Table 8. Escherichia coli Counts of Samples Obtained at Surimi Processing Steps of the Old Plant[a]

#	Mince	Wash	Refine	Dehydrate	Surimi
1	<3	<3	<3	<3	<3
2	<3	<3	<3	<3	<3
M	<3	<3	<3	<3	<3

[a] E. coli counts reported as the most probable number (MPN) per gram.
# = Sampling day.
M = Median value for the two sampling days.

Table 10. Microbiological Profile of Mince and Surimi Samples Obtained from Two Processing Plants[a]

Genus	New Plant		Old Plant	
	Mince	Surimi	Mince	Surimi
Flavobacterium	43	26	43	37
Pseudomonas	39	23	16	8
Acinetobacter	5	3	9	2
Aeromonas	2	24	4	5
Lactobacillus	3	15	0	2
Enterobacteriaceae	2	5	0	0
Alcaligenes	2	1	5	2
Arthrobacter	0	1	17	39
Moraxella	0	0	2	1
Yeast	2	0	1	0
Unidentified	3	2	5	3
NCI[b]:	61	110	81	92
APC[c]:	1.8×10^4	1.1×10^5	7.8×10^4	1.4×10^5

[a] Data for the bacterial genera are shown as a percentage in the sample.
[b] NCI = Number of colonies identified.
[c] APC = Aerobic plate count (number of bacteria per gram).

Table 13. Microbial Counts of Pollock Fillets and Samples[a] Obtained at Surimi Processing Steps of Two Surimi Plants

Sample	New Plant			Old Plant		
	APC	TC	FC	APC	TC	FC
Holding Tank	--[b]	--	--	$<2.0 \times 10^1$	<3	<3
Whole Pollock	$<6.9 \times 10^1$	<3	<3	nd	nd	nd
Fillet + Skin	nd	nd	nd	2.8×10^4	16	<3
Fillet - Skin	2.4×10^3	<3	<3	9.3×10^3	7	<3
Fillet (Belt 1)	9.2×10^3	<3	<3	--	--	--
Fillet (Belt 2)	9.1×10^4	240	<3	--	--	--
Mince #1	1.0×10^4	23	<3	8.2×10^3	<3	<3
Mince #2	6.5×10^4	43	<3	--	--	--
Drain	3.6×10^4	4	<3	--	--	--
Wash	4.7×10^4	9	<3	2.5×10^4	23	<3
Refiner	2.8×10^5	93	<3	1.2×10^5	43	<3
Dehydrator	1.8×10^6	460	<3	4.6×10^5	1100	<3
Surimi	3.0×10^6	460	<3	2.0×10^6	>1100	<3

[a] APC = Aerobic plate counts, TC = total coliforms, and FC = fecal coliforms (as *E. coli*). APC are reported as the number of bacteria per ml (for holding tank water), per cm^2 (for whole pollock) and per gram (for all other samples). TC and FC are reported as the MPN per ml (for holding tank water), per cm^2 (for whole pollock) and per gram (for all other samples). The data for whole pollock and the fillets, with or without skin, are the means for duplicate samples. The processing steps were sampled during the course of a processing day at each plant.

[b] The blank values reflect processes that were absent for each surimi plant. nd = not determined.

Table 15. Temperatures Recorded During Surimi Processing at the New Plant[a]

Sampling Site	Temperature (°C)						Mean °C
Ambient (air by mincers)	3.6	4.0	5.0	4.5	4.5	--[b]	4.3±0.5
Mince 1	2.2	1.5	2.5	2.5	3.5	--	2.4±0.7
Mince 2	2.5	1.5	2.0	2.5	2.5	--	2.2±0.5
Drain	2.7	2.2	1.8	1.8	2.0	--	2.1±0.4
Wash	3.0	2.0	2.0	2.5	3.0	--	2.5±0.5
Refiner	3.0	5.0	3.0	6.1	--	--	4.3±1.5
Pt. 1[c]	--	--	--	--	3.0	3.1	3.1±0.1
Pt. 2	--	--	--	--	--	4.0	4.0
Pt. 3	--	--	--	--	--	6.4	6.4
Pt. 4	--	--	--	--	**11.5**	9.4	**10.5±1.5**
Dehydrator	4.5	4.6	4.2	4.5	5.0	4.5	4.6±0.3
Surimi	7.6	8.0	7.5	8.0	**10.0**	8.3	8.2±0.9
Ambient (air by surimi)	5.7	6.5	4.0	3.6	5.0	4.5	4.8±1.1

[a] Temperatures were recorded at the plant during two shifts on three consecutive days of surimi processing. The temperatures that are above 10°C are highlighted in boldface.

[b] Not sampled.

[c] Points (Pt.) 1 through 4 are located at the refiner where Pt. 1 is located nearest the inlet and Pt. 4 is located nearest the exit.

Table 17. Generation Times for Bacteria Isolated from Surimi[a]

Growth Medium:	CAYE			CAS	
Growth Temp (°C):	8	12	25	12	25
Bacterium	Generation Times (hr)[b]				
Pseudomonas fluorescens	3.0	1.7	0.9	2.3	1.1
Pseudomonas sp.	3.2	2.1	0.8	2.6	1.9
Serratia fonticola	4.4	2.3	0.8	4.1	1.9
Serratia sp.	3.6	2.0	0.8	6.5	2.8
Aeromonas sp.	3.1	1.5	0.8	11.0	3.8

[a] Pure cultures were grown in 300 ml side-arm flasks containing 60 ml of either casamino acids and yeast extract broth (CAYE) or sodium caseinate broth (CAS). The cultures were shaken at 150 rpm throughout the incubation period.

[b] Generation times were calculated from the logarithmic phase of bacterial growth as measured by optical density at 560 nm.

Table 19. Microbial Counts at Various Locations Within a Surimi Plant[a]

Location	APC	TC	FC
Conveyor Belt (near filleting machines)	1.4×10^2	<3	<3
Edge of Floor Drain (near filleting machines)	6.7×10^2	<3	<3
Shelf on Post (near minced fish washers)	1.8×10^3	<3	<3
Interior of Hose (near minced fish washers)	<5	<3	<3
Inner Side of Curtain (near refining machine)	<10	<3	<3
Under Stairwell (near forming machine)	<10	<3	<3

[a] Aerobic plate counts (APC) are reported as the number of bacteria per cm^2. Total coliforms (TC) and fecal coliforms (FC) are reported as the most probable number (MPN) of bacteria per $10 cm^2$, except for the hose data which are reported as MPN per 6.5 cm^2. Swab samples were obtained in the older plant after surimi production had been halted for several months.

Table 21. Microbial Counts in Harbor Water Near Fish Processing Plants[a]

Location	APC	TC	FC
Marine Way East (east of plants)	3.7×10^2	4	<3
Ferry Terminal (between plants)	1.3×10^3	4	9
Near Island (across from plants)	8.0×10^3	<3	<3
Floatplane Dock (between plants)	5.5×10^2	<3	<3
Fish Meal Facility (west of plants)	3.4×10^3	43	23
Buskin River Mouth (west of plants)	3.1×10^3	<3	<3

[a] Aerobic plate counts (APC) are reported as the number of bacteria per ml. Total coliforms (TC) and fecal coliforms (FC) are reported as the most probable number (MPN) of bacteria per ml. Water samples were obtained after surimi production had been halted for several months.