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The microbiology of cooked prawns and shrimps on retail sale

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SUMMARY

In an inter-laboratory survey, 148 samples of cooked prawns and shrimps were obtained at the point of sale to the consumer. Salmonellae and *Vibrio parahaemolyticus* were not detected. *Yersinia enterocolitica* was isolated from three samples. Results for total viable count and presence of *Escherichia coli* and *Staphylococcus aureus* complied well with available guidelines for imported cooked prawns, suggesting that the risk of food poisoning from retail samples of these foods in the South of England is minimal.

INTRODUCTION

Crustaceae are generally free from contamination with the common food-borne pathogens at the time of harvesting. However, they may become contaminated during subsequent handling. Shrimps and prawns are often cooked before retailing, and shelling may take place. The peeled fish may then be breaded or battered. Coliforms, staphylococci and other mesophilic organisms may be introduced during processing. The widespread practice of freezing these fish has improved availability, resulting in an increased occurrence of shrimps and prawns as components of meals.

The International Commission on Microbiological Specifications for Foods (ICMSF) has published recommendations for the microbiological quality of this type of cooked food before it reaches the retailer (ICMSF, 1974). Using a 3-class plan, they recommend that acceptable samples should have a total count of less than 10^6/g at 35 °C, the most probable number (MPN) of faecal coliforms should be less than 4/100 g, and samples should contain less than 10^3/g *Staphylococcus aureus* and 10^2/g *Vibrio parahaemolyticus*. Occasional samples would be acceptable if total count was less then 10^7/g, MPN of faecal coliforms was less than 400/100 g, and *Staph. aureus* was less than 2×10^3/g. In the United Kingdom, guidelines have been put forward relating to imported frozen cooked prawns (Report, 1975). These guidelines allow unconditional release of the batch of imported prawns if viable counts of 5 sample units of the batch are less than 10^5/g at 37 °C, they contain less than 10/g *Escherichia coli* and 10^3/g *Staph. aureus*, and salmonellae are not detected. Conditional release is allowed with advice to use immediately on thawing if 4 of 5 sample units have total counts less than 10^6/g and the fifth count does

not exceed 5×10^6/g; also one sample might contain 10–100/g E. *coli* and 10^3–10^4/g *Staph. aureus*. Extensive sampling has been carried out on imported prawns since the introduction of these guidelines (Gilbert, 1982). However, little information is available concerning the quality of these fish at the point of consumption.

A survey has been carried out by three neighbouring public health laboratories to assess the microbiological quality of cooked prawns and shrimps when available to the consumer.

MATERIALS AND METHODS

Sampling

Samples of cooked shrimps and prawns were obtained by members of the environmental health departments from supermarkets, fishmongers, catering establishments and open stalls in markets and on the seafront. The fish originated from at least 12 countries in addition to the United Kingdom. Samples were transported to the local laboratory and examined on the day of purchase. Sampling took place between April and August 1982 and between January and April 1983.

Microbiological methods of examination

Preparation of samples

At least 20 g of sample was weighed, sufficient sterilized 0·1 % peptone in water added to form a 1/10 dilution, and the sample blended in a Colworth 'Stomacher 400' (A. J. Seward, Bury St Edmunds, Suffolk). Serial decimal dilutions were made in 0·1 % peptone from this 1/10 suspension.

Total viable count (TVC)

Total viable counts were performed using the surface drop method (Miles & Misra, 1938) modified according to Thatcher & Clark (1968). Plates were incubated at 30 °C for 2 days.

Detection of organisms

Lactose-fermenting coliforms

A 0·5 ml sample of the 1/10 homogenate was applied to the surface of two violet red bile agar (Oxoid), MacConkey agar (Oxoid) or Teepol lactose agar plates, and the plates incubated overnight at 37 °C. Lactose-fermenting colonies were counted.

Escherichia coli

Lactose-fermenting coliforms isolated at 37 °C were subcultured to test for gas production and formation of indole in peptone water at 44 °C after overnight incubation. Colonies that were positive for both tests were confirmed as *E. coli*. Alternatively, 0·5 ml of the 1/10 homogenate was applied to the surface of two Teepol lactose agar plates which were incubated at 44 °C overnight. Any lactose-fermenting colonies were then tested for indole production in peptone water after overnight incubation at 44 °C.

