Incidence and Low Temperature Survival of Coagulase Positive Staphylococci in Fishery Products*

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Coagulase-positive staphylococci was found to be absent in all the frozen samples of lobsters, cuttle fish, cat fish, seer fish and red snapper examined. Coagulase-positive staphylococci were present in 38% of the cooked frozen shrimps and only 16% of the samples had staphylococci count more than 100/g. In the case of headless, peeled and deveined, peeled undeveined shrimps, the incidence of the organism was 6, 12 and 16% respectively. The study indicated that the incidence of coagulase-positive staphylococci is not a serious problem in frozen fishery products processed in this country. There was remarkable difference in the rate of destruction of coagulase-positive staphylococci in raw and cooked shrimps during freezing and frozen storage.

From the public health point of view, coagulase-positive staphylococci are important in fishery products. Contamination of food with coagulase-positive staphylococci could cause gastroenteritis as the organism, growing in food materials in considerable numbers, secretes an exotoxin (Jay, 1978).

Fish caught from the open sea does not contain coagulase-positive staphylococci (Ridley & Slabyj, 1978). Bryan (1973), Liston (1980), Sumner *et al.* (1982) and Hobbs (1982) maintain that fish handlers are the main source of contamination of the processed product with coagulase-positive staphylococci. Thus, this organism is a useful indicator of hygiene in a process involving human handling (ICMSF, 1978; Liston, 1980 and Hobbs, 1982). The incidence of caogulase-positive staphylococci has been found to be comparatively higher in cooked fishery products, evidently due to the additional human handling after cooking and the inherent behaviour of the organism to grow competitively in substrates containing a very small number of competing micro-organisms (Appleman *et al.*, 1964; Carroll *et al.*, 1968; Neufeld, 1971). Only very little information is available on the incidence of coagulase-positive staphylococci in fishery products processed in India. Similarly, there is lack of information on the behaviour of the organism in fishery products during freezing and frozen storage. Raj & Liston (1961) observed that the organism showed a sevenfold reduction during the 130 days of storage at - 17.8°C followed by little change thereafter till the end of the study which extended upto 393 days. No other work seems to have been done on the behaviour of coagulase-positive staphylococci at sub-zero temperatures. Hence detailed studies were carried out on the incidence and low-temperature viability of coagulase-positive staphylococci in fishery products and the results obtained are summarised in this paper.

Materials and Methods

All the samples examined were collected at random from the fish processing factories situated at Cochin. About 50 g of fishery products were collected for bacteriological analysis. The swabbing technique of Tanner

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(1950) was followed to determine the bacterial load on fish contact surfaces. Water and ice samples were aseptically collected in sterile bottles. The samples were transported to the laboratory in ice under aseptic conditions and were analysed within 3 h after their collection. During the time between the arrival of the samples in the laboratory and starting of the bacterial examination, the samples were kept at refrigerated temperatures (4–5°C). Coagulase-positive staphylococci in the samples were enumerated as per the method given in IS: 2237 (1971) using Baird-Parker medium (Oxoid). Difco plasma was used for coagulase test.

Survival during freezing and frozen storage were studied using ten strains of coagulase-positive staphylococci. Strain Nos. 10345 and 936 were obtained from the National Collection of Type Cultures, London and Kings Institute, Madras, respectively. Strain Nos. 1 to 8 were isolated from frozen shrimps examined in the present study. Substrates used for inoculation were (i) fresh peeled and deveined shrimps and (ii) peeled, deveined and cooked shrimps.

On the previous day of the inoculation, the strains were separately inoculated into sterile brain heart infusion broth and were incubated at 37°C for 24 h. The cultures were centrifuged for 15 min at 5000 rpm and the liquid was aseptically decanted off. Ten ml of sterile isotonic saline was then added to the sediment, the contents of the tube were shaken to get a uniform suspension of the cells in the saline and this was centrifuged again for another 10 min at 5000 rpm. The sediment was once again washed with isotonic saline, centrifuged and the cells resuspended in 10ml of fresh saline. The suspension was diluted 10^5 times using sterile isotonic saline.

