Microbial Survey of Imported Shrimp[1]

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INTRODUCTION

Importation of frozen shrimp is a multimillion dollar industry in the United States. In 1970, imports accounted for 53% of the U.S. shrimp supply (218.7 million pounds) valued at over 200 million dollars (Anonymous, 1971). These shrimp are often suspected of contributing excessive microbial loads as well as enteric pathogens to the processed shrimp products. However, little or no data has been reported to support these charges. Most bacteriological surveys (Green 1949a,b,c,d; Williams et al. 1952a,b; Silverman et al. 1961 and Carroll et al. 1966) have been conducted using only domestic fresh and frozen products. This study was designed to provide data on the microbial quality of imported frozen raw shrimp.

METHODS

Forty-six imported frozen samples representing 17 countries and four domestic fresh samples were collected from three Florida seafood plants (Table 1). The imported samples were of two types: frozen headed (24 samples) and frozen peeled and deveined (P + D) samples. Fresh samples were usually analyzed within 4 hr of receipt, while frozen samples were stored at -20C until thawed for analyses.

A total of four subsamples from each sample were prepared for analyses as follows: three 50 g subsamples were homogenized separately by Waring blender for 2 min in the following diluents: 450 ml phosphate buffer, 50 ml distilled water and 225 ml double strength gram-negative enrichment broth (GN). The buffered phosphate water homogenate was used to inoculate standard plate count agar (SPC); trypticase sulfite neomycin agar (TSN) for *Clostridium perfringens;* trypticase soy broth with 10% sodium chloride (10% TSB) for coagulase positive staphylococci; lauryl sulfate tryptose broth (LST) for coliforms and *E. coli;* Kenner's fecal streptococcal agar (KF) for enterococci; and antibiotic potato dextrose agar (APD) for yeasts and molds. The distilled water homogenate was used for measuring the pH of the shrimp tissue immediately after preparation. *Salmonella* and *Shigella* (SS) analyses began with enrichment in the GN broth homogenate. This homogenate was washed into a sterile flask with 225 ml sterile distilled water and incubated for 24 hr at 32C. The fourth subsample (25 g) was homogenized with 225 ml glucose-salt-teepol broth (GSTB) for 2 min in a Waring blender for *Vibrio parahemolyticus* analysis.

Total plate counts were conducted by the American Public Health Association (APHA) method (American Public Health Association, 1970). Triplicate pour plates per dilution were prepared with SPC agar using 1 ml aliquots of serially diluted buffered homogenate. Incubation was at 22C for 5 days.

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TABLE 1
Distribution by Country of 50 Imported and Domestic Shrimp Samples

Country	Number of Samples
India	11
Panama	7
United States	4
Venezuela	4
Mexico	3
Saudi Arabia	2
French Guiana	2
Trinidad	2
Guatemala	2
Pakistan	2
El Salvador	2
Thailand	2
Costa Rica	2
Guyana	1
Taiwan	1
Iran	1
Kuwait	1
Honduras	1

Yeasts and molds were isolated on APD agar (Koburger, 1968). Triplicate pour plates per dilution were prepared with 1 ml aliquots of the serially diluted buffered homogenate and incubated for 5 days at 22C.

Coagulase positive staphylococci were selectively enriched by inoculation of 1 ml aliquots of the serially diluted phosphate buffered homogenate into a 3-tube most probable number (MPN) series of 10% TSB. Loopfuls from 10% TSB tubes showing turbid growth after incubation for 48 hr at 32C were streaked onto Vogel Johnson agar (VJ). The VJ plates were then incubated for 48 hr at 32C. Coagulase tests were performed on the isolated colonies obtained from the VJ plates as the final confirmatory step.

The APHA method for examination of seawater and shellfish (American Public Health Association, 1970) was followed for total coliform and fecal coliform analyses. Incubation temperatures were, however, changed to 32C instead of the recommended 35C.

Presumptive enumeration of enterococci was on triplicate KF agar pour plates. Ten representative colonies were picked from countable plates of each sample and confirmed as enterococci if they were capable of growth in brain heart infusion broth (BHI) containing 6.5% salt, growth at 10 and 45C in 3 days, and were capable of reducing 0.1% methylene blue milk. Confirmed enterococci counts were calculated by multiplying the percentage of the ten colonies from each sample which were confirmed as enterococci times the presumptive count.

Clostridium perfringens was recovered on triplicate TSN agar pour plates anaerobically incubated for 24 hr at 45C in gas-pak Brewer jars. Nonmotile, obligately anaerobic cultures capable of reducing nitrate to nitrite and producing a stormy fermentation in iron milk medium were reported as confirmed *Clostridium perfringens*.

A double selective enrichment procedure was used for the isolation of SS.

