Fish. Technol. (1980) 17, 35-39

Bacteriology of Spoilage of Canned Prawns

V. NARAYANAN NAMBIAR*
Central Institute of Fisheries Technology, Cochin-682 029

Spoilage characteristics of different types of bacteria isolated from bacteriologically defective cans and processing factory environs were studied by inoculating pure cultures into sterile prawn meat. The pattern of spoilage, namely, production of off odour, bulging of the cans and disintegration of meat were observed. Data on spoilage under aerobic as well as anaerobic conditions are presented. Most of the cultures produced some kind of spoilage, though differences were observed in the extent of spoilage produced by different types of bacteria. Gram positive spore formers were found to be the major spoilers and the extent of spoilage was more with mixed cultures.

Canned prawns undergo spoilage due to bacterial action and as per the present standards they should be examined for commerical sterility before being exported. Commercial sterility may be defined as that condition in which all *Clostridium botulinum* spores and all other pathogenic bacteria, as well as more heat resistant organisms, which if present, could produce spoilage under normal conditions of storage and distribution have been destroyed (Denny, 1970). Evancho *et al.* (1973) have specified the conditions necessary for sterility testing of heat processed canned foods and Denny (1970, 1972) described the methods for the determination of commercial sterility of low-acid canned foods. Chaudhuri *et al.* (1970) have studied the factors controlling sterility in canned prawns and stressed the necessity of strict hygienic conditions in the processing centres for reducing the bacterial spoilage of canned prawns. Bacterial spoilage of canned foods may occur due to under processing or post-process reinfection due to defects in seam and rough handling. Many cases of food poisoning are due to post-process contamination. Nambiar & Iyer (1970) have made a detailed study of the type of micro-organisms present in bacteriologically defective canned prawns. As is the case with spoilage of fish (Adams *et al.*, 1964), it is very unlikely that all the bacteria are equally active in spoilage. Though the bacteriology of spoilage of fish muscle had been studied extensively (Shewan *et al.*, 1960; Lerke *et al.*, 1963; Adams *et al.*, 1964; Herbert *et al.*, 1971), very little information is available on the bacteriology of spoilage in canned prawns. Farber & Ferro (1956) have made a report on the volatile reducing substances and volatile nitrogen compounds in relation to spoilage in canned fish and Tanikawa *et al.* (1967) have studied the bacterial action involved in can swelling and blackening of canned baby clams. Put *et al.* (1972) have reviewed the mechanism of mircrobial leaker spoilage of canned foods. Mc Meekin (1975, 1977) has studied the ability of pure cultures of bacteria to produce strong off odours in chicken breast muscle and chicken leg muscles. Much work has not been done on the ability of pure cultures to produce spoilage and off odours in canned prawns. The present study was undertaken to collect information on the ability of pure cultures of bacteria isolated from bacteriologically defective cans and processing factory environs to produce spoilage of canned prawns.

Materials and Methods

Fresh prawns *(Metapenaeus dobsoni)* of uniform size were obtained from the landing places in and around Cochin. The material was cleaned, washed, peeled and deveined manually. The deveined meat was washed thoroughly in potable water and blanched in 7% brine containing 0.2% citric acid for four minutes (Varma *et al.*,

*Present address: Bombay Research Centre of Central Institute of Fisheries Technology, Bombay-400 005

1969). After draining off the brine, the blanched meat was immediately cooled under a fan and packed. For spoilage studies under aerobic conditions 50 g of the blanched material was packed in 150 ml conical flasks with 50 ml of 3% brine containing 0.2% citric acid, plugged with cotton, sterilized at 115.2°C for 18 min, cooled and used for further studies. For spoilage studies under anaerobic conditions good quality cans of 301 x 206 were used. The cans were washed properly. 128 g of blanched meat was packed with 90 ml of 3% brine containing 0.2% citric acid. After steam exhausting for 10 min, the cans were double seamed and sterilized at 115.2°C for 18 min. Immediately after autoclaving the cans were taken out, cooled in chlorinated cooling water (5 ppm available chlorine), wiped dry and used for further studies.

