FIRTA 75/9

AN EXAMINATION OF BACTERIOLOGICAL, CHEMICAL AND PHYSICAL ASPECTS OF HANDLING PRAWNS

FINAL REPORT

for

the project

Studies of the bacteriology of prawns

N.C. Gillespie

Queensland Fisheries Service
Southern Fisheries Research Centre
Deception Bay

26 February 1980

1. Introduction

The object of the project 'Studies on the Bacteriology of Prawns' was to examine the effect of current handling and storage techniques on the bacteriological and biochemical quality of prawns. This objective was extended to include the development of a simple quality test for prawns. In addition, considering developments in the export of whole prawns, an investigation was undertaken to assess the effect of current handling techniques on their structural integrity. In this way factors affecting recovery of whole prawns for export were determined and procedures that would minimize losses were investigated. Consequently, this report will deal with bacteriological, chemical and physical aspects of prawn quality, with particular reference to prawns handled in an unfrozen or "wet" condition.

2. Bacteriological aspects of handling prawns

(a) Effects of handling prawns on fishing vessels

When captured, prawns are usually associated with a large number of other marine species, many of which exude large quantities of slime or are associated with filth. This causes heavy contamination of the prawns on the sorting table which usually have bacterial counts ranging from 100,000/g to 1,200,000/g. Heavy washing with a strong jet of water or in a tank of circulating clean water subsequently reduces the range of bacterial counts to 1.0×10^3/g to 1.8×10^4/g, effectively reducing the bacterial load by 99%.

...2...

The type of bacteria initially present varies greatly with locality. Banana prawns (Penaeus mergiensus) freshly caught in the Gulf of Carpentaria have a bacterial population consisting predominantly of Acinetobacter spp. However, those caught in south-east Queensland have an initial bacterial flora consisting chiefly of Vibrio spp.; bacteria from either of these genera do not appear to be significant in prawn spoilage.

Following immersion in refrigerated seawater (RSW) at -1°C the bacterial count of prawns drops for 1-2 days, then remains stationary for up to 8 days. During this time, the bacteria initially present are replaced by ones able to grow at 0°C and sub-zero temperatures. The better the conditions of low temperature storage, the longer the lag period before bacterial levels increase. Because the temperature of RSW tanks on board a prawn trawler normally may sometimes range up to 5°C (sometimes 10°C), the lag period before bacterial numbers increase may, in practice, be much shorter than 8 days.

The bacterial count of the refrigerated seawater itself begins to increase 1-2 days earlier than that of the prawns. Odours produced in the liquid phase by bacteria growing on substances leached from the prawns can often be quite powerful and precede odour development in the prawns themselves.

Once bacterial numbers curve upwards, odour production proceeds very rapidly and only three to four days need elapse before spoilage occurs. Once the bacterial count of

...3...

the prawns has reached 500,000/g, the remaining shelf life is very limited. Even though odours are not detectable at this stage, any lapses in temperature control will allow the rapid development of spoilage odours.

Storage experiments showed that when bacterial numbers reach 1,000,000/g, the bacterial flora usually consists of four different types of organisms: Pseudomonas spp. able to grow at low temperatures but with a lesser ability to produce spoilage odours; Alteromonas spp. similar to A. putrefaciens which, while lacking in the ability to produce H_2S are able to produce ammonia from arginine (present in high concentrations in prawns), trimethylamine from trimethylamine and a variety of sulphydryl compounds comprising a significant proportion of the spoilage odours of prawns; Arthrobacter spp. which principally split into two groups, based on the ability to produce trimethylamine.

The presence of sodium sulphite does not appear to significantly affect bacterial development, even at very high concentrations. However, sodium sulphite solution has an acid pH and this tends to reduce the numbers of Alteromonas spp. responsible for spoilage odours. Dipping prawns in sodium sulphite with free SO_2 levels of 3,000ppm for 30 seconds, whilst effective against black spot, has little effect on bacterial numbers of spoilage. Bacterial development in the dip solution itself is rapid and for this reason dip solutions must be renewed frequently.

Cooking on board vessels does not appear to affect the pattern of bacterial development. Whilst the cooked

...4...

prawns are "pastuerised" by the boiling water, they are immediately recontaminated with bacteria from the seawater used to cool them, as well as all of the implements and containers used to handle them. Bacteria causing spoilage of cooked prawns are of similar identity to those of uncooked prawns. However, because they are a cooked product, contamination with bacteria such as Staphylococcus aureus, Vibrio parahaemolyticus and Escherichia loli is of concern not only from the public health point of view, but also because of the necessity to meet bacteriological standards. Uncooked prawns are rarely submitted to bacteriological testing, the idea being that any harmful bacteria will be destroyed during cooking.