A 10 ml aliquot from the GN enrichment broth homogenate incubated for 24 hr at 32C was pipetted into 90 ml tetrathionate broth (TT). The TT broth was incubated 24 hr at 32C. Loopfuls of this broth were then streaked onto three plates each of xylose lysine desoxycholate (XLD) and SS agars, and both sets of media were incubated for 24 hr at 32C. Representative isolated colonies on these media were then confirmed using American Organization of Agricultural Chemists (AOAC) methods (Anonymous, 1967). The final confirmatory test for *Salmonella* was a slide agglutination test with polyvalent somatic antiserum. *Salmonella* cultures were then sent to the Florida State Department of Health for species determination.

Vibrio parahemolyticus was enriched in the GSTB homogenate for 24 hr at 32C. Loopfuls of this homogenate were then streaked onto three plates of previously prepared thiosulfate-citrate-bile-salts agar (TCBS) and incubated at 32C. Suspected *Vibrio* colonies were confirmed biochemically.

Lower incubation temperatures and mildly selective enrichments were used whenever possible in this study to reduce the combined stresses of temperature and the action of inhibitory agents on the debilitated micro-organisms which survived the rigors of freezing. For this reason, an incubation temperature of 32C was used in place of the usually recommended 35C for many of the above analyses. Also, the total plate count was determined after 5 days at 22C since this temperature produced higher counts than 35C.

RESULTS

Imported frozen headed shrimp samples as a group had lower total plate counts, fecal coliform counts and enterococci than the P + D samples. The four fresh samples had greater numbers of coagulase positive staphylococci and higher coliform counts than either headed or P + D samples.

Total plate counts: Fifty percent of all the samples had total plate counts greater than 1,200,000 per gram, with the range being from 1,800 to 30,000,000 per gram. Imported P + D samples had the largest average and geometric mean count of all three groups of samples (Table 2). While the headed samples had a high average count of 3,200,000 per gram, this group also had the lowest geometric mean count (420,000 per gram). Seventeen P + D samples (77%) had total plate counts greater than 1,000,000 per gram compared to only nine headed samples (38%). Also, 55% of the P + D samples had counts greater than 2,000,000 per gram, while only 33% of the headed samples recorded counts greater than this number.

Yeasts and molds: The average yeast and mold count for all samples was 750 colony forming units (CFU) per gram, with a range of 10 to 17,000 CFU per gram. Greater than 95% of the P + D samples and 87% of the headed samples had less than 1,000 yeasts and molds per gram. The sample with the 17,000 CFU per gram exhibited a musty and earthy odor.

Staphylococci: Coagulase positive staphylococci MPN's were uniformly low, ranging from 0 to 230 per gram for all samples. Headed samples had the lowest numbers, while fresh samples had the greatest numbers of staphylococci (Table 2). Twelve headed (50%) and nine P + D samples (41%) were negative for coagulase positive staphylococci, whereas 46% of headed and 42% of P + D samples recorded MPN's from 1 to 100 per gram.

Coliforms: Coliform data was divided into total coliform MPN's and fecal coliform MPN's. Total coliform MPN's ranged from 0 to 4,300 per gram (Table

TABLE 2
Some Quality Observations on 50 Imported and Domestic Shrimp Samples

	Frozen Headed (24)	Frozen P + D (22)	Domestic (4)
	organisms per g		
Total Count			
range	1,800-30,000,000	130,000-9,300,000	250,000-1,600,000
average	3,200,000	3,300,000	840,000
geometric mean	420,000	1,900,000	670,000
Staphylococcus			
range	0-150	0-230	0-230
average	18	48	60
Coliforms			
total			
average	80	62	2,000
range	0-1,100	0-430	0-4,300
fecal			
average	1.8	2.6	62
range	0-40	0-43	0-230
Enterococci			
average	6,300	27,000	6,700
range	0-120,000	0-340,000	93-19,000
Yeasts & molds			
average	1,000	380	1,100
geometric mean	190	230	1,000
range	10-17,000	30-1,600	640-1,400
	pH		
pH			
range	7.1-8.0	6.7-7.5	7.1-7.7
average	7.4	7.1	7.4

2) with an average of 230 per gram for all samples. Twenty samples were negative for any coliforms, while only one imported headed sample had an MPN greater than 1,000 per gram. Fresh samples had the highest total coliform numbers of all samples tested.

Fecal coliform MPN's were lower than the total coliform MPN's (Table 2). The overall range was from 0 to 230 per gram with maximum MPN's of 40 and 43 per gram for headed and P + D samples, respectively. Only eight samples contained fecal coliforms. *E. coli* was isolated from four samples, *Enterobacter aerogenes* from six samples and both microorganisms from two samples. Imported headed and P + D samples had a 10-fold lower average MPN than fresh samples.

Enterococci : Confirmed enterococci counts averaged 16,000 organisms per gram, with a range of 0 to 340,000 organisms per gram (Table 2). P + D samples,

as a group, had 10-fold higher counts than either headed or fresh samples. Seven samples (14%) were negative for enterococci, and of these, six were headed samples and one was a P + D sample. Twenty-three headed samples (96%) had 10,000 or less enterococci per gram compared to only 12 P + D samples (55%).