Pure cultures of bacteria isolated from bacteriologically defective cans, as well as processing factory environs were used for this study (Nambiar & Iyer 1970, 1971, 1973). Representative cultures having differences in one or more biochemical characteristics were selected from the isolates. The cultures, other than *Clostridium* sp. were maintained on nutrient agar slants and subcultured and tested for purity every fortnight. Suspensions of these cultures were prepared by harvesting 48-72 h nutrient agar slant cultures using normal saline. The suspensions were diluted with normal saline to give a count of approximately 10^6 cells per ml. In the case of *Clostridium* the cultures were maintained in cooked meat medium. For use, stock cultures were inoculated into thioglycollate broth and the cells from 48-72 h cultures were separated by centrifuging at 3000 rpm for 5 min at room temperature (28± 1°C), washed and suspended in normal saline to give a count of approximately 10^6 cells per ml. In addition to the 200 pure cultures, 120 mixed cultures prepared by mixing two cultures were also used in the study. Fifteen strains of *Bacillus* sp., four gram negative rods, two gram positive cocci and two gram negative cocci were used in the preparation of mixed cultures. They were mixed in the order of one *Bacillus* with one gram negative rod or one *Bacillus* with a *Cocci*. Equal volumes of suspensions of the individual cultures containing 5×10^6 cells per ml were mixed to give a final count of 1×10^6 cells per ml.

In the case of prawns packed in conical flasks, one ml each of the bacterial suspension (other than *Clostridium)* was inoculated into two flasks using a sterile graduated pipette, a strip of lead acetate paper was inserted into each flask, without touching the contents, under aseptic conditions plugged with cotton and stored at room temperature (28±1°c) for two weeks. The contents were examined for changes in odour, colour, texture, pH and the lead acetate paper was observed for blackening, if any. The contents were shaken to observe disintegration.

The cans packed with prawns were inoculated with the bacterial cultures by piercing a hole in the lid of the can using a sterile sharp needle. One ml each of the suspension was inoculated into a set of two cans using a sterile syringe. The hole was closed by soldering. The cans were incubated at room temperature for 2 weeks and observed for bulging, if any, opened and the contents examined. Odour, texture, colour, pH and disintegration of the contents were noted. The bacteria from the cans were examined for their morphology.

In another set of experiments, the prawns in conical flasks were inoculated with bacterial suspensions and steamed for 5 min and further incubated at room temperature as above, to study whether the bacteria could survive steaming for 5 min. Suspensions of bacterial strains were inoculated into the cans just before retorting and the cans were incubated and observed as before, to study the effect of autoclaving on the bacterial strains.

The effect of prolonged incubation of the cans on the survival, growth and spoilage characteristics of the different types of bacteria was also studied by inoculating the bacterial suspensions into the cans and incubating the cans for 1 year. A few cans were examined every month for spoilage and changes in the morphology of the bacteria.

Results and Discussion

The total number of bacterial strains and their types used in this study are shown in Table 1. The spoilage pattern of all the cultures except the *Clostridium* under aerobic conditions is presented in Table 2. The pattern of spoilage like production of off odour, stale odour, hydrogen sulphide, pH changes and disintegration of the meat are presented in terms of percentages of each type of bacteria showing such changes. Disintegration of meat was observed in all cases except by a few cocci, gram negative rods and mixed cultures. It was comparatively high with gram positive spore formers and with mixtures of spore formers with gram negative rods. Although all the spore formers which were components of the mixed cultures produced hydrogen sulphide when used alone, only six of them did so when mixed with gram negative rods and none produced hydrogen sulphide when mixed with gram positive and negative cocci. Similarly, all the spore formers which produced off odour when used alone did not do so when mixed with other cultures. Only eight spore formers when mixed with a gram negative rod and two spore formers when mixed with a gram negative cocci produced off odour. It may be observed from the results that none of the gram negative rods, gram positive and negative cocci produced hydrogen sulphide or off odour when used alone. In the case of five spore formers when mixed with the two gram positive cocci, no change was observed. These changes in the pattern of spoilage with mixed cultures may be either due to the inhibition of the particular reaction or due to the competitive overgrowth of the less reactive culture.