The bacterial suspension in appropriate quantities varying from 0.1 to 10 ml was added to 500 ml of sterile water contained in sterile basins. The water was stirred and the material, whether raw or cooked to be inoculated was kept immersed in this bacterial suspension for 20 min. The water was then completely drained off and samples were drawn for the determination of bacterial count. The inoculated material, after being placed in suitable cartons, was frozen at –40°C for 2½ h. The frozen slabs were separately wrapped with polythene paper and were stored in a deep freezer at – 20°C. At regular intervals, the samples from these slabs were aseptically drawn for the enumeration of the coagulase-positive staphylococci.

Results and Discussion

Incidence of coagulase-positive staphylococci on the utensils and on the palms of workers is given in Table 1. The organism was isolated from 10% of the swab samples of the surface of the utensils examined. Compared to utensils, the palms of workers showed higher incidence (34%) of coagulase-positive staphylococci. Only 1% of the water and 3% of the ice samples were found to be contaminated with coagulase-positive staphylococci as shown in Table 2. Table 3 details the incidence of coagulase-positive staphylococci in various frozen fishery products. Coagulase-positive staphylococci was found to be absent in all the samples of frozen lobsters, cuttle fish, cat fish, seer fish and the red snapper examined. A notable observation was the higher incidence (38%) of coagulase-positive staphylococci in cooked frozen shrimps. In the case of headless shell-on (HL), peeled and deveined (PD) and peeled undeveined (PUD) shrimps the incidence of the pathogen was 6, 12 and 14% respectively.

The incidence of coagulase-positive staphylococci on the surfaces of the utensils used in the processing line has great public health significance. Obviously, the material processed in the factory may also get contaminated from this source. As thoroughly cleaned and disinfected utensils used in the fish processing factories are expected to be free from bacteria of public health significance (Iyer *et al.*, 1966), the incidence of this organism on the utensils in the present study is definitely due to external contamination. The results indicate the necessity for a more scientific cleaning schedule to be followed in the factory. Contamination of the utensils can also take place as a result of their handling by unclean workers and bacteriologically defective water and ice used in the processing line. The incidence

Table 1. *Incidence of coagulase-positive staphylococci on the utensils used and on the palms of the workers employed in fish processing factories*

Nature of samples	No. of samples analysed	No. of samples positive for coagulase-positive staphylococci	No. of samples containing coagulase-positive staphylococci within the given range			
			Less than 10 per cm^2	11–50 per cm^2	51–100 per cm^2	Above 100 per cm^2
Utensils	150	15 (10)	143 (95)	6 (4)	0 (0)	1 (1)
Swabs from palms of workers	50	17 (34)	33 (66)	9 (18)	3 (6)	5 (10)

Note: 1. Percentages are given in parenthesis
2. For the purpose of calculating the percentage, fractions above 0.5 have been rounded off to the next whole number and fractions below 0.5 have been ignored.

Table 2. *Incidence of coagulase-positive staphylococci in the water and ice used in fish processing factories*

Nature of samples	No. of samples analysed	No. of samples positive for coagulase-positive staphylococci
Water	120	1 (1)
Ice	120	4 (3)

Note: 1. Percentages are given in parenthesis.
2. Fractions above 0.5 have been rounded off to the next whole number and fractions below 0.5 have been ignored.

of coagulase-positive staphylococci on the palms of workers as well as the water and ice, reported in the present study, fully justifies this assumption.

The isolation of coagulase-positive staphylococci from 34% of the palms of workers must be viewed seriously. Human beings are the main reservoir of this organism (Gilbert, 1974; ICMSF, 1978; Mossel, 1982) and the isolation of this organism from palm of workers has been accepted as a good indicator of worker's hygiene (Cann, 1977; Liston, 1980; Hobbs, 1982; 1983). In the present study, it was observed that some of the shrimp-handlers suffered from skin eruptions on their palms due to constant handling of shrimps and ice and these workers invariably harboured coagulase-positive staphylococci on their palms. Similar skin eruptions among fish handlers have also been reported by Shewan (1971). In a recent study, Sumner *et al.* (1982) have reported the occurrence of coagulase-positive staphylococci on the palms of 52% of shrimp handlers in Sri Lanka. Incidence of coagulase-positive staphylococci on the palms of workers emphasise the need for improvement in their personnel hygiene. In view of the presence of this pathogen in some of the water and ice samples, it is recommended that the water used for processing and ice manufacture may be chlorinated to a residual level of 10 ppm and the ice blocks be stored and handled in such a way that bacterial contamination could be reduced to the minimum.