In this regard it must be noted that there are very few sanitizers available which are effective when used with saltwater. Despite the enforcement of certain facilities such as wash basin and toilets on export registered vessels, these improvements are meaningless if the product handling surfaces are in an unclean condition. It is important that the fishing industry encourage local detergent or sanitizer manufacturers to develop cleaners and sanitizers that are compatible with saltwater and thus are effective on board trawlers.

(b) Effect of handling and processing on shore

Any problems with bacteriological spoilage on shore are usually subordinate in importance to those caused as a result of black spot and shell damage. However, where spoilage does occur, it is related to excessive times of

...5...

storage in the large eskies used for transporting prawns on land. However, the speed at which losses can occur due to the loss of integrity of the prawns and black spot when prawns are stored using this method, prompts most processors to turn them over very rapidly.

In general, the bacteriological quality of raw prawns for processing or packaging is good and counts vary principally in the range 40,000/g to 600,000/g. About 16% of vessels in a survey of boats supplying wet banana prawns in the Gulf of Carpentaria landed prawns with counts ranging from 3 million to 100 million/g. These higher counts can be attributed to poor washing and inadequate or faulty refrigeration.

Whilst public health bacteria such as Staphylococcus aureus and E. coli are usually not monitored in the raw product, it is significant to note that the most significant source for contamination with these types of bacteria was found to be the freezing moulds used for packing prawns for freezing by plate freezer. During a survey of one prawn processing operation it was found that prawn cutlets with coliform counts of about 20/g suffered gross contamination when packed into moulds and afterwards showed levels of about 20,000/g. Although most machinery and surfaces are thoroughly cleaned and sanitized, the freezing moulds quite often are neglected between freezing cycles, the only cleaning usually being a quick treatment with a hose. It is stressed that these moulds should be thoroughly washed using a good detergent/sanitizer between cycles to assure against

...6...

bacterial contamination.

The production of breaded prawn cutlets lends itself to bacteriological problems. Breaded prawn cutlets sometimes may show coliform levels up to 50,000/g. In these cases it is often found that the batter contributes large numbers through contamination in the containers used to mix the batter, from improperly cleaned batter circulation equipment and from the batter mix itself. Milk powder is a major ingredient in commercial batter mixes and can carry significant numbers of coliform bacteria as well as creating peculiar cleaning problems due to the formation of "milk stone". Consequently, prawn processors should set certain bacteriological specifications for the manufacturers of batter mix as follows:

Total viable count	50,000/g
Mould	100/g
Yeasts	10/g
Coliform negative in 1 g.	

Also, a strict cleaning regime should be daily applied to the bread and batter machines, with complete dismantling and brushing of all components. A chlorine-type sanitizer should be circulated in the unit before use.

Cooked prawns are subject to bacteriological surveillance in international trade and their handling requires strict sanitation on board vessel and in land operations as well. They are easily contaminated by unsanitized bins, by weighing trays previously used for raw prawns or fish and by the ungloved hands of personnel. Consequently, the

...7...

handling of cooked prawns for export needs to be strictly supervised to ensure they are not contaminated by E. coli or Staphylococcus aureus which will render them unacceptable for export.

3. Chemical aspects of handling prawns

(a) Black spot

Black spot, or melanosis is the most obvious chemical change occurring during the storage of prawns. Adequate practices have been developed to minimize the problem on board fishing vessels, so this will not be discussed here. The development of black spot in frozen prawns being thawed for processing however, remains a problem on occasions. During the course of this project, it was observed that the control of this problem depends upon design of the thaw tank. Factors affecting black spot formation are time, temperature and exposure to air. With these factors in mind, the thaw tank used for thawing blocks of frozen prawns, needs to be fitted with a chilled water supply coupled with a thermostatically regulated solinoid valve to maintain thaw tank temperature at about 10°C. In addition, the tank should circulate the water at sufficient velocity to thaw an entire block of prawns within 20 minutes. Loading and unloading rates must be matched to processing capacity to avoid bottlenecks.

(b) Trimethylamine and total volatile nitrogens

Levels for trimethylamine (TMA) and total volatile nitrogen (TVN) are routinely included in product specifications for seafoods in general. The limit of acceptability

...8...

for prawns in some sectors of the Australian marketing system has been established at 5 mg TMA and/or 30 mg TVN/100 g of flesh. Work during the course of this project showed that prawns stored in brine or in RSW rarely attained these levels. In storage experiments, 8 mg TVN/100 g of flesh was not exceeded, even after 18 days storage. High levels were only obtained when an extreme effort was made to produce a putrid soup. An additional factor was the ratio of prawns to liquid, but in general leaching of volatile basic compounds under normal conditions of transport and storage in the prawning industry is sufficient to render tests for TMA or TVN irrelevant to our situation. A similar situation applies for cooked prawns. In general, determinations of compounds such as TMA and TVN merely corroborate poor organoleptic ratings on spoiling product. They are mainly applicable in cases where legal action may be taken but are not practical for routine quality control of prawns. These findings are similar to those of several overseas workers who examined the suitability of TMA and TVN for the quality control of prawns.