Table 1. *Microorganisms tested for spoilage characteristics*

Type of bacteria	No. of strains
Gram positive spore formers	
(a) *Bacillus* sp.	80
(b) *Clostridium* sp.	34
Gram positive non-spore forming rods	24
Gram positive cocci	24
Gram negative cocci	12
Gram negative rods	26
Total	200

Table 3 presents the spoilage pattern under anaerobic conditions. Among the gram positive spore formers all the *Clostridium* produced off odour and bulging of the cans whereas no bulging was observed in the case of *Bacillus* and only 5% of them produced off odour, the rest producing stale odour. Although none of the cultures other than the *Clostridium* produced bulging of the cans when used alone, six strains of the *Bacillus* produced bulging when used along with a gram negative rod. Similarly, whereas only four *Bacillus* produced off odour when used alone, eight of them produced off odour when mixed with two gram negative rods. This may be due to the combined effect of the components of the mixed cultures under anaerobic condi-

Table 2. *Spoilage pattern under aerobic conditions**

Type of bacteria	No. of strains	Percentage showing Stale odour	Off odour	H_2S production	pH change	Disintegration
Bacillus sp.	80	75.0	25.0	90.0	25.0	100.00
Gram positive non-spore forming rods	24	100.0	Nil	50.0	16.5	100.00
Gram positive cocci	24	80.0	Nil	Nil	Nil	80.0
Gram negative rods	26	75.0	Nil	Nil	Nil	75.0
Gram negative cocci	12	85.0	Nil	Nil	Nil	85.0
Mixed cultures	120	85.0	8.0	20.0	25.0	93.0

*Bacterial cultures inoculated into prawn meat packed in conical flasks

Table 3. *Spoilage pattern under anaerobic conditions**

Type of bacteria	No. of strains	Percentage showing Stale odour	Off odour	Bulging	Disintegration
Bacillus sp.	80	95.0	5.0	Nil	100.0
Clostridium sp.	34	Nil	100.0	100.0	100.0
Gram positive non-spore forming rods	24	66.6	Nil	Nil	50.0
Gram positive cocci	24	50.0	Nil	Nil	50.0
Gram negative rods	26	100.0	Nil	Nil	100.0
Gram negative cocci	12	75.0	Nil	Nil	75.0
Mixed cultures	120	80.0	13.0	5.0	98.0

*Bacterial cultures inoculated into prawns packed in cans

tions. In the case of cans which showed bulging, the colour of the brine changed black and the meat became pale. Small changes in pH were also observed in bulged cans whereas in all other cases the pH was 6. Though disintegration was produced by all the *Bacillus*, *Clostridium*, gram negative rods and 98% of the mixed cultures, it was 75% in the case of gram negative cocci and only 50% in the case of gram positive non-spore forming rods and gram positive cocci. Similarly, changes in odour was observed with 66.6% of gram positive non-spore forming rods, 50% of gram positive cocci and 75% of gram negative cocci, whereas it was almost 100% with other cultures. Disintegration of the meat was very high with *Clostridium*, moderate with *Bacillus* and mixed cultures and comparatively less with other cultures. Though a few cultures did not produce any change in the contents, all cultures survived in the cans as the respective strains could be isolated from the cans.

Results of the experiments carried out to study the effect of steaming after inoculation of bacteria into prawn meat showed that except 60% of the *Bacillus* and 50% of the mixed cultures,others did not produce spoilage as they were all destroyed during steaming. Even in the case of cultures which showed growth, a delay was observed in the onset of spoilage. Production of stale odour and disintegration of meat were the changes observed in these cases.

None of the cultures survived sterilization and no changes were observed in the can contents when the cultures were inoculated into the cans before sterilization. Prolonged incubation of the cans after inoculation did not show any change in the pattern of spoilage,. The cultures were found to survive such incubation without any change in their morphology.