Salmonella, Shigella, Clostridium and Vibrio: Only one sample (P + D) contained *Salmonella.* This isolate was identified as *S. lexington.* A *Clostridium perfringens* count of three organisms per gram was recorded for a headed sample, while *Vibrio parahemolyticus* and *Shigella* were not detected in any of the samples.

Shrimp tissue pH: Shrimp pH values ranged from 6.7 to 8.0 for all samples (Table 2) with no apparent relationship to microbial counts.

DISCUSSION

Table 2 shows that both headed and P + D samples had average total plate counts in excess of 3,000,000 per gram. However, the geometric mean count data showed that P + D samples actually had 10-fold higher counts than headed samples, while headed and fresh samples had comparable counts. The higher counts for P + D samples can be explained in several ways. First, there is considerably more handling during processing of P + D samples than headed samples. Poor sanitary habits of workers and/or improperly sanitized machine surfaces may have been sources of contamination. Secondly, during removal of the exoskeleton the natural shrimp microflora may have been partially replaced by land microorganisms (Pedraja, 1970). These contaminants, as well as some of the remaining natural flora, may then more easily attack the shrimp tissue. However, removal of the exoskeleton should have resulted in reduced microbial numbers.

The total count data is in agreement with other studies. Approximately 31% of the samples in this study had counts of 500,000 per gram or less compared to 36% for Kachikian et al. (1959); 36% for Silverman et al. (1961) and 39% for Carroll et al. (1966). Also, the range of counts for this study was smaller than that reported by Kachikian et al. (1959), but larger than that reported by Silverman et al. (1961). Freezing appeared to have had a debilitating effect on yeasts and molds (as it undoubtedly had on other organisms), since the counts for frozen samples were lower than the counts for fresh samples. However, it should be noted that only four fresh samples were used in this study.

Coagulase positive staphycoccal MPN's were low with 22 samples recording negative counts. One should consider, however, the possibility that the MPN's were low because these microorganisms are easily overgrown by competing organisms during enumeration.

The significance of enterococci in a raw frozen shrimp product is not known with any certainty. Varga and Anderson (1968) showed that enterococci are capable of multiplying in lobster and fish residues on inadequately sanitized machine surfaces. Extremely high counts would, therefore, lead one to suspect poor sanitary procedures. It should be noted that 96% of the headed samples had counts less than 10,000 enterococci per gram as compared to only 59% of the P + D samples.

Total coliform MPN's indicate that the imported headed and P + D samples analyzed had markedly lower counts than fresh samples. This undoubtedly is the result of freezing on these organisms. Both *Enterobacter aerogenes* and *E. coli* were isolated in this study, their low numbers indicating that fecal contamination was minimal.

Only one sample (P + D) was found to contain *Salmonella*, and *Shigella* was not detected in any of the samples. These results may indicate that the salmonellae and shigellae were too debilitated to survive the selective enrichments. It is more likely, however, that the data is correct, indicating little enteric pathogen contamination in these imported products.

The importance of *Clostridium perfringens* as a food poisoning organism has been widely demonstrated in recent years. The detection of only one positive sample at a level of three organisms per gram is not surprising since the vegetative cells of this organism are very susceptible to freezing. From the data it would appear that the organism is, therefore, of little importance in these imported frozen samples.

Vibrio parahemolyticus, while not demonstrated in these samples, has been isolated from shrimp and other shellfish by several researchers. This organism is also extremely susceptible to freezing and this fact may account for our failure to isolate it from the samples examined.

Shrimp tissue pH did not appear to be a useful indicator of shrimp quality as pH values did not correlate well with total plate counts. This indicates that factors other than bacterial activity will affect the pH of shrimp tissue.

SUMMARY AND CONCLUSIONS

Headed samples, as a group, had lower total plate counts, fewer fecal coliforms and fewer enterococci than P + D samples. P + D samples had fewer yeasts and molds, fewer total coliforms and a lower average pH than headed samples. The four fresh samples had more yeasts and molds, more coagulase positive staphylococci and more coliforms and fecal coliforms than the frozen imported samples. P + D samples had the highest average enterococci counts while headed and fresh samples had approximately equal counts.

On the basis of the results described above, a number of conclusions can be drawn. The principle one being that imported shrimp, as well as domestic, entering a plant must be considered as potential sources of inoculum for equipment and product. While the levels of contamination were generally low (except for total counts), the presence in these products of organisms of public health significance must be taken into consideration during the handling of the product to avoid cross contamination. Salmonellae and coliforms usually can be adequately controlled by the use of chlorine rinses, however, the more resistant nature of the staphylococci to this sanitizing agent makes it imperative that the product entering the plant contain low levels of this particular organism.

Since the complete history of the products analyzed in this study is not known, it is rather difficult to speculate as to what would be an acceptable total count on the basis of this work.

A number of factors contribute to the microbial load on shrimp; i.e., normal flora, water quality, icing and sanitation. The most important ones being adequate icing and sanitation. If imported shrimp are to continue contributing to the U. S. domestic market, steps to improve the microbial quality of these shrimp must be instituted.

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