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Bacterial Pathogens in Processed Frozen Fish Samples from Pune City

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ABSTRACT

Amongst non-vegetarian foods, fish foods include various alive and preserved fishes. The preserved fish and their products include loose and packaged salted, dried and frozen. The frozen fishes and their products are mainly export grade and in very little quantity they are used in local markets. Many times such processed frozen fishes are rejected due to presence of undesirable microorganisms and pathogens. In the present study processed frozen fish samples from local market of Pune city were collected and used for detection and isolation of bacterial pathogens. The fish samples collected were L-Sardine (*Sardinella longiceps*), Mackerel (*Rastrelliger kanagurta)*, Croaker (*Johnius spp.*), Ribbon fish (*Lepturacanthus savali*), Lizard fish (*Nodus indicus*), Itoyori (*Nemipterus peroni*) and L-Jacket (*Oligoplites saurus*). The enrichment and selective media were used to detect and isolate the pathogens in the processed frozen fish samples collected. The bacterial pathogens found in these fish samples were *E. coli, Salmonella paratyphi-B, Staphylococcus aureus, Bacillus cereus and Vibrio parahaemolyticus.* The attention is needed to control and remove such pathogens from food as it is alarming at safety point of view of consumers.

Key words: *Processed frozen fish, Bacterial pathogens, Pune city*

Introduction

Globally, in general and in India particularly, huge quantities of foods are spoiled every year especially by microorganisms and cause incalculable losses to producers, handlers, stockiest and industrialists. Many times packaged foods are unapproved and hence rejected as they harbor pathogenic and other microorganisms. Amongst non vegetarian foods fish is very valuable source of food for mankind. The flesh of fish is composed of proteins, fats, minerals, vitamins, iodine, phosphorus and it is easily digestible due to low percentage of connective tissues. India has a continental sea shelf of about 59.7 million hectare of which only 20% is explored. India is one of ten largest fish producing nations, but has a share of little over 3% of total fish production of the world. The seafood processing industries were established during world war-II (MPEDA, 2014).

The processed frozen fishes are mainly exported but small quantity is consumed locally.Many times improper handling, processing and packaging leads to harboring of various pathogens in it. As reported by many workersthat the fish is the most common food to cause variety of cases of illnesses caused by microorganisms in consumers which include Enteric Viruses, Protozoan Parasites, bacteria like *Yersinia enterocolitica, Campylobacter, Listeria monocytogenes, E. coli, Salmonella shigella* and *Vibrio* spp., *Staphylococcus aureus* and *Bacillus cereus* (Fellows *et al.,* 1992; Smith

et al., 2005; Novak *et al.,* 2005; Watkins *et al.,* 2008). It was reported that the growth of seafood industry and increased seafood consumption has posed challenges of spoilage and disease caused by microorganisms and estimated losses due to spoilage are 25% annually (Toranzo *et al.,* 2005). While it was reported that the hurdle technology to control microbial growth and pathogens which includes the combination of different preservation methods and processes to inhibit microorganisms (Terebiznik *et al., 2000*). The practices of detection and isolation of pathogenic microorganisms in the processed frozen fishes need to be done regularly in the places where they are consumed locally to prevent dissemination of food borne diseases.

Materials and Methods

Details of fish samples collected

The fish samples in the form of packets were collected from local market of Pune city, Maharashtra, India and details are as below:

Isolation of pathogenic bacteria from fish samples

Attempts were made to isolate following bacterial pathogens which are commonly reported (Buzby *et al.,* 1996; WHO, 2002) in the processed frozen fishesand using standard enrichment media, isolation media and procedures (Cruickshank *et al., 1985*) using the Laminar Air Flow. The identification of these isolates was done in the laboratory on the basis of their morphological, cultural and biochemical properties (Cruickshank *et al.,* 1985; Holt *et al.,* 1994). Each fish sample was processed for pathogen isolation in triplicates.

Media used for isolation of bacterial pathogens from frozen fish samples were the Mac-Conkey's and Hichrome agar (Hi-Media manual, 2010) for *Escherichia coli,* Mannitol Salt agar for *Staphylococcus aureus,* Wilson and Blair's medium for *Salmonella spp,* Nitrate agar for *Bacillus cereus and* Vibrio agar for *Vibrio parahaemolyticusin* triplicate for each fish sample. The results were compared and concluded with reference to International standards (MPEDA, 2014).

Results and Discussion

The fish samples in the form of packets were collected from local market of Pune city for the detection and isolation of pathogens.

Bacterial pathogens from frozen fish samples with respective media

The Growth of isolated pathogenic bacteria on the respective selective growth media plates is as below:

Attempts were made to isolate above bacterial pathogens (Table 3, Photo plates 2 and 3) which are commonly reported in the processed frozen fishes and using standard enrichment and isolation media. The identification of these isolates was done in the laboratory on the basis of their morphological, cul-

Table 1. Details of fish samples collected

Sr. No.	Local name of the fish	Number of samples collected	Packet Size (g)	Scientific name of the fish
1	L-sardine	5	100	*Sardinella longiceps*
2	Ribbon fish	5	100	*Lepturacanthussavali*
3	Lizard fish	5	100	*Synodus indicus*
4	Itoyori fish	5	100	*Nemipterusperoni*
5	Croaker	5	100	*Johniusspp*
6	L-Jacket fish	5	100	*Oligoplites saurus*

Table 2. Enrichment and isolation of pathogens from frozen fish samples

Sr. No.	Pathogen to be isolated	Enrichment medium used (100mL)	Amount of fish sample used (g)	Incubation temperature and time
1	*Escherichia coli*	Mac-Conkey's broth	5	37 ^{0}C for 24 to 48- h
2	*Staphylococcus aureus*	Mannitol Saltbroth	5	
3	*Salmonella spp*	Tetrathionate broth	5	
4	*Bacillus cereus*	Nitrate broth	5	
5	*Vibrio parahaemolyticus*	Peptone water pH – 9.0	5	