Evidently, the coagulase-positive staphylococci isolated from the different fishery products in the present study have originated mainly from the palms of workers. Isolation of this organism from 34% of the palms of workers strengthens this view. The higher incidence of the organism in cooked frozen shrimps compared to the

Table 3. *Incidence of coagulase-positive staphylococci in different fishery products*

Type of product	No. of samples analysed	Incidence of coagulase-positive staphylococci	Coagulase-positive staphylococci more than 100/g
Frozen shrimps, headless shell-on	130	8 (6)	4 (3)
Frozen shrimps, peeled and deveined	150	18 (12)	9 (6)
Frozen shrimps, peeled undeveined	100	14 (14)	8 (8)
Frozen shrimps, cooked and peeled	180	68 (38)	29 (16)
Frozen lobsters, headless shell-on	6	0 (0)	0 (0)
Frozen cuttle fish	50	0 (0)	0 (0)
Frozen squids	30	5 (17)	2 (7)
Frozen red snapper	1	0 (0)	0 (0)
Frozen cat fish	8	0 (0)	0 (0)
Frozen seer fish	40	0 (0)	0 (0)

Notes: 1. Percentages are given in parenthesis
2. For the purpose of calculating the percentage, fractions above 0.5 have been rounded off to the next whole number and fractions below 0.5 are ignored.

Table 4. *Details of incidence of coagulase-positive staphylococci in fishery products*

Type of psoducts	Percentage of samples containing coagulase-positive staphylococci within the given range of					
	0 per g	1–100 pe gr	101–200 per g	201–500 per g	501–1000 per g	Above 1000 per g
Frozen shrimps, headless shell-on	94	2	1	3	0	0
Frozen shrimps, peeled and deveined	88	6	5	1	0	0
Frozen shrimps, peeled but undevined	86	9	5	0	0	0
Frozen shrimps, cookcd and peeled	62	22	7	4	4	1
Frozen squids	83	11	0	6	0	0

Table 5. *Viability of coagulase-positive staphylococci in peeled and deveined raw and cooked shrimps during freezing at -40°C and subsequent storage at -20 °C*

Strain No.	Substrate in which inoculated	Count/g		Count/g during cold storage on completion of					
		Before freezing	After freezing	1st month	2nd month	3rd month	4th month	5th month	6th month
936*	RS	5.25×10^4	2.63×10^4	1.24×10^4	7650	900	380	0	0
	CS	1.64×10^4	1.47×10^4	1.25×10^4	8550	5550	1320	360	30
10345**	RS	1.42×10^5	7.66×10^4	3.33×10^4	9800	2500	450	0	0
	CS	4.56×10^4	4.24×10^4	3.24×10^4	6760	3400	1160	200	0
1	RS	9428	5000	3260	750	170	0	0	0
	CS	1.14×10^4	9860	5600	2000	1100	600	30	0
2	RS	1.17×10^4	6000	3300	1200	540	180	0	0
	CS	1.24×10^4	1.04×10^4	6850	1850	300	50	0	0
3	RS	1.54×10^4	8480	3450	1500	770	20	0	0
	CS	1.49×10^4	1.35×10^4	6850	2500	200	40	0	0
4	RS	1.48×10^4	8950	3200	1870	670	110	0	0
	CS	1.83×10^4	1.71×10^4	1.05×10^4	9000	2500	650	50	0
5	RS	3.54×10^4	1.83×10^4	9500	5800	2100	250	0	0
	CS	6.87×10^4	6.29×10^4	2.11×10^4	1.65×10^4	8500	2300	650	200
6	RS	1.22×10^4	7500	4220	1130	660	210	0	0
	CS	1.14×10^4	9500	6700	1850	250	50	20	0
7	RS	1450	850	320	100	30	0	0	0
	CS	4935	4500	1840	850	610	30	0	0
8	RS	5.07×10^4	2.69×10^4	1.07×10^4	9300	4150	340	0	0
	CS	5.36×10^4	4.81×10^4	3.61×10^4	7050	1050	250	0	0

