

STAPHYLOCOCCAL ENTEROTOXINS, ENTEROTOXIGENIC STAPHYLOCOCCI AND FROZEN FISH PRODUCTS

By

S. Sanjeev and P.K. Surendran
Central Institute of Fisheries Technology
Cochin 682029, India

ABSTRACT

One hundred and eight frozen fish products consisting of cooked, picked crab meat, prawns and ready to fry fish cutlet collected from cold stores situated in and around Cochin were examined for staphylococcal enterotoxin A, B, C, D and enterotoxigenic staphylococci. All the frozen fish products were found free from enterotoxins. *Staphylococcus aureus* was isolated from eighty six samples (79.63%) and its load varied from 55 to 1.1×10^6 CFU/g. Ninety eight *Staphylococcus aureus* strains out of one hundred and forty strains tested were found enterotoxigenic and produced enterotoxins A, B, C and D either singly or in combinations. RPLA method was used for the detection of enterotoxins but the enterotoxigenicity of *Staphylococcus aureus* isolates was not tested for enterotoxin E in this study because the RPLA kit does not contain the reagents for its testing. Enterotoxin A and D were detected more often than others. Seventy four samples contained (68.52%) enterotoxigenic staphylococci.

INTRODUCTION

Staphylococcal food poisoning is one of the most common types of foodborne illness and results from the ingestion of enterotoxin produced during growth of enterotoxigenic strains of *Staphylococcus aureus* in food (Anon., 1986). The detection of preformed staphylococcal enterotoxins in foods is therefore important in epidemiological studies of outbreaks of food poisoning and in routine quality appraisal monitoring of foods during manufacture (Rose *et al.*, 1989). Foods involved in staphylococcal food poisoning are often proteinaceous products which are subjected to contamination by the organism at the post-cooking stage. Fish products are good proteinaceous substrata which can support the growth of *Staphylococcus aureus* strains under favourable conditions. The foods involved are usually those which have been cooked or heat processed or treated with a high level of salt. The heat or the high salt content destroys or retards the growth of competing saprophytes and thus leaves a free field for staphylococci which happen to contaminate the food at the time of handling and processing.

Though few studies have been carried out in recent years on the incidence and detection of staphylococcal enterotoxins in dairy products, meat products, etc. (Batish and Chander, 1987; Park and Szabo, 1986; Rose *et al.*, 1989) no such information in respect of fish and fish products is available. Hence, the present study was undertaken to gather information regarding the incidence of staphylococcal enterotoxins A, B, C and D and enterotoxigenic strains of staphylococci in fish products especially frozen fish products.

MATERIAL AND METHODS

One hundred and eight frozen fish products (65 cooked picked crabmeat, 34 prawns and 8 fish cutlets (ready to fry)) collected from cold stores situated in and around Cochin, were examined for the incidence of staphylococcal enterotoxins A, B, C, D and enterotoxigenic staphylococci. The samples were collected in polythene bags and brought to the laboratory and subjected to analysis within 3 to 4 hours. The products were prepared for bacterial analysis by grinding a 10 g sample with 9 ml sterile normal saline. Serial dilutions were made with normal saline and 0.2 ml of the appropriate dilutions were spread on the surface of plates containing Baird Parker medium (Difco) in duplicate for *Staphylococcus aureus*. The plates were then incubated at 37°C for 48 hours. The black and shiny colonies with narrow white margin surrounded by clear zones were regarded as presumptive *Staphylococcus aureus*. These colonies were confirmed by plasma coagulase test (Leviene, 1960). The ICMSF (1978) method was followed for the determination of total bacterial count (TBC).

One part of frozen fish product was homogenized with one part sterile normal saline for testing enterotoxin. A sample of 10 ml of the homogenate was centrifuged at 900 g at 4°C for 30 minutes. The supernatant was filtered through a 0.2 µm membrane filter (Millipore) and the filtrate collected.

Reversed passive latex agglutination (SET-RPLA) kit was used for the detection of toxin in fish product extracts and culture fluids. The samples were tested and the results interpreted as per the protocols provided by the manufacturer (Oxoid).