(c) Hypoxanthine accumulation in prawns

Hypoxanthine is regularly proposed in the literature as an index of quality of various seafoods. The formation of this compound from the degradation nucleoside phosphates present in the prawn flesh. One of these substances, inosine monophosphate (IMP) is a major flavour potentiator and in combination with animo acids, is responsible for the sweet distinctive flavour of fresh prawns. The degradation of

...9...

IMP to inosine and then to hypoxanthine coincides with the loss of fresh flavour. During the course of this project the accumulation of hypoxanthine in both banana prawns (Penaeus merguiensis) and eastern king prawns (P. plebejus) was studied.

The rate of hypoxanthine accumulation in king prawns was greater than banana prawns but the peak levels of about 1.8 u mole/g in each case were higher than those reported overseas for Pandalus and Pandalopsis spp. The hypoxanthine levels reach a peak and begin to fall after about 10 days in P. plebejus but continue to rise in P. merguiensis even after 16 days.

Consequently, it appeared that significant changes in hypoxanthine levels were occurring between 0 and 10 days storage, suggesting that this compound may form the basis for a simple test to determine the previous storage history of prawns.

(b) Development of a simple test for hypoxanthine

Various attempts had been made by overseas workers to abbreviate the analysis of fish samples for hypoxanthine but most have required expensive laboratory equipment. However, the publication of an enzyme strip method in 1976 by American workers laid the foundation for the development of a simple test for prawns. Whilst removing the need for test tubes, solutions and other equipment, the original method necessitated the preparation of neutralized acid extracts. The production of these extracts is usually the lengthiest part of an analysis and requires some laboratory equipment. Consequently, a great deal of effort was expended

...10...

during this project deriving a method of sample preparation whereby the hypoxanthine concentration could be determined without any complex laboratory manipulations.

It has been found that it is possible to produce a sample extract in phosphate buffer that is compatible with the enzyme strip. The extract is derived simply by homogenizing the sample in a household blender and dipping the strip immediately in the homogenate. This method has proved very simple and accurate, with very good correlation being found with spectrophotometric assays performed in the normal manner. The efficacy of the test in the field has been proven by trials conducted in Karumba under very rough conditions.

Unfortunately, the hypoxanthine levels found in prawns during the first five days of storage are so low that the enzyme strip is insensitive to changes during this period and it is quite common to experience false high readings at this stage.

In addition, as the levels accumulated by prawns are only about a third those found in fish, the sensitivity of the hypoxanthine test for prawns is also much less. Another problem with the strip method is the stability of the reagent in the strip itself. It is found that the strips will remain true for only two weeks, after which they must be constantly recalibrated against hypoxanthine standards. Correspondence with scientists working with enzyme strips at the Unilever Research Laboratory in the UK suggests that they are having similar problems.

...11...

Consequently, it must be stated here that the enzyme strip procedure is not sufficiently developed to enable its application on a routine basis at present. The problems with sensitivity and stability suggests that a strip formulated somewhat differently from the original may need to be developed. This work is to continue at the Deception Bay Laboratory of the Queensland Fisheries Service.

4. Physical aspects of handling prawns

(a) Effect of handling on board vessel

Apart from black spot, or freezer burn, shell damage or loss of the structural integrity is one of the principal reasons why prawns do not reach export standard. Considering that premium returns result from the export of whole prawns, especially tiger prawns, it is important to identify those areas where shell damage occurs or where the attachment of the head is loosened.

Freezer boats should not experience any problem with the landing of prawns suitable for packing as whole prawns, provided that reasonable care is taken and the refrigeration is operating satisfactorily.

However, boats with only "wet" chilling facilities do produce a more fragile product. Damage occurring during vessel motion contributes mainly to this, as well as natural aging on storage. During the course of this work it was shown, that under controlled storage conditions at $-2^{o}C$, in static RSW, prawns begin to lose their structural integrity after 5-6 days. Because of vessel motion, this period is reduced to 3-4 days with the recovery of whole

...12...

prawns being reduced significantly after 2-3 days storage. The Queensland Fisheries Service submitted a research proposal to the Fishing Industry Research Committee for 1979, entitled "Evaluation of methods to assure the quality of prawns in wet storage" which was to investigate the use of containerization to reduce this damage. Subsequent to this proposal being refused financial support, the Queensland Fish Board performed trials which showed that containerizing prawns in lidded baskets on board vessels has an extremely significant effect on the physical quality of both raw and cooked prawns. The concept of containerization involves the packaging of prawns in open meshed lidded baskets (the 8 kg baskets used on some freezer boats in Northern Australia are excellent for this purpose). They are then dipped in sodium sulphite solution and stored in the RSW tanks. Upon unloading, the baskets are packed into the large insulated "eskies" now universal in the prawning industry and transported to the processing plant. The containerization concept completely eliminates the 3-4 scoop-transfers that occur presently, substantially minimizes damage due to vessel motion and speeds up unloading operations by use of conveyors. It also allows the development of mothership operations for wet vessels by facilitating the transfer of product. This would greatly extend the range and mobility of wet boats.