Staphylococcus aureus

A 0·5 ml sample of the 1/10 homogenate was applied to the surface of two Baird-Parker agar plates (Baird-Parker, 1962), and the plates incubated at 37 °C

for 2 days. Typical black, shiny colonies surrounded by a zone of opalescence or clearing were counted, subcultured and confirmed as *Staph. aureus* by testing for coagulase and/or deoxyribonuclease production.

Salmonella spp.

A 50 ml volume of the 1/10 homogenate was added to 50 ml of double-strength selenite broth and incubated at 37 °C for 2 days. The selenite broth was then subcultured to the selective media currently used by each laboratory for detection of salmonellae. Suspect colonies of *Salmonella* spp. were subjected to biochemical and serological testing.

Vibrio parahaemolyticus

A 50 ml volume of the 1/10 homogenate was added to 50 ml of double-strength salt colistin broth, incubated overnight at 37 °C, and subcultured to thiosulphate citrate bile salts agar (TCBS, Oxoid). After incubation at 37 °C overnight, suspect colonies were subjected to testing by published methods for the identification of *V. parahaemolyticus* (Furniss, Lee & Donovan, 1978).

Yersinia spp.

A 25 g portion of the sample was homogenized with 225 ml of 1% buffered peptone water (Oxoid), incubated at 4 °C for $2\frac{1}{2}$–3 weeks, and subcultured to CIN agar (Yersinia Selective agar base plus Yersinia Selective Supplement, Oxoid). CIN plates were incubated at 30 °C for 24 h. Suspect colonies were confirmed as *Yersinia* spp. by standard methods (Cowan, 1974). Confirmed strains were sent to Leicester Public Health Laboratory for biotyping and serotyping.

Other organisms

Other organisms were identified from total count plates using standard techniques.

RESULTS

A total of 129 samples of cooked prawns and 19 samples of cooked shrimps were examined. Six shrimp samples were potted, 2 shrimp and 1 prawn sample were tinned, and at least 95 samples had been frozen.

The distribution of total counts obtained after incubation at 30 °C for 48 h is shown in Fig. 1. The counts of 63·5% of samples were less than 10^5/g, and nearly 85% of samples had counts less than 10^6/g. The mean $\log_{10}$ (TVC/g) was 4·89, with a range of 2·4–8·78. No seasonal difference could be found in counts, probably due to the large number of frozen samples. Counts from peeled fish (mean $\log_{10}$ (TVC/g) = 5·05) were only slightly higher than those obtained from whole fish (mean $\log_{10}$ (TVC/g) = 4·75).

V. parahaemolyticus and *Salmonella* spp. were not detected. The occurrence of lactose-fermenting coliforms, *E. coli* and *Staph. aureus*, is shown in Table 1.

The incidence of coliforms in fish samples in the summer months was almost double that found in fish sampled in the winter months, and peeling also contributed to the occurrence of coliforms. However, the incidence of *E. coli* was low throughout the investigation. Coliforms were not found in any sample with

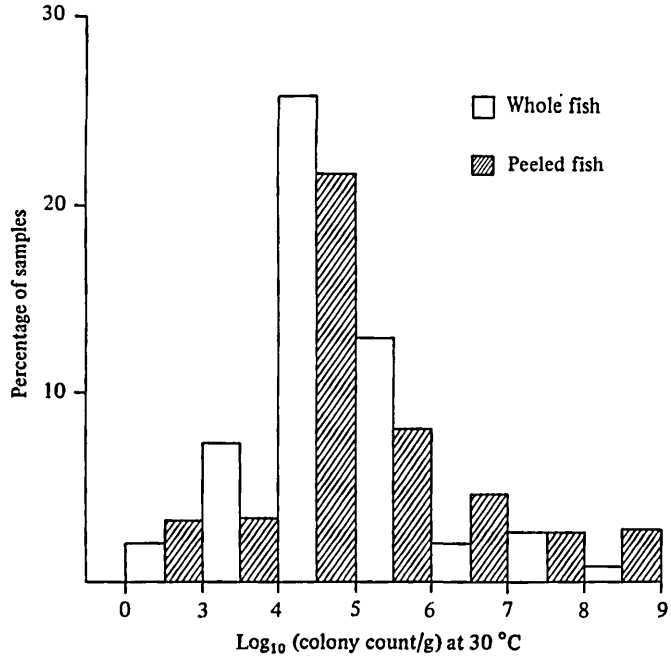

Fig. 1. Distribution of colony counts at 30 °C from 148 samples of cooked prawns and shrimps.

a viable count less than 10^4/g, but occurred in 70 % of samples with viable counts exceeding 10^6/g. The presence of *Staph aureus* was not related to the viable count of the sample; this organism occurred in products with total counts ranging from 10^2 to 10^8/g. Levels of *Staph. aureus* did not exceed 10^4/g. Incidence did not appear to depend on season or shelling.