RESULTS AND DISCUSSION

Total bacterial count, *Staphylococcus aureus* count and the incidence of staphylococcal enterotoxins in frozen fish products are given in Table 1. TBC of 68 samples (62.97%) of frozen fish products were more than 1.0×10^6 /g. Compared to other frozen fish products cooked, picked crabmeat had a higher TBC. *Staphylococcus aureus* was isolated from 98.46% of crabmeat, 73.53% of prawns and 66.67% of fish cutlet. In crabmeat, the *Staphylococcus aureus* load varied from 237 CFU/g to 1.1×10^6 CFU/g, in prawns it varied from 15 CFU/g to 903 CFU/g and in fish cutlet it varied from 100 CFU/g to 1.0×10^3 CFU/g. All frozen fish products were found free from staphylococcal enterotoxins A, B, C and D even though the *Staphylococcus aureus* load was up to 1.1×10^6 CFU/g.

It has been established that enterotoxin production at detectable levels (> 0.1 ng/g) in foods occurs only when growth reaches approximately 10^6 CFU/g (Bautista *et al.*, 1988; Bryant *et al.*, 1988; Evenson *et al.*, 1988; Noletto and Bergdoll, 1980; Woolaway *et al.*, 1986). In prawn homogenate and crabmeat homogenate, the minimum detectable level of enterotoxins by SET-RPLA kit was 0.30 ng/g and 0.6 ng/g respectively (Sanjeev and Surendran, 1992).

Enterotoxigenicity of *Staphylococcus aureus* strains isolated from various frozen fish products are given in Table 2. In an earlier study on dried fish products, 23 (88.46%) out of 26 *Staphylococcus aureus* isolates were found enterotoxigenic (Sanjeev *et al.*, 1985). In contrast to these observations on fishery products, a lower proportion of *Staphylococcus aureus* isolates from milk and meat in this country were found enterotoxigenic (Rao, 1976,

1977a, 1977b; Varadaraj and Nambudiripad, 1982). It appears from the above that *Staphylococcus aureus* isolates from fish products are more often enterotoxigenic and consequently more hazardous than similar isolates from milk and meats.

Incidence of enterotoxigenic staphylococci in various frozen fish products are tabulated in Table 3; 68.52% of frozen fish products harboured enterotoxigenic staphylococci. All fish cutlet samples were found free from enterotoxigenic staphylococci.

Distribution of enterotoxins among 98 enterotoxigenic staphylococci are indicated in Table 4. The majority of enterotoxigenic strains in the present study produced enterotoxin A followed by enterotoxin D. A similar trend was observed in respect of isolates from dried fish products by Sanjeev *et al.* (1985). These results are very interesting when compared with the preponderance of enterotoxin C producing strains in milk, meat and human carriers reported by Rao (1976, 1977a and 1977b) and enterotoxin B producing strain in Khoa reported by Varadaraj and Nambudiripad (1982). The majority of *Staphylococcus aureus* isolates recovered from cases of bacterial food poisoning in India were found to produce enterotoxin C, either alone or in combination with others (Rajalakshmi and Rajyalakshmi, 1982). In this connection, it is interesting to recall the predominance on enterotoxin D producing strain in frozen foods in USA (Casman *et al.*, 1967) and predominance of enterotoxin A producing strain in meat products in UK (Simkovicova and Gilbert, 1971; Payne and Wood, 1974).

The inability of staphylococci to compete successfully with other food bacteria has been established and reviewed by several workers (Minor and Marth, 1976; Hurst, 1973; Genigeorgis and Riemann, 1979). Most of the bacteria found in food suppresses staphylococci though some may exhibit stimulatory effects. Suppression of staphylococci growth is in general followed by suppression of enterotoxin production. If conditions are not favourable enterotoxin may be absent even in the presence of growth to well over 10^7 cells/g (Metaxopoulos *et al.*, 1981; Tatini *et al.*, 1976; Niskamen and Nurmi, 1976; Baran and Stevenson, 1975). The previous studies have shown that detectable level of enterotoxins A-D could be produced by enterotoxigenic staphylococci only in sterile fish products and not in raw fish products or cooked fish products in the presence of natural bacterial flora (Sanjeev and Surendran, 1993). However, the high incidence of enterotoxigenic staphylococci in frozen fish products must be viewed with caution.

The true incidence of staphylococcal food poisoning is far greater than is reflected by the number of outbreaks that are actually reported. The real problem with Staphylococcal food poisoning are not associated with food processors alone, but are also associated with mishandling of food in food service establishments and in home. Staphylococci can be destroyed easily but the enterotoxin can survive practically all food processing.

As stated by Minor and Marth (1972), we have all the information needed to prevent outbreaks of staphylococcal food poisoning that originate in food service establishments and in the home. If these problems could be eliminated, the number of persons suffering from this illness would drop to a negligible level.

