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MICROBIOLOGICAL QUALITY OF FROZEN LOBSTER AND CUTTLE FISH

by

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INTRODUCTION

The marine product export trade which has a history just over a decade is a well established industry in Sri Lanka. The total foreign exchange earnings from this trade has passed the 615 million rupee target for 1984. Among the frozen marine products exported from Sri Lanka, Shrimp, Lobsters and Cuttle fish are the most important. To maintain and enhance the reputation of the Sri Lankan seafood industry in the overseas market, attention was focused on the code of hygienic practice for processing lobsters and shrimps and establishing standards for the end-product quality. Quality control and examination of foods usually include counting and identifying microorganisms that can cause food poisoning.

Shellfish products like lobster and cuttle fish are very perishable and spoilage occurs relatively rapidly. Literature on chemical quality of squid and cuttle fish indicate a high level of amino nitrogen in their flesh (Velankar & Govindan, 1957). High amino nitrogen levels encourage the rapid growth of bacteria. As the processing of squid and lobsters require a lot of handling, the probability of the product becoming contaminated with microflora is greater. Health hazards like food poisoning and disease caused by pathogens are associated with such products and have been found to be responsible for 6% of the reported out breaks of staphylococal intoxication. *Staphylococcus aureaus* is known to survive freezing and frozen storage better than most vegetative bacteria (Bryan, 1973).

Vibrio paraphaemolyticus and *Clostridium botulinum* are organisms that are naturally found in freshly caught fish and shellfish in tropical waters (Cann, 1977). Other organisms such as *E.coli, Staphylococcus* aureus and *Salmonella* which are isolated from shell-fish are contaminants due to handling conditions on the decks, beaches, transportation etc.

Microbial quality studies on our frozen lobsters and cuttle fish is non existent and therefore this study was undertaken to ascertain the microbial levels of these products and also the presence and levels of organisms of public health significance such as *Staphylococcus aureus, Vibrio parahaemolyticus* and *Salmonella.*

MATERIALS AND METHODS

Collection of samples

Samples of lobsters and cuttlefish were collected from the processing factories. Lobsters were collected from five factories, A,B,C,D and E and cuttlefish were collected from three factories X, Y and Z. At each sampling occasion five samples of frozen lobster/cuttle fish were collected aseptically, into polytene bags, wrapped and secured with rubber bands and transported to the laboratory in an insulated box. Sampling was carried out on two occasions for lobsters and three occasions for cuttlefish and a total

of 50 samples of lobsters and 45 samples of cuttlefish were analyzed for total plate count (TPC/g), total coliforms (MPN/g), *Staphylococcus aureus* (/g) and *Salmonella* (/25g). Cuttlefish samples were analyzed for *Vibrio parahaemolyticus* (MPN/g) as well.

Bacteriological analysis

The analysis for total plate count, total coliform count *Staphylococcus aureus* count *V.paraphemoloyticus* count and the presence of *Salmonella*, were carried out according to the microbiological test methods of the Sri Lanka Standards Institution.

RESULTS

Lobster

The total plate count varied from $3.0x10^4$/g to $3x10^4$/g. A majority of the samples (74%) fell in the range 10^5-10^6/g (Table 1); 6% of the samples had counts of less than 10^5 whereas 8% of samples had a high count of 10^7 - 10^8/g range. All the samples from factories E and C had counts below 10^6/g.

Staphylococcus aureus was found to be 50/g in 40% of the samples and a total of 52% had counts 100/g. The highest range of 10^3- 10^4/g was recorded in 6% of the samples. Factory E had 80% of samples in the 100/g range and all the samples were below 10^3/g. All samples from factories A,B and C too were less than 103/g. In contrast, factory D had 30% of samples in the range 10^3-10^4/g and only 20% of samples below 100/g.

Total coliforms were found to be <3/g in 18% of the samples and 10^3/g in 2% of the samples. All samples in factory E had total coliforms below 100/g. Factories B and C too had counts 100/g and of this 50% and 20% respectively were found to be <3/g. The majority of the samples had counts in the 3-100g (76%) range. One sample from factory had a count over 10^3/g.

