

Interim Report on Project "Studies on Fish
Spoilage in Queensland"

72/3 interim report.
Final report could not
be located.

1. Financial Report

Expenditure 1st July to 30th November

Travelling	\$111.55
Operating	\$573.27
Capital	-

2. Experimental Report

(a) Introduction

The main aim of this project has been to produce a method for the detection and enumeration of bacteria capable of producing spoilage odours on fish. As described in the previous report, the achievement of this object has involved the successful accomplishment of some intermediate goals which were:-

(i) The isolation and selection of bacteria capable of producing spoilage odours on fish.

(ii) The identification and characterization of these bacteria and their comparison with non-spoilage types.

(iii) The confirmation of the activity of the selected spoilage bacteria in fish spoilage using gas chromatographic analysis of head space volatiles.

All phases of this work have been completed. A new method for the detection and selective enumeration of spoilage bacteria has proved to be highly successful. The method should be an extremely valuable tool to all phases of the fishing industry and indeed any food industry where proteinaceous foods are being handled. As the method is extremely simple, it can be used by industry personnel with little prior knowledge of bacteriology.

An attempt was made to use similar principles for a simple objective test for determining fish quality. However, this proved less successful than had been hoped and the idea was discarded. Instead the use of head space gas chromatography as a

tool for use in fishery problems has been and is being developed further. Specific details of these phases of the project are provided in the various sections of the report.

This document is intended to be an interim report only and a comprehensive collation of all the information obtained will be presented in July, 1976. This will include copies of all publications derived from this work. Much of the information is being collated for a paper entitled "Fish Spoilage - causes and effects" to be presented at the Fisheries exposition in September, 1976.

(b) Bacteria responsible for spoilage of fish

Approximately 1,200 bacterial isolates have been screened for their ability to cause fish spoilage. As was stated in the previous report, those most active in fish spoilage appeared to be members of the genus Pseudomonas. Isolates from this group were divided into two groups; those capable of producing spoilage odours and those which did not.

The two groups, totalling 154 isolates, were then subjected to a massive testing programme involving 160 separate biochemical and nutritional tests in order to characterize and identify them as well as determine characteristics that could be used to distinguish spoilage from non-spoilage organisms.

Computer analysis of the results of this section of the investigation reveals the following:-

(i) Pseudomonas isolates from fishery sources can be divided into two large groups, both of which contain spoilage and non spoilage bacteria. The first group, which contains all organisms resembling Ps. fragi and Ps. fluorescens includes bacteria producing fruity odours when grown on fishy substrates. The second group includes a well defined cluster of organisms which produce very strong sulphhydryl-type odours. This cluster shows some similarity to Ps. putrefaciens, an organism that has been implicated in the deterioration of a wide range of foodstuffs including meat and chicken as well as cutting oils

used in industry.

(ii) Organisms producing fruity odours show no outstanding characteristics which distinguish them from other pseudomonads and appear to belong to the fluorescent group of pseudomonads even though they do not produce a fluorescent pigment. Gas Chromatographic analysis of spoiling fish indicates that the fruity esters produced by these organisms, are not important in early spoilage but are present in advanced decay.

(iii) Organisms resembling Ps. putrificiens are found in proportions ranging from 20%-60% at the time of spoilage and seem to be the most important spoilage bacteria in terms of the organoleptic defects produced. They can be separated from other pseudomonads by their ability to produce copious amounts of sulphhydryl compounds during growth.

The method for the specific enumeration of spoilage bacteria described later is therefore based on the understanding that the most serious spoilage organisms are capable of producing sulphhydryl compounds.

(c) A Method for the specific detection and enumeration of fish spoilage bacteria

Methods previously proposed for determining numbers of hydrogen-sulphide producing organisms by various workers have included the use of iron salts in a bacteriological medium. Production of black colonies indicates the presence of hydrogen sulphide and implicates that particular organism in fish spoilage. Trials with these types of methods showed them to be completely unsatisfactory and the results very difficult to interpret.

Instead, another approach to the detection of sulphhydryl producing organisms was used to produce a test that could be performed by industry personnel with a minimum of training. The materials needed are as follows:

- (i) normal nutrient agar in a petri dish

- (ii) Glass spreaders for spreading a dilution of the sample over the surface.
- (iii) A disc of cellophane (unlacquered) cut to fit the inside of the petri dish.
- (iv) A disc of 9 cm filter paper (glass fibre)
- (v) Reagent solution

After performing a normal surface plate count on the sample and counting the number of colonies, the cellophane disc is pressed gently onto the surface of the plate and the disc of filter paper placed on top. The filter paper is then moistened with about 2 ml of reagent solution. Sulphydryl producing spoilage bacteria produce coloured spots on the filter paper due to reaction of volatiles with the reagent solution. The paper is then peeled off the plate and the spoilage bacteria counted. Thus a total count and a spoilage count can be performed on the same plate.

The method would be particularly valuable for internal quality control by processors of fresh fish and for assessing the storage history of fresh or frozen fish products without the need for elaborate testing procedures.