Table 1

Total bacterial count, *Staphylococcus aureus* count and the incidence of Staphylococcal enterotoxins in frozen fish products

Name of sample	No. of samples examined	No. of samples with TBC/g (range)				No. of samples with <i>Staphylococcus aureus</i> count/g (range)						Enterotoxins			
		$\leq 2 \times 10^5$	2.1×10^5 to 9.9×10^5	1×10^6 to 9.9×10^6	$> 1 \times 10^7$	0-100 (%)	101 to 999 (%)	1×10^3 to 9.9×10^3 (%)	1×10^4 to 9.9×10^4 (%)	1×10^5 to 9.9×10^5 (%)	$> 1 \times 10^6$	A	B	C	D
Cooked, pickled and frozen crabmeat	65	4	11	33	17	1 (1.54)	7 (10.77)	37 (56.92)	15 (23.08)	4 (6.15)	1 (1.54)	-	-	-	-
Frozen prawn	34	6	12	16	-	23 (67.65)	7 (20.59)	4 (11.76)	-	-	-	-	-	-	-
Frozen fish cutlet (ready to fry)	9	3	4	1	1	3 (33.33)	5 (55.56)	1 (11.11)	-	-	-	-	-	-	-
Total	108	13	27	50	18	27 (25.00)	19 (17.59)	42 (38.89)	15 (13.89)	4 (3.70)	-	-	-	-	

Table 2

Enterotoxigenicity of *Staphylococcus aureus* strains isolated from various frozen fish products

Name of samples	No. of <i>staphylococcus aureus</i> strains isolated	No. of enterotoxigenic <i>Staphylococcus aureus</i> strains	Percentage (%)
Cooked, pickled and frozen crabmeat	118	89	75.42
Frozen prawns	15	9	60.00
Frozen fish cutlet (ready to fry)	7	-	-
Total	140	98	70.00

Table 3

Incidence of enterotoxigenic staphylococci
in various frozen fish products

Name of sample	No. of samples examined	No. of samples revealing <i>Staphylococcus aureus</i>	Percentage (%)	No. of samples revealing enterotoxigenic staphylococci	Percentage (%)
Cooked, picked and frozen crabmeat	65	64	98.45	54	83.10
Frozen prawns	34	16	47.10	20	58.80
Frozen fish cutlet (ready to fry)	9	6	66.67	-	-
Total	108	86	79.63	74	68.52

Table 4

Distribution of enterotoxin(s) among
98 enterotoxigenic *Staphylococcus aureus* strains

Enterotoxin(s) pattern	Positive No.	Isolates (%)
A	19	19.39
B	5	5.10
C	7	7.14
D	13	13.27
AB	2	2.04
AC	7	7.14
AD	7	7.14
BC	3	3.06
BD	2	2.04
CD	7	7.14
ABC	3	3.06
ABD	6	6.12
ACD	5	5.10
ABCD	12	12.24

REFERENCES

- Anon. 1986. Food borne disease surveillance in England and Wales, 1984. Br.Med.J., 293, 1424-7
- Baran, W.L. and Stevenson, K.E. 1975. Survival of selected pathogens during processing of a fermented turkey sausage. J.Food Sci., 40, 618-20
- Batish, V.K. and Chander, H. 1987. Occurrence of *Staphylococcus aureus* and their preformed enterotoxins in frozen dairy products. Australian J. of Dairy Technology, 42, 22-4
- Bautista, L., Gaya, P., Medina, M. and Nunez, M. 1988. A quantitative study of enterotoxin production by sheep milk staphylococci. Appl.Environ.Microbiol., 54, 566-9
- Bryant, R.G., Jarvis, J. and Guilbert, G. 1988. Selective enterotoxin production by a *Staphylococcus aureus* strain implicated in a foodborne outbreak. J.Food Prot., 54, 566-9
- Casman, E.P., Bennett, R.W., Dorsey, A.E. and Issa, J.A. 1967. Identification of a fourth staphylococcal enterotoxin D. J.Bacteriol., 94, 1875-82
- Evenson, M.L., Hinds, M.W., Bernstein, R.S. and Bergdoll, M.S. 1988. Estimation of human dose of staphylococcal enterotoxin A from a large outbreak of staphylococcal food poisoning. Int.J.Food Microbiol., 7, 311-6
- Genigeorgis, C. and Riemann, H. 1979. Food processing and hygiene. In: H. Riemann and Bryan, F. (Eds). Food-borne Infections and Intoxications. Academic Press, N.Y., 613-713
- Hurst, A. 1973. Microbial antagonism in foods. Can.Inst.Food Sci.Technol., 6, 80-90
- ICMSF (International Commission on Microbiological Specification for Foods). 1978. Microorganisms in Foods I. their significance and methods of enumeration. Second edition. University of Toronto Press, Toronto, London, 115-8
- Leviene, M. 1960. An introduction to the laboratory technique in bacteriology. The MacMillian Co., New York
- Metaxopoulos, J., Genigeorgis, C., Fanelli, M.J., Franti, C. and Cosma, E. 1981. Production of Italian dry salami. I. Initiation of staphylococcal growth in salami under commercial manufacturing conditions. J.Food Protect., 44, 347-82
- Minor, T.E. and Marth, E.H. 1972. *Staphylococcus aureus* and staphylococcal food intoxications, A Review-IV. Staphylococci in meat, bakery products and other foods. J.Milk Food Technol., 35, 288-341