Salmonella was not detected in any of the samples during this study.

Table 1: **Percentage of Frozen Lobster Samples of Different Ranges of Bacterial Counts**

Factor	A	B	C	D	E	ALL
Range						
Total Plate Count						
10^5	20	-	-	-	10	6
10^5-10^6	50	70	100	60	90	74
10^6-10^7	10	20	-	30	-	12
10^6-10^8	20	10	-	10	-	8
Staph.aureus Count						
100	70	40	50	20	80	52
10^2-10^3	30	60	50	50	20	42
10^3-10^4	-	-	-	30	-	6
Total Coliforms Count						
<3	20	50	20	-	-	18
3 - 10^2	70	50	80	80	100	76
10^2-10^3	10	-	-	10	-	4
10^3	-	-	-	10	-	2

Cuttle Fish

Vibrio parahaemolyticus and *Salmonella* were not isolated from any of the samples tested during this study. Total counts ranged from $1.5x10^5$/g to 2.1×10^8/g. A majority (48.8%) of the samples fell within the range 10^6-10^7/g followed by 28% in the range 10^7-10^8/g (Table 2). 20% of the samples were in the range 10^5- 10^6/g and a small percentage (2.2%) had counts 10^8/g Factory X had counts within 10^5-10^6/g and 10^6-10^7/g range with only one sample in the 10^7 - 10^8 /g Samples from factory Z were in contrast, mainly found (66.6%) in the range 10^7 -10^8g. The counts in the range>10^8 were found only in this factory.

In factory X, 60% of the samples had *Staph.aureus* counts of 50/g. The rest of the samples had counts ranging from 50-150/g. Factories Y and Z had high *Staph.* counts with a majority in the ranges 10^2-10^3/g and 10^3-10^4/g respectively. Over 40 % of the total samples fell within the 10^2-10^3/g range, followed by 33.35 in the 100/g and 24.45 in the 10^3-10^4/g range. One sample from factory Z had counts>10^4/g.

The total coliform counts were very low in the samples from factory X with 60% less than 3/g and 40% in the range 3-100/g. As shown in table 2 the coliform counts in the samples from factory Z were very high with 60% of the samples with counts of greater than 10^3/g. Samples from factory Y too were fairly high with 26.6% in the range 10^2-10^3/g and 13.37 with a count more than 10^3/g.

Table 2: Percentage of Frozen Cuttlefish samples of Different` Ranges of Bacterial Counts

Factory Rang	X	Y	Z	ALL
Total Plate Count				
10^5-10^6	46.6	13.3	0	20.0
10^6-10^7	46.6	73.3	26.6	48.8
10^7-10^8	6.6	13.3	66.6	28.8
>10^8	0	0	6.6	2.2
Staph. aureus Count				
100	80.0	6.6	13.3	33.3
10^2-10^3	20.0	66.6	33.3	40.3
10^3-10^4	0	26.6	46.6	24.4
>10^4	0	0	6.6	2.2
Total Coliform Count				
3	60.0	0	0	20.0
3 - 100	40.0	60.0	26.6	42.2
10^2-10^3	0	26.6	13.3	13.3
>10^3	0	13.3	60.0	24.4

DISCUSSION

The total bacterial counts of frozen lobsters which varied from 10^4-10^7/g, is higher than the values quoted by Sumner *et al;* (1972) in New Zealand (10^2-2.33 x 10^6/g) and Yapp (1978) in Australia ($1.6x10^3$ to $2.5x10^4$/g). However, these counts are comparable with the counts on frozen prawns in Sri Lanka as reported by Sumner *et al;* (1982).

The total bacterial counts of frozen cuttlefish ranging from $1.5x10^5$-$2.1x10^8$ are higher than those found in the lobsters. This may be due mainly to the fact that cuttlefish caught in the north, north-western and north-eastern areas need longer hours of transportation whilst lobsters caught mainly in the south reach the processing factories in Colombo in a relatively shorter time. The processing technique for cuttle fish also need more handling than lobsters. The lobster shell protects its fish (or muscle) from being exposed to bacteria in comparison to the cuttlefish which has no such protection.