Unfortunately the procedure does not detect organisms producing fruity odours but under local conditions these do not seem to be as significant as the sulphydryl type. According to recent overseas work, the method should also detect most of those organisms capable of reducing trimethyanine oxide to trimethylamine, the compound which causes the fishy, ammonia like odour sometimes characteristic of spoiling fish.

(d) Headspace gas chromatography of seafood products

In the course of this project the analysis of the volatiles emitted by various samples of volatiles were analysed by gas chromatography, yielding fundamental information of great worth. At the same time however, it was realized that the method could be of use to the fishing industry for investigation or solution of everyday quality problems.

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For example, the technique revealed that the products of spoilage that increase in greatest amount during the storage of fish are methyl mercaptan, dimethyl sulphide, dimethyl disulphide, trimethylamine and, ethyl acetate. Many other compounds are present, but the magnitude of change in the above volatiles places them above the others in significance. With this knowledge, fish samples or prawns with defects can be quickly analyzed to determine whether the fault is due to microbial growth or other causes.

Also, properties of some products such as the "iodine" taint in some fish and prawns can be easily monitored using this technique.

Work during the final six months will concentrate on refinements and simplification of the system so that samples can be treated more quickly.

(e) A simple test for determining fish quality

Investigations for a simple quality test for seafoods centred on determinations of sulphhydryl compounds. The procedure would have had to be quicker and yield results better than accepted tests such as that for trimethylamine or total volatile nitrogen. Two different approaches were used:

- (1) A quick filter paper-cellophane technique similar to that developed for the rapid test for spoilage bacteria and
- (2) A more refined technique involving the preincubation of a piece of fish flesh and flushing with a set volume of gas into a chromogenic solution which changed colour in the presence of sulphhydryl groups. The change in absorbance was then read on a spectrophotometer.

Approach 1. Although differences in the colouration were evident during storage life, the interpretations of colour changes were as subjective as an organoleptic judgement would have been.

Approach 2. Changes in absorbances were obtained during the storage life of the product. However, as with determinations of trimethylamine, total volatile nitrogen etc, spoilage changes were

evident organoleptically before marked increases in absorbance were detected using the procedure. Also, as no time was saved over that needed for those accepted tests this approach was abandoned.

It appears as though any test for freshness or spoilage based on the endproducts of bacterial activity could never be as rapid or improve substantially the assessment of a trained grader. For this reason, it is planned to cease attempts to improve this type of test for fish and fish products.

Instead, in the project "Bacteriology of prawns", tests based on changes brought about by tissue enzymes are being used to determine freshness by measurements of hypoxanthine and other compounds. Changes in these substances begin earlier after death and increase at a reasonably constant rate during storage at a rate dependent on the temperature. Consequently, this method would give earlier indication of the quality of the product than those used in this project.

(f) Publications

As a result of investigations in connection with this project the following paper has been published:

Gillespie, N.C. and MacRae, I.C. (1975) The bacterial flora of some Queensland fish and its ability to cause spoilage. J. Appl. Bact. 39, 91.

and the following papers are in the course of preparation:

Gillespie, N.C., English, N.J., and MacRae, I.C. (1975) Identification and characterization of Pseudomonas spp. isolated from spoiling fish using computer methods. J. Appl. Bact. (to be submitted).

Gillespie, N.C. and MacRae, I.E. (1975) Definition of the roles played by various Pseudomonas spp. in causing fish spoilage using headspace gas chromatography. J. Appl. Bact. (in preparation).

Gillespie, N.C. and MacRae, I.C. (1975) A method for the detection

and selective enumeration of bacteria producing sulphhydryl compounds from spoiling fish. J. Appl. Bact. (to be submitted).

(g) Conclusion

The principle objective of this project to produce a method for the detection of spoilage bacteria has been accomplished. At the same time, valuable information on the bacteriological state of the fishing industry as well as methods for its study has been obtained. Summarized briefly the results of the investigation have revealed the following:-

- (i) The initial bacterial flora of fish caught in Queensland waters consists chiefly of bacteria incapable of causing spoilage.
- (ii) Fish on the market floor have widely varying bacterial counts and the composition of the bacterial flora varies much more drastically than the freshly caught product.
- (iii) Potential spoilage organisms are greatly reduced in numbers by the washing procedures performed concurrently with processing. Contamination after filleting can greatly reduce the shelf life of fish fillets.
- (iv) Spoilage bacteria belong principally to the genus Pseudomonas. Those causing fruity odours are strongly related to Ps. fluorescens and Ps. fragi while those causing sulphhydryl type odours are similar to Ps. putrificiens.
- (v) The most serious defects are associated with high concentrations of sulphhydryl compounds mainly, methyl mercaptan, dimethyl sulphide and dimethyl disulphide.
- (vi) A method allowing the simultaneous determination of total count and spoilage bacteria count on a sample was developed.
- (vii) Headspace gas chromatography has been found to be a very useful tool in investigations into organoleptic defects of seafoods.