Note: * Strain obtained from M/s Kings Institute, Guindy, Madras (India)

** Strain from the National Collection of type Cultures, London. All the other strains were isolated from frozen shrimps in the present study.

RS = Peeled and deveined raw shrimps

CS = Peeled and deveined cooked shrimps

uncooked types is due to the handling after cooking and the inherent behaviour of coagulase-positive staphylococci to grow and thrive competitively in such substrates like cooked shrimps containing a very small number of microorganisms (Mossel, 1982). The incidence of coagulase-positive staphylococci was higher in shrimps compared to fishes. This may be due to a far greater degree of human handling involved in shrimp processing compared to the processing of fish.

Cann (1977) reported that 30% of the cooked frozen shrimps imported by the U.K. contained more than 100 *Staph. aureus*/g with the counts ranging from 50–19,000/g. Sumner *et al.* (1982) noted that 32, 35 and 67% of HL, PD and cooked shrimps respectively processed in Sri Lanka showed the presence of more than 100 *Staph. aureus*/g. Compared with these results, staphylococci counts in the shrimp samples examined in the present study are lower. According to the International Commission on Microbiological Specifications for Foods (ICMSF, 1978), 2000 numbers of *Staph. aureus*/g of cooked frozen shrimps are permitted. An 'Administrative Specification' in the U.K. insists that the staphylococci count in the frozen shrimps should be less than 104/g (Hobbs, 1976). The Australian and New Zealand Specifications of cooked frozen shrimps imported to these countries permit 1000 *Staph. aureus*/g (Sumner *et al.*, 1982). Ninety nine per cent of cooked frozen shrimps analysed in the present study contained staphylococci less than 1000/g (Table 4). Further, the organism was not detected in any of the samples of frozen lobsters, cuttle fish, cat fish, seer fish and red snapper. Therefore, in fishery products processed in this country, coagulase-positive staphylococci does not pose any serious problem.

Table 5 illustrates the viability of coagulase positive staphylococci during freezing at –40°C and further storage at –20°C. There was a considerable difference between the destruction rate of this organism in raw and cooked shrimps. In cooked shrimps, the percentage of destruction during freezing at –40°C was between 8 and 15%. There was a gradual decrease in the count during subsequent storage at – 20°C and in about 6 months, most of the test strains lost viability. In raw shrimps, the percentage of destruction during freezing ranged between 40 and 50%. Compared to cooked frozen shrimps, the rate of destruction of the organism during subsequent frozen storage was rapid in raw frozen shrimps with the result that, in about 5 months, all the strains studied lost their viability. This difference in the behaviour of coagulase-positive staphylococci in raw and cooked shrimps may be due to some inherent factor influencing the viability of the organism in the different substrates. The rapid destruction rate of coagulase-positive staphylococci during frozen storage may be due to the slow enzymic action of the natural bacterial flora, active even at low temperatures. Studies on the viability of coagulase-positive staphylococci are scanty and no such work seems to have been done in shrimps. Raj & Liston (1961) observed only a ten fold reduction of *Staph. aureus* suspended in fish homogenate within a period of 13 months at -16.8°C. Evidently, this observation is quite different from the results of the present work. Perhaps the difference in the test strains and suspending medium may be the reason for the difference in the results. Different species of the same genus and possibly even the different strains of a particular species of bacterium may vary in their resistance properties of this kind.

Results of the present study show that freezing is not the remedy for poor handling of the product. The only reliable way to produce bacteriologically safe fishery products is to use raw material having low numbers of bacteria but no pathogens and to pack them under the most hygienic conditions.

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