The total bacterial counts of fish and shellfish caught in the different environments reported to vary considerably. The fish taken from cold or temperate waters have lower counts than those taken from tropical waters (Cann, 1977 Shewan,1977). Therefore a count of about 10^5-10^6/g does not indicate that the product is of poor quality and is only a reflection of the natural condition in the tropics. Counts in excess of 10^7/g appear to indicate poor handling holding and processing of products.

The *Staphylococcus aureus* count in lobsters range from 50/g- 10^4/g, while those in cuttlefish range from 50/g 10^4/g. The cuttlefish show higher *Staph.* counts as the processing requires a lot of handling (cleaning the skin, removal of cuttle bone, viscera, ink sac etc;), where as in lobsters only the tail butt is exposed and handling is limited to washing the shell and removal of the head. Certain types of *Staph.aureus* produce a toxic substance. Whereas the *Staphylococcus* itself is destroyed by the heat of pasteurisation and normal cooking procedures, the toxic is more resistant to heat, and is destroyed on boiling for at least 30 mins (Hobbs and Gilbert, 1979). *Staph.aureus* is frequently found in the nose and the skin of man and it finds its way to food due to poor personal hygiene and manufacturing practices.

The total coliform counts of frozen lobsters and cuttlefish ranged from less than 3/g to $1.1x10^3$g majority (94%) of counts in lobsters were below 100 and only 62.2% of cuttlefish samples fell within this range.37.7% of cuttlefish samples had counts over 10^2 (Table 1 and 2). The coliform count faecal coliform count was used in standards as an indicator of poor sanitary conditions (Sumner *et al;* 1972). Coliforms are not naturally found on fresh fish and shellfish but are the result of contamination from decks, beaches and later on even in the processing factories. Thorough washing and good manufacturing practices such as clean tables, utensils and the use of gloves by the workers etc, can remove most of the bacteria as well as coliforms from the products, as is evident from the lobster results of factory E and cuttlefish results of factory X. The use of coliforms as an index of sanitary quality has been questioned by many who found this group susceptible to freezing and frozen storage (Hartman, 1960 and Larkin et al; 1956).

International standards and standards of many seafood importing countries have included *E.coli* count in the sea foods such as cuttlefish and lobsters (Appendix I). *E.coli* is a natural inhabitant of the intentional flora of man and animal and certain serotype cause diarrhoea in adults (Hobbs and Gilbert 1979).

Vibrio parahaemolyticus is naturally found on fish and shellfish. (Cann, 1977). *V. parahaemolyticus* is well established as the major cause of food poisoning in Japan and has been repeatedly isolated from

Japanese seafoods and environmental samples (Sakasaki, 1965). During the study on cuttlefish *V. parahaemolyticus* was not detected in any of the samples examined. *V.parahaemolyticus* is sensitive to heat. It is also reported to be sensitive to cold temperature (Jonson and Liston, 1973). This could be the reason for the absence of *V. parahaemolytics*.

Salmonella is not normally present in fish and shellfish caught in the open areas of the seas, although, they may be present in shellfish taken from estuarine and penned-off coastal waters (Cann 1974). The presence of *Salmonella* may be indicative of subsequent contamination due to poor handling practices and personal hygiene. However, during these studies, *Salmonella* was not detected.

Considering the microbiological limits set in the proposed Sri Lanka Standards for frozen lobsters and cuttlefish (Appendix I) it appears that the frozen lobsters can conform to the standards more frequently than frozen cuttlefish.

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Appendix I

Microbiological Limits in proposed Sri lankan Lobster and Cuttle fish standards

Test	Raw M	Quick M	Frozen C
1) Aerobic plate counts/g	106	107	3
2) Total coliforms (MPN)/g	20	400	3
3) Staphylococcus aureus/g	500	5000	3
4) Salmonella/25g	Nil	Nil	Nil

m -Bacterial limit below which a count is acceptable for any sample unit

M -Bacterial limit above which a count is unacceptable for any sample unit

C - Maximum allowable number of sample units yielding values between m and M.