Yersinia spp. were sought in 61 samples collected during the winter months, and three strains of *Y. enterocolitica* were isolated. All strains belonged to Wauters' biotype 1 (Wauters, 1970). Two strains belonged to serotype 0:6,30 and were isolated from whole prawns bought on separate occasions from the same fishmonger. The third strain belonged to serotype 0:7 and was isolated from whole frozen prawns originating from Greenland. In addition, *Y. ruckeri*, the causative organism of redmouth disease in some fish, was detected in one further sample of shelled prawns as a result of its cross-reactivity with salmonella 0:6,7 antiserum.

The bacterial flora of all samples received by one laboratory was determined from the total count plate. The incidence and predominance of different groups of organisms in these 86 samples is shown in Table 2, and the relationship between viable count and predominant organism or groups of organisms which constitute at least 50 % of the total flora is shown in Table 3. Gram-negative bacilli, micrococci and streptococci were the most common groups of organisms encountered. The predominance of gram-negative bacilli was not related to viable count. When micrococci or streptococci were predominant, the viable count was usually less than 10^6/g. When the viable count exceeded 10^6/g, the flora consisted mainly of gram-negative bacilli, but at lower counts the flora were more mixed, with greater proportions of micrococci and streptococci.

Table 1. *Incidence of specified organisms in cooked prawns and shrimps (organisms detected in 0·1 g by direct surface plating)*

Organism	Type of sample	Season	Log_{10} (count/g) 1·0–1·99	2·0–2·99	3·0–3·99	4·0–4·99	5·0–5·99	Number of occurrences	Number of samples
Lactose-fermenting coliforms	Whole	Summer	—	3	4	—	—	7	27
	Peeled	Summer	2	3	2	1	1	9	14
	Whole	Winter	5	3	—	—	—	8	52
	Peeled	Winter	4	3	4	1	1	13	55
E. coli	Whole	Summer	—	1	—	—	—	1	27
	Peeled	Summer	2	—	—	—	—	2	14
	Total	Winter	—	—	—	—	—	0	107
Staph.	Whole	Summer	5	—	2	—	—	7	27
	Peeled	Summer	3	—	—	—	—	3	14
aureus	Whole	Winter	9	—	—	—	—	9	52
	Peeled	Winter	8	4	2	—	—	14	55

Table 2. *The incidence and predominance of different types of bacteria in* 86 *samples of cooked prawns and shrimps* (*predominant organisms* ⩾ 50 % *total flora*)

Type of bacteria	Occurrence (%)	Predominant organism* (%)
Gram-negative bacilli	76	53
Micrococci	65	20
Streptococci	55	9
Lactobacilli/coryneforms	21	0
Aerobic spore-bearers	10	0

* Eighteen per cent of samples did not have a predominant group of organisms.

Table 3. *Relationship between viable count and predominant organism in cooked prawns and shrimps* (*predominant organisms* ⩾ 50 % *total flora*)

Predominant organisms	Log_{10} total viable count/g of product						
	2·0–2·99	3·0–3·99	4·0–4·99	5·0–5·99	6·0–6·99	7·0–7·99	8·0–8·99
Whole fish							
Gram-negative bacilli	—	6	15	5	—	—	—
Micrococci	1	—	1	2	1	—	—
Streptococci	1	2	2	1	—	—	—
Peeled fish							
Gram-negative bacilli	—	—	6	2	4	4	4
Micrococci	—	2	8	2	—	—	—
Streptococci	1	—	1	—	—	—	—

DISCUSSION

The consumption of prawns and shrimps is increasing in the U.K. and their availability is no longer restricted by transporting time since the advent of freezing. These items are particularly popular as ingredients of dishes at banquets and buffets, which usually involve catering for large numbers of people. When a food poisoning episode occurs, shellfish items are regarded with considerable suspicion.