Although canning of prawns involves heat treatment which is sufficient to destroy enzymes, micro-organisms and their toxins, canned prawns nevertheless undergo microbial spoilage under certain conditions. The canning operation may fail in two respects. First, if the cans are not subjected to sufficient heat to effectively sterilize the contents, the product may spoil on storage. This type of spoilage is known as under processing and the factors contributing to this are (a) faulty retort operation, (b) exceptional heat resistance of some bacteria present in the material, (c) excessive microbial contamination of the raw material, where the heat treatment may not be sufficient to reduce the contamination to commercial sterility level and (d) inefficient cooling of the cans after retorting which may favour the growth of thermophilic organisms surviving the heat treatment. In all these cases, the bacteriological analysis of the cans may usually show a single type of organism, especially of the spore forming type, except in cases of gross under-processing where a mixed culture may be observed. The second type of spoilage is due to post process reinfection wherein micro-organisms from the surrounding environment may gain access to the contents through leaks in the container. This type of spoilage is called

leaker spoilage and is the most significant type of spoilage in canned foods. The major factor contributing to this type of spoilage is seam defects either in the construction of the can or due to straining of the seams during processing, owing to the sudden fluctuations in the pressure outside the cans during sterilization and cooling, or during rough handling. Water used for cooling the cans after retorting is the major source of contamination due to leakage. As the cooling water is often chlorinated, bacteria other than the spore formers may be absent and hence in this type of spoilage also a single type of bacteria of the spore forming type may be observed, except in cases of excessive contamination of cooling water and rough handling of the cans. Nambiar & Iyer (1970, 1971) have observed that gram positive spore formers are predominant in bacteriologically defective cans though other types are observed occasionally. Though in the spoilage of fish muscle gram negative organisms of the *Pseudomonas* and *Achromobacter* are the major spoilers as reported by Adams *et al.* (1964), the results of the present investigation show that gram positive spore formers are the major spoilers in canned prawns. It is also observed that other types of organisms produce spoilage in canned prawns, though to a lesser degree. Majority of the bacterial cultures produce changes in the odour and texture of the material though no visible change in the external appearance of the cans is produced. The difference observed in the extent of spoilage caused by organisms other than gram positive spore formers under anaerobic conditions may be due to the unfavourable condition for the growth and proliferation of these organisms. It is also observed that with mixed cultures the extent of spoilage is more when compared to that of pure cultures.

The present study shows that bacteria, if present in sufficient numbers, irrespective of their type, can cause spoilage of canned prawns and hence the bacteriological examination of canned prawns for commercial sterility is all the more important. To prevent bacterial spoilage of canned prawns, it is necessary to ensure that the cans are given adequate heat treatment and good quality water is used for cooling.

Thanks are due to Shri K. Mahadeva Iyer, Scientist-in-Charge of Microbiology Division, for his keen interest and valuable suggestions and to Shri G. K. Kuriyan, Director, Central Institute of Fisheries Technology, Cochin for permission to publish the paper.

References

Adams, R., Farber, L. & Lerke, P. (1964) *Appl. Microbiol.* 12, 277

Chaudhuri, D. R., Iyer, T. S. G. & Pillai, V. K. (1970) *Fish. Technol.* 7, 73

Denny,C.B. (1970) *J.Ass. agric. chem.* 53, 713

Denny,C.B. (1972) *J. Ass. agric. chem.* 55, 613

Evancho, G. M., Ashton, D. H. & Brisky, E. J. (1973) *J. Fd Sci.* 38, 185

Farber, L & Ferro,M.(1956)*Fd Technol.*10,191

Herbert, R. A., Hendrie, M. S., Gibson, D. M. & Shewan, J. M. (1971) *J. appl. Bact.* 34, 41

Lerke, P., Adams, R. & Farber, L. (1963) *Appl. Microbiol.* 11, 458

Mc Meekin, T. A. (1975) *Appl. Microbiol.* 29, 44

Mc Meekin, T. A. (1977) *Appl. Environ. Microbiol.* 33, 1244

Nambiar, V. N. & Iyer, K. M. (1970) *Fish. Technol.* 7, 116

Nambiar, V. N. & Iyer, K. M. (1971) *Fish. Technol.* 8, 215

Nambiar, V. N. & Iyer, K. M. (1973) *Fish. Technol.* 10, 6

Put, H. M. C., Van Doren, H., Warner, W. R. & Kruiswijk, J. Th. (1972) *J. appl. Bact.* 35, 7

Shewan, J. M., Hobbs, G. & Hodgkiss, W. (1960) *J. appl. Bact.* 23, 463

Tanikawa, E., Motohiro, T. & Akiba, M. (1967) *J. Fd Sci.* 32, 231

Varma, P. R. G., Chaudhuri, D. R. & Pillai, V. K. (1969) *Fish. Technol.* 6, 134