Photoplate 1. Fish sample Packets of processed frozen fishes

tural and biochemical properties. Total 71 different bacterial pathogens were isolated from 30 processed frozen fish samples collected (Table 3). Statistically, out of 71 bacterial pathogenic isolates 44 (62%) were *Escherichia coli* isolates. In the 44 *Escherichia coli isolates,* 3 (6.81%) were obtained on the Hicrome agar medium (pathogenic *E coli.*), *Staphylococcus aureus* isolates were 11(15.49 %) out of total isolates having 3 (27 %) coagulase positive. The *Salmonella* isolates were 7 (9.86%) in number of total isolates obtained in which 2 (28.57 % each were *S.typhi*, *S.paratyphi*-B) and 3 (33.33%) were *S. paratyphi-A.* The 5 (7.0 %) were *Bacillus cereus* of which 2 (40 %) were hemolytic in nature While *Vibrio parahaemolyticus* were 4 (5.6 %) of the total pathogenic isolates (Photoplate 3). From all the 30 fish samples *E coli* were obtained from all while other pathogens were isolated from 16 (53.33 %) fish samples. From these 53.33% of the samples, 4 (25%) each were L Jacket, L Sardine, 5 (31.25 %) of Croaker, 1 (6.25 %) each of Ribbonfish, Lizard andItoyori fishes. The pathogenic isolates used for further studies were Hicrome*E. coli* (pathogenic), Coagulase positive *S. aureus* (pathogenic), *S.paratyphi- B* (pathogens),hemolytic *B.cereus* (pathogenic) and *V. parahaemolyticus*. The numbers obtained in case of *E. coli* and Coagulase positive *S. aureus* were > 2-3/sample while detection of *S.paratyphi- B* and *V. parahaemolyticus* indicated that those fish sampleswere of reject grade (Tables 3 and 4).

It was reported that *Escherichia coli* 0157:H7 (Hicrome *E.coli*), *Staphylococcus aureus, Bacillus cereus, Salmonella spp., Campylobacter jejuni*, *Listeria monocytogenes*, *Vibrio parahaemolyticus, Clostridium perfringens* and *Clostridium botulinum* are involved in seafood borne illness (Buzby *et al., 1996*).

It was mentioned that food borne diseases cause millions of deaths every year all over the world and mainly due to undercooked and contaminated foods (Novak *et al.*, 2005).

In spite of various precautions are being taken with respect to foods like frozen fishes, regarding

Photoplate 2. Bacterial pathogens from frozen fish samples with respective media

E. coli S. aureus S. paratyphi-BB. cereus V. parahaemolyticus
Mac-Conkey's Mannitol Salt Wilson and Blair's Nitrate Vibrio agaragaragagaragar

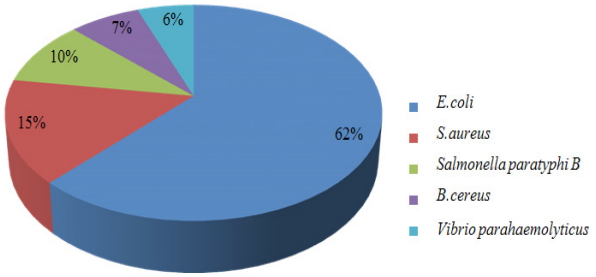
Table 3. Bacterial pathogens isolated from processed frozen fish samples

Sr. No.	Pathogen Type	Number of pathogens obtained, type of fish, Number of samples used
1	*Escherichiacoli*	44-isolates (3 on Hicromeagar), 30 samples of all six types of fishes
2	*Staphylococcus aureus*	11-isolates (3 -coagulase positive in nature) from 5 fish samples: two of Ljacket, two of Croaker and one of Lizard fish
3	*Salmonella spp*	7-isolates (*S. typhi*-2, *S. paratyphi*-A-3, *S.paratyphi*-B–2) From 6 fish samples: two of L Sardine, one each of L jacket, Itoyori, Croaker and one of Lizard fish
4	*Bacillus cereus*	5- isolates (2- hemolytic innature), from 3 fish samples: one each of L Sardine, L jacket and Ribbon fish
5	*Vibrio parahaemolyticus*	4-isolates (hemolytic innature), from 3 fish samples: two of L Sardine and one of Croaker
	Total	71

Table 4. International standards and regulations with respect to bacterial pathogens and other bacteria in frozen fish: (6): (Standards are based on fish samples of 25-g each)

Organism	Bacterial count/detection of bacteria	Grade
1) *E. coli*	>3 or one sample>40	Reject
2) *Salmonella* spp	Detected	Reject
3) *Vibriocholerae* and *V. parahemolyticus*	Detected	Reject
4) Coagulase positive *Staphylococci*	>2/or one sample >10000	Reject

(*The SPC for fish samples ranged from 10^2 to 10^4)



Photoplate 3. The percentage of types of pathogens from total pathogens isolated

cleanliness and sanitation, processing and handling, packaging and storage, use of preservatives,the prevailing climatic conditions (high temperature and moisture content) cause survival and increase in number of common bacterial pathogens in fish like *E.coli*, coagulase positive *Staphylococci*, *Salmonella*, *Vibrio*, Sulphate reducing *Clostridia* and aerobic mesophilic and psychrophilic bacteria and hence rejection in export process is a problem for India (MPEDA, 2018).

Conclusion

Ecofriendly and effective methods of preservation of processed frozen fishes is need of time to prevent dissemination of pathogens and to prevent the rejection of fish samples in export.

Conflicts of Interests

There are no conflicts of interests among authors

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