- Minor T.E. and Marth E.H. 1976. Staphylococci and their significance in foods. Elsevier, New York
- Niskamen, A. and Nurmi, E. 1976. Effect of starter culture on staphylococcal enterotoxin and thermonuclease production in dry sausage. Appl. Environ. Microbiol., 31, 11-20
- Noieto, A.L. and Bergdoll, M.S. 1980. Staphylococcal enterotoxin production in the presence of non-enterotoxigenic staphylococci. Appl. Environ. Microbiol., 39, 1167-71
- Park, C.E. and Szabo, R. 1986. Evaluation of the reversed passive latex agglutination (RPLA test kits for detection of staphylococcal enterotoxins A, B, C and D in foods. Can. J. Microbiol., 32, 723-27
- Payne, D.N. and Wood, J.M. 1974. The incidence of enterotoxin production in strains of *staphylococcus aureus* isolated from foods. J. Appl. Bact., 37, 319
- Rajalakshmi and Rajyalakshmi, K. 1982. Types of enterotoxin production by *Staphylococcus aureus* isolated from cases of food poisoning. Indian J. Med. Res., 76
- Rao, C.C.P. 1976. Enterotoxigenic staphylococci in goat meat. J. Food Sci. Technol., 13, 264
- _____, 1977a. Enterotoxigenic staphylococci in Milk. Indian J. Microbiol., 17, 106
- _____, 1977b. Enterotoxigenic staphylococci in raw meats. J. Food Sci. Technol., 14, 224
- Rose, S.A., Bankee, P. and Stringer, M.F. 1989. Detection of staphylococcal enterotoxins in dairy products by the reversed passive latex agglutination SET-RPLA kit. Int. J. Food Microbiol., 8, 65-72
- Sanjeev, S., Mahadeva Iyer, K., Arul James, M. and Panduranga Rao, C.C. 1985. Enterotoxigenic staphylococci in dried fishery products from Cochin area. J. Food Sci. Technol., 22, 295-8
- Sanjeev, S. and Surendran, P.K. 1992. Evaluation of reversed passive latex agglutination test kits for the detection of staphylococcal enterotoxins A, B, C and D in fishery products. J. Food Sci. Technol., 29, 311-2
- _____, 1993. Production of staphylococcal enterotoxins A, B, C and D in fish products and its detection by reversed passive latex agglutination (SET-RPLA) kits. Presented in International Symposium on "nutrients and Bioactive Substances in Aquatic Organisms", held in Cochin during 16-17 September 1993
- Simkovicova, M. and Gilbert, R.J. 1971. Serological detection of enterotoxin from food poisoning strains of *Staphylococcus aureus*. J. Med. Microbiol., 4, 19

- Tatini, S.R., Lee, R.Y., McCall, W.A. and Hill, W.M. 1976. Growth of *Staphylococcus aureus* and production of enterotoxins in pepperoni. J.Food Sci., 41, 223-5
- Varadaraj, M.C. and Nambudiripad, V.K.N. 1982. Staphylococcal incidence in market Khoa and their enterotoxin production. J.Food Sci.Technol., 19, 53
- Woolaway, M.C., Bartlett, C.L.R., Wieneke, A.A., Gilbert, R.J., Murrell, H.C. and Aureli, P. 1986. International outbreak of staphylococcal food poisoning caused by contaminated lasagna. J.Hyg.Cam., 96, 67-73