However, at present, trawlers with no freezer storage extend their time at sea by adding salt to the RSW so that a lower temperature can be reached without freezing up the refrigerated tanks. This often leads to the prawns becoming

...13...

frozen. Whilst freezing decreases hydraulic damage from vessel motion, the slow freezing that prawns undergo in the fortified RSW leads to shell separation and loosening of the heads upon thawing. Consequently, although prawns hard frozen in salt fortified RSW are attractive in appearance upon unloading, significant changes can be noticed when they are thawed:

(i) The flexible hinge between the third and fourth large tail segments is stretched, the membrane forming the connection to the shell being pulled away (although still attached) resulting in a separation between the two segments. If the tail is tightly curled to rigor mortis, the membrane separation is more severe along the back of the tail, allowing the fourth segment to overlap the third when the prawn is straightened, giving it a mutilated appearance.

(ii) The lower flexible joint between the tail and the head is stretched and weakened. Prawns frozen in refrigerated brine have a head with an exaggerated kicked-up appearance. When they are thawed the heads are separated from the tail by 2-3 mm more than those of normal unfrozen prawns. This creates greater pressure on the upper retaining membrane, often distending the connection of this membrane to the head. This creates the impression of a loosened head and predisposes the prawn to greater damage during transport.

...14...

Each of these changes appears to be the direct result of slow freezing in the brine tanks. Such damage can be reduced if RSW tanks are operated at not lower than -2° to $-3^{\circ}C$. There may be some need to add some additional salt because of the inherent inaccuracy of thermostats in association with a static system, but there should be no need to raise the salt concentration above 4.5%.

(b) Effect of processing and handling on shore

Retaining physical quality requires that handling of the prawns be kept to a minimum, and is "gentle" when it occurs. Areas where whole prawns suffer damage during inspection and packaging in a typical processing plant include:

(i) Dropping onto the inspection conveyor from the wash tank elevator.

(ii) Dropping from the inspection conveyor onto the grader elevator.

(iii) Dropping from the elevator onto the top pan of the roller grader.

(iv) Jostling caused by the "anti-piggy-backing" knobs on the roller grader.

(v) Dropping from the roller grader to the chute of the roller grader.

(vi) Violent "knock-out" procedures when removing frozen prawn blocks from the moulds.

The effects of problem areas (i), (ii), (iii) and (v) can be reduced by using smooth flexible chutes positioned at an angle appropriate to lessen the impact of dropping vertically onto a horizontal surface. Removal of the knobs on the rollers of a roller grader (by machine grinding)

...15...

greatly enhances recovery of whole prawns graded in this manner. Belt graders can also be used for grading whole prawns as they cause minimal damage.

5. Conclusions

(i) Bacterial spoilage or growth should not be a problem for prawns stored at zero and sub-zero temperatures for 8 days and less.

(ii) Alteromonas putrefaciens appears to be significantly involved in spoilage when it occurs.

(iii) The presence of sodium sulphite has little effect on bacterial development but does affect the composition of the bacterial flora.

(iv) A pattern of spoilage occurs on cooked prawns similar to that of uncooked ones.

(v) Contamination of cooked prawns with Staphylococcus aureus, Vibrio parahaemolyticus and Escherichia coli can only be controlled by the adoption of effective sanitation programs.

(vi) Estimations of trimethylamine and total volatile nitrogen do not give a satisfactory index of prawn quality because of leaching.

(vii) Hypoxanthine gives virtually the only hope of a simple quality test for prawns. However, even with the simplified sample preparation procedures developed during this project, the enzyme strip method is still not ready for routine use in the industry.

(viii) Containerization of prawns greatly reduces shell damage and simplifies handling and storage.

...16...

(ix) Operating RSW tanks at below -3°C causes damage to the structural integrity of the prawn through slow freezing.

(x) Recoveries of whole prawns in processing plants can be boosted by a few simple procedures.

6. Publication of results

Detailed technical results will be published in the "Journal of Applied Bacteriology" and "Journal of Food Science". Information of practical interest to the fishing industry will be submitted to the editor of "Australian Fisheries" in the near future.