Between 1965 and 1980, 16 food poisoning outbreaks in England and Wales were attributed to prawns/scampi (Turnbull & Gilbert, 1982). Of these 7 episodes were attributed to the presence of enterotoxin-producing *Staph. aureus*, 5 incidents to the presence of *V. parahaemolyticus* and 2 outbreaks to the presence of *Salmonella* spp. In the period 1981–2, an outbreak of food poisoning caused by salmonella was attributed to prawns, although the food was not tested (Public Health Laboratory Service, 1983). At least 4 other outbreaks of unknown aetiology were attributed to the consumption of prawn dishes during that time. Recently in the Netherlands, *Shigella flexneri* type 2 was isolated from affected persons after they had eaten frozen cooked peeled small shrimps or prawns imported from Asia (PHLS Communicable Disease Surveillance Centre, unpublished data). Salmonellae and *V. parahaemolyticus* have also been isolated from prawns imported from Malaysia,

India and occasionally the Mediterranean area. Despite this, the number of food poisoning incidents caused by prawns and shrimps represents only a very small proportion of all food poisoning episodes.

In general, specimens of prawns and shrimps tested in this survey complied well with the guidelines relating to release of imported frozen cooked prawns into the U.K. The total viable counts at 30 °C of 85% of samples were less than 10^6/g. Although 22% of samples contained *Staph. aureus*, none contained more than 10^4/g and less than 3% of samples contained more than 10^3/g. Only 2% of samples contained *E. coli*, and levels of *E. coli* exceeded 100/g in only one sample. These results also comply with limits specified by the ICMSF; over 90% of samples in this survey satisfy these requirements. An increase in total count would be expected by the time the fish reached the consumer unless they were eaten immediately after defrosting. Also, viable counts performed at 30 °C are generally higher than those obtained at 35–37 °C. Handling and peeling might be expected to contribute to higher counts and to introduce mesophilic spoilage and pathogenic organisms. However, contamination with *E. coli* and *Staph. aureus* generally appears to be minimal. High levels of lactose-fermenting coliforms are probably due to temperature abuse, as coliforms were not found in fish with counts less than 10^4/g, but their presence indicates some post-processing contamination.

It is of interest to note that the microbial population of cooked prawns and shrimps comprises mainly gram-negative bacilli, particularly as total counts increase. In a previous survey (Greenwood *et al.* 1984) the microbial flora of most 'ready to eat' products examined was composed predominantly of gram-positive cocci and bacilli. The microflora associated with raw prawns and shrimps from temperate waters is composed predominantly of gram-negative bacilli (Beckers *et al.* 1981). Cooking of prawns and shrimps tends to be minimal in order to preserve texture and flavour, and may not necessarily result in total destruction of the bacterial population. Also subsequent cooling and handling allows recontamination of the fish with psychrophilic bacteria.

The significance of the recovery of *Y. enterocolitica* from three samples of whole cooked prawns is not clear. This organism is recognized as a food-borne pathogen, but its epidemiology is not yet clear. Yersiniae are capable of survival and multiplication at refrigeration temperatures. Many strains of *Y. enterocolitica* are able to produce heat-stable enterotoxin under aerobic conditions between 10 °C and 28 °C (Robins-Browne, Barson & Koornhof, 1981) and enterotoxin production also occurs at refrigeration temperature in some strains (Kapperud & Langeland, 1981). It is possible that ingestion of pre-formed toxin might cause illness if consumed in sufficient quantity, particularly if the food was allowed to remain at ambient temperature before consumption. It has also been shown that strains of yersiniae grown at low temperatures are much more efficient in adhering to the intestinal epithelial cells (Martinez, 1983), and that several serotypes possess invasive properties similar to those described for salmonellae and shigellae (Carter, 1981). Further studies are required to elucidate the role of yersinias in the pathogenesis of gastroenteritis.

Despite the time lapses and extra handling involved during the retail process, most samples of cooked prawns and shrimps examined in this survey satisfied the guidelines used by the United Kingdom for the microbiological quality of imported

frozen cooked prawns, and the risk of food poisoning from these fish appears to